The impact of hypoxia on in vivo glucose uptake in a hypoglycemic fish, *Myoxocephalus scorpius*

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MacCormack TJ, Driedzic WR. The impact of hypoxia on in vivo glucose uptake in a hypoglycemic fish, *Myoxocephalus scorpius*. *Am J Physiol Regul Integr Comp Physiol* 292: R1033–R1042, 2007. First published September 28, 2006; doi:10.1152/ajpregu.00308.2006.—The mechanisms controlling carbohydrate utilization in teleost fish are poorly understood, particularly in the heart. Tissue glucose uptake and cardiovascular characteristics were measured in the short-horned sculpin, *Myoxocephalus scorpius*, a species exhibiting low blood glucose levels, during normoxia and hypoxia to assess the role of adenosine receptors in the control of glucose uptake and anaerobic metabolism. As expected, hypoxia exposure (300 min at 2 mg/l dissolved oxygen) resulted in a bradycardia and plasma lactate accumulation, but glucose uptake rates did not change in heart, brain, gill, spleen, and white muscle. Plasma glucose-to-intracellular glucose ratios indicated that glucose uptake was the rate-limiting step in glucose utilization. The majority of intracellular glucose was unphosphorylated, however, suggesting that hexokinase is also important in controlling the tissue glucose gradient. During hypoxia, the cholinergic blocker atropine resulted in tachycardia but did not significantly change tissue glucose uptake rates or heart and brain adenosine levels. In contrast, the combined treatment of atropine and an adenosine receptor blocker [8-(p-sulfophenyl)theophylline] during hypoxia increased heart glucose uptake to levels fivefold higher than normoxic fish, with no additive effects on cardiovascular parameters. Significant tissue lactate accumulation was observed in this group of fish, signifying that adenosine receptors may depress anaerobic metabolism, even though tissue adenosine accumulation was absent during hypoxia. White muscle accumulated glucose during normoxia, suggesting the presence of gluconeogenic pathways or active uptake mechanisms not previously described in this tissue.

2-deoxyglucose; adenosine receptor; atropine; short-horned sculpin

CIRCULATING GLUCOSE LEVELS vary widely among teleost fish species (6), and glucose homeostasis is not tightly regulated as it is in mammals (28). In vivo glucose utilization under normoxic conditions has been studied in the heart and locomotor muscle of exercising carp (*Cyprinus carpio*; Ref. 45) and rainbow trout (*Oncorhynchus mykiss*; Ref. 44) and in a number of tissues in the brown trout (*Salmo trutta*; Refs. 4, 5). These species have plasma glucose concentrations between 3 and 10 mmol/l, typical of the levels observed in many fish. Other species, like the Antarctic eelpout (*Pachycara brachycephalum*) and short-horned sculpin (*Myoxocephalus scorpius*), have only 0.2–0.5 mmol/l plasma glucose (14, 42). The characteristics of tissue glucose uptake and the regulation of carbohydrate metabolism have not been examined in fish with naturally low blood glucose levels. Tissues, such as the heart and brain, which remain active under hypoxia, upregulate anaerobic metabolic pathways and are reliant on carbohydrate fuels for ATP production. Hypoxia potentiates in vitro glucose uptake in both the American eel (*Anguilla anguilla*) and the Atlantic cod (*Gadus morhua*) heart (7, 35). Although this demonstrates the capacity for increased glucose uptake in fish hearts, it is difficult to predict the importance of this phenomenon in vivo. Glucose uptake rates were ~90–130 nmol·g⁻¹·min⁻¹ in in vitro heart preparations from eel (35) and cod (7), whereas, in vivo uptake rates in carp, rainbow trout, and brown trout hearts were only 5–10 nmol·g⁻¹·min⁻¹ (5, 44, 45).

In fish, the potential for increased heart glucose uptake has not been demonstrated in vivo. Plasma glucose can increase during hypoxia in many species of fish, including rainbow trout (43), Atlantic cod (33), European eel (*Anguilla anguilla*) (18), and several species of armored catfish (25, 26). Changes in plasma glucose during hypoxia are potentially important in determining the rate of tissue glucose utilization, an issue that has not been addressed in in vitro glucose uptake studies.

Glucose enters the cell through facilitated glucose transporters (GLUTs), and, once inside, it is phosphorylated to glucose-6-phosphate (G-6-P) by hexokinase (HK). In the brown trout, a large part of the glucose taken up by the heart and brain in vivo remains unphosphorylated (5), indicating that HK is a potentially limiting factor controlling the rate of glucose utilization. In ventricular strip preparations from cod, however, virtually all of the glucose taken up was phosphorylated (7), suggesting that HK activity is not rate limiting. Information on tissue glucose gradients, in conjunction with data on the intracellular factors controlling glucose utilization, would be valuable in clarifying the precise mechanisms regulating carbohydrate metabolism in fish.

Hypoxia leads to cardiac and brain adenosine accumulation in mammals. Adenosine interacts with specific cell surface receptors that subsequently cause vasodilation and increase fuel and oxygen delivery to sensitive tissues. Adenosine also influences hypoxic cardiac metabolism in mammals by enhancing glycolytic flux (46), increasing glucose uptake (1), and decreasing myocardial oxygen consumption (19). Hypoxic adenosine accumulation does not seem to occur in teleosts (24, 25); however, a number of studies suggest that it may influence fish carbohydrate metabolism. The blocking of adenosine receptors leads to an apparent activation of anaerobic metabolism in hypoxia-exposed rainbow trout and hagfish (2) and increases the excretion of ethanol in anoxic crucian carp (31). Adenosine can also affect cardiovascular performance (40); therefore, it is unclear whether it directly

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influences metabolism or whether alterations are a consequence of other physiological effects such as changes in cardiac workload.

The objective of this study was to characterize tissue glucose uptake, intracellular glucose levels, and the mechanisms influencing these factors in short-horned sculpin. In doing this, we present the first measurements of the gradient of glucose from plasma to the intracellular compartment in a fish. We investigated the possibility that low blood glucose levels are associated with differences in the characteristics of tissue glucose uptake relative to fish that exhibit higher glucose levels. For the first time, we assess the impact of hypoxia on in vivo tissue glucose uptake in a fish. We determined the influence of a hypoxic bradycardia on tissue glucose uptake and lactate and adenosine levels by injecting sculpins with atropine before hypoxia. We hypothesized that fish treated with atropine would exhibit higher adenosine levels and glucose uptake rates during hypoxia. To separate the impact of changes in heart rate (fH) from a possible direct influence of adenosine on carbohydrate metabolism, a group of atropine-treated fish were also given the adenosine receptor blocker 8-p-(sulphophenyl)theophylline (8SPT) before hypoxia, and their cardiovascular and glucose uptake characteristics were determined.

MATERIALS AND METHODS

Short-horned sculpin (Myoxocephalus scorpius; n = 24, body mass \(833 \pm 53\) g) were captured locally and held in aerated seawater (8 \(\pm 2\) °C). Fish were fed to satiation weekly with chopped herring. Fish were also fitted with a cannula at the junction of the ventral aorta and bulbus arteriosus for serial blood sampling and isotope and drug injections. A 100-cm length of PE-50 tubing was heat shaped to aorta and bulbus arteriosus for serial blood sampling and isotope and drug injections. A 100-cm length of PE-50 tubing was heat shaped to aorta and bulbus arteriosus for serial blood sampling and isotope and drug injections.

Experimental system. The experimental system consisted of a covered, temperature-controlled (8 \(\pm 0.4\) °C) tank containing 35 liters of water, oxygenated by an air pump supplemented with 100% O2 when necessary. Dissolved oxygen (DO2) was monitored by pumping water across a DO2 electrode (YSI model 95D; Yellow Springs Instruments, Yellow Springs, OH) with the use of a submersible pump that also circulated the water in the tank. DO2 was reduced in <5 min by gassing with 100% N2 and maintained within 4% of the desired level by gassing with 100% O2. Excessive movement of the fish in the system was restricted by an adjustable, perforated plastic box to avoid entangling the flow probe lead and cannula.

Surgical procedures. Fish were anesthetized with 80 mg/l eugenol (Sigma, St. Louis, MO), positioned on their right side on a surgery table, iced, and ventilated with cold 50 mg/l eugenol solution. The rostral end of ventral aorta was accessed via the opercular cavity and fitted with an ultrasonic flow probe (model 2.0SB or 2.5SB; Transonic, Ithaca, NY) as previously described (24). Blood flow was monitored by a Transonic T106 flowmeter interfaced with a PowerLab data acquisition system (ADInstruments, Castle Hill, NSW, Australia).

Fish were also fitted with a cannula at the junction of the ventral aorta and bulbus arteriosus for serial blood sampling and isotope and drug injections. A 100-cm length of PE-50 tubing was heat shaped to conform tightly to the body and opercular cavity of the fish. The cannula was curved \(\approx 90\)° toward the head of the fish \(\approx 10\) mm from the tip to match the anatomy of the ventral aorta. A 5-mm length of 22-gauge polished stainless steel tubing was attached to the tip of cannula to aid in insertion. The cannula was bubbled at \(\approx 20, 65,\) and 120 mm from the tip. The ventral aorta was held stationary with blunt forceps and punctured by a 23-gauge needle reshaped to remove the cutting edge. The cannula was then implanted \(\approx 6\) mm into the vessel. Minimal bleeding was evident when the vessel was punctured, and it stopped immediately when the cannula was inserted. The cannula was secured inside the opercular cavity by suturing once behind the first bubble, allowing it to pivot with each heart beat. If the cannula was not correctly shaped to the fish’s body or if it was not allowed to move with the vessel, the tip would adhere to the wall of the aorta and prevent blood sampling. The cannula was subsequently routed with the flow probe lead and secured to the ventral and dorsal aspects of the animal with sutures behind the bubbles in the tubing.

Fish were allowed to recover for \(\approx 40\) h in the experimental system before trials were carried out. Water was aerated, maintained at 8°C, and replaced after 24 h to maintain quality. During recovery, the cannula was attached to a syringe pump infusing heparinized saline (100 IU/ml) at 0.25 ml/h to prevent clotting. Hematocrit was monitored after surgery, and animals exhibiting low hematocrit levels (below 20%) or blood loss were not used in the study. Blood flow parameters were recorded overnight to attain resting values.

Glucose uptake measurements. Glucose uptake rates were measured with radiolabeled 2-\(\text{-}\text{deoxyglucose}\) (2-DG) by a modification of the method of Blasco et al. (5). The duration of each experiment was 300 min. Fish were injected with trace amounts of 2-\(\text{[3H]}\text{DG (15.8 \(\mu\)Ci/kg)}\) and the extracellular marker [\(\text{L-14C}\)] glucose (LG) (4.5 \(\mu\)Ci/kg) (Perkin-Elmer, Boston, MA) in saline (2.5 ml/kg) 60 min after the trial was initiated. After injections, the cannula was flushed with three volumes of saline. Blood samples (200 μl) were collected before isotope injections \([\text{time} (t) = 60\) min] and at 15, 30, 60, 120, 180, and 240 min after the injection. Plasma was immediately separated by centrifugation, and 20 μl were collected and counted for radioactivity in a Tri Carb 2100TR scintillation counter (Packard, Downer’s Grove, IL) after the addition of scintillation fluid (Ecolumine; ICN Biomedical, Costa Mesa, CA). Remaining plasma was quickly frozen in liquid nitrogen and stored at \(\approx 80°C\) for subsequent analysis. The total volume of blood sampled in any trial \((<2\) ml) was small relative to total blood volume and did not appear to significantly affect hematocrit.

At the termination of experiments, fish were quickly removed from the system and killed by a blow to the head, and the spinal cord was severed. Brain, heart (ventricle), spleen, and white muscle were collected and immediately frozen in liquid nitrogen. White muscle was taken from behind to the dorsal fin, \(\approx 2\)–3 cm above the lateral line. Gill arches were then dissected, and the lamellae were scraped with a razor blade to remove the soft tissue, which was frozen as above. Samples of all tissues were retained for determinations of water content by drying to a constant mass in a vacuum oven.

Normoxia and hypoxia treatment. For control experiments \((n = 6),\) an initial blood sample was taken \((t = 60\) min), and fish were injected with isotopes and exposed to normoxic conditions (9.4 mg/l DO2) for an additional 240 min. Blood samples were taken at the intervals described above. For hypoxia treatments \((n = 6),\) a blood sample was taken during normoxia (9.4 mg/l, \(t = 0\) min) to determine resting glucose levels. Oxygen was then reduced to 2.0 mg/l over 60 min, with fish being held for 30 min at 5.6 and 3.8 mg/l during the step down. Blood was sampled when DO2 reached 2.0 mg/l \((t = 60\) min), and then isotopes were injected. Fish were held at this level of DO2 for 240 min, and blood samples were collected at the intervals indicated above. Short-horned sculpin exposed to deeper levels of hypoxia exhibit frequent struggling and rapid loss of equilibrium (24). Cardiorespiratory parameters and temperature were continuously monitored, and recordings were collected just before each change in DO2 and at 60-min intervals while the fish were at 2.0 mg/l.

Effect of atropine treatment on glucose uptake and adenosine levels. To assess the effects of fH on glucose uptake and adenosine levels during hypoxia, animals were treated with the ACh receptor inhibitor atropine before hypoxia exposure \((n = 6).\) Animals were injected with 1.2 mg/kg atropine sulfate (Sigma) through the cannula 1 h before DO2 levels were reduced. This concentration of atropine completely abolishes the hypoxic bradycardia exhibited by this species (16). Fish were then subjected to the above hypoxia treatment and blood sampling regime.
Effect of adenosine receptor blockade. The effects of adenosine receptor blockade on cardiovascular and glucose uptake characteristics in hypoxia-exposed fish treated with atropine were assessed (n = 6). Cardiovascular characteristics are available for only four of six fish treated with atropine plus the adenosine receptor inhibitor 8SPT because of technical problems with flow probes. Fish were injected with 8SPT (10 mg/kg in saline) (21) and atropine (1.2 mg/kg) (t = 0 min) and held for 60 min before being exposed to the hypoxia treatment and blood sampling regime used in the previous experiments. In preliminary work, a number fish injected with 8SPT or the related drug aminophylline became highly agitated and died within 1 h of treatment. Those included in the analysis behaved normally and did not appear agitated.

Biochemical analysis. Tissue samples were homogenized in ice-cold perchloric acid (6%) and centrifuged, and the supernatant was removed and neutralized with 5 mol/l K2CO3. The sample was cold perchloric acid (6%) and centrifuged, and the supernatant was removed and neutralized with 5 mol/l K2CO3. The sample was centrifuged, and the supernatant was removed and frozen in liquid N2. For plasma glucose determinations, plasma samples were deproteinized by addition of two volumes of perchloric acid and centrifuged, and the supernatant was removed. All extracts were stored at −80°C until use.

Plasma glucose and tissue glucose and G-6-P levels were measured spectrophotometrically (model DU 640; Beckman Coulter, Fullerton, CA) using a G-6-P dehydrogenase-HK-based assay described previously (7). Plasma lactate levels were determined at t = 60 min (before hypoxia exposure) and at t = 300 min for all fish. Lactate levels in plasma and tissue samples were measured spectrophotometrically (model DTX880 microplate reader; Beckman Coulter) by following the reduction of NAD+ to NADH (340 nm) in a glycine-hydrizine buffer. Adenosine levels were determined in heart and brain tissue extracts by HPLC coupled to UV detection (24).

Tissue 2-DG and 2-DG-6-P were separated by ion-exchange chromatography according to Clow et al. (7). Sample extracts (200 μl) were applied to a small column (bed volume of 0.5 ml) containing DEAE-Sephacel. Successive 2.0-ml washes of distilled water and 0.5 mol/l HCl were used to elute 2-DG and 2-DG-6-P, respectively. Samples of crude tissue extracts and both column fractions were counted for radioactivity after the addition of scintillation fluid.

Data analysis and statistics. Calculations for glucose uptake rates were modified from Blasco et al. (5). Plasma specific activity (dpm/nmol glucose) over time was fitted to a three-parameter exponential decay curve using SigmaPlot (version 8.0). 2-DG in the extracellular compartment (2-DGECF) was calculated as follows

\[
2-DG_{ECF}(\text{dpm/ml tissue H}_2O) = \frac{dpm^{[14]C}LG/ml tissue H_2O}{dpm^{[14]C}LG/ml plasma at 240 min} \times dpm^{[3]H}2-DG/ml tissue H_2O
\]

Counts (in dpm) of [14C]LG per milliliter plasma were stable after 60 min. For this reason, the value of dpm [14C]LG per milliliter plasma at 240 min (the time of tissue sampling) was chosen for extracellular space corrections. Corrections utilizing the area under the decay curve of dpm [14C]LG per milliliter plasma (5) included [14C]LG counts before they reached equilibrium with the extracellular space, hence underestimating the size of this compartment. Tissue water and ECF volume estimates were consistent among animals; however, it is possible that not all of the fluid was removed from the tissue samples before analysis. This is an important consideration in heart, where the trabecular spaces of the ventricle may retain fluid after the sample is blotted dry. Individual calculations of tissue glucose uptake account for these differences, and potential overestimates of ECF volume will not impact glucose uptake rates.

The denominator represents the area under the plasma specific activity decay curve. Because [14C]LG was nearly undetectable in brain tissue, corrections for extracellular space were not made in calculations of brain glucose uptake rates. The rates of brain glucose uptake measured here represent net glucose transport across the blood-brain barrier rather than cellular transport within the brain.

Whole animal glucose turnover was calculated according to Dunn and Hochachka (13)

\[
\text{Glucose turnover} = \frac{\text{dose}[^{3}H]-2-DG(\text{dpm/kg})}{\int_{0}^{240} \text{dpm}[^{3}H]-2-DG(\text{dpm/nmol glucose})}
\]

The affinity of transport mechanisms and HK for glucose and 2-DG may differ on a tissue- or condition-specific basis (39). Determinations of the absolute rate of tissue glucose uptake require corrections with a “lumped constant” specific to each tissue and physiological condition. This constant is determined in vitro and is based on differences in the affinity of transport mechanisms and HK for glucose and 2-DG. Because the affinity for 2-DG is different for GLUTs and HK, this constant can change under physiological conditions, depending on which step is rate limiting for tissue glucose utilization (9). The lack of complex physiological influences in vitro may result in the determination of a lumped constant unrepresentative of the true in vivo situation. The values presented here are uncorrected and should be considered indexes of net glucose uptake and not absolute rates.

Cardiac glucose uptake occurs via GLUTs and is dependent on glucose delivery from the circulation. The term “percent glucose extraction” describes the relationship between changes in tissue glucose uptake and not absolute rates.

\[
\text{Percent glucose extraction} = \frac{\text{cardiac output} \times \text{plasma glucose(mmol/ml)} \times \text{cardiac output(mL g heart}^{-1} \cdot \text{min}^{-1})}{100}
\]
Percent glucose extraction 

\[
\text{Percent glucose extraction} = \frac{\text{glucose uptake (nmol g}^{-1}\text{240 min}^{-1})}{\text{glucose delivery (nmol g}^{-1}\text{min}^{-1})} \times 100\% 
\]

Data are expressed as means ± SE throughout. Stroke volume was determined by dividing Q (ml kg}^{-1}\text{min}^{-1}) by \(f_{H} \) (beats/min). Comparisons of cardiorespiratory parameters and plasma glucose concentrations between treatment groups were done by repeated-measures ANOVA with a Bonferroni correction for multiple comparisons (SPSS 13.0). Comparisons of data within treatment groups were achieved with one-way ANOVAs and Tukey’s honestly significant difference post hoc tests. Adenosine, lactate, glucose, and G-6-P content and glucose turnover rates were subjected to a similar analysis. Where indicated, Student’s \(t\)-tests were used for a priori comparisons. Transformations of percent glucose extraction data and intracellular-to-plasma glucose ratios were not necessary because errors were normally distributed. Mean values of intracellular-to-plasma glucose ratios were tested for differences from the hypothesized equilibrium value of one using a \(t\)-test. Statistical comparisons giving \(P\) values of <0.05 are considered significant.

RESULTS

Cardiovascular responses. The characteristics of \(f_{H}\), Q, and stroke volume during normoxic and hypoxic conditions (Fig. 1) were similar to those observed previously under these conditions (24). Atropine treatment resulted in a significant and immediate increase in \(f_{H}\) to ~26 beats/min during normoxic conditions. As expected, atropine abolished the bradycardia observed in hypoxia-exposed animals. During hypoxia exposure, Q in atropine-treated animals was significantly higher than in untreated hypoxic fish after 30 min at 2.0 mg/l DO\(_2\) (\(t = 90\) min). Throughout the hypoxia exposure, atropine-treated fish exhibited cardiovascular characteristics similar to those injected with atropine alone.

**Plasma glucose levels and glucose uptake.** Plasma glucose concentrations in normoxic fish without pharmacological treatment \((0.233 ± 0.054 \text{ mmol/l; n} = 12)\) were similar to levels noted previously \((14)\) and did not change significantly over time (Fig. 2). Atropine-treated fish exhibited noticeably more variation in plasma glucose levels than the other treatment groups. When resting levels \((t = 0\) min) were compared with those after hypoxia exposure \((t = 300\) min) using Student’s \(t\)-tests, plasma glucose concentrations were significantly higher in hypoxia-treated, atropine-treated, and atropine + 8SPT-treated fish. Whole animal glucose turnover rates were not different among treatment groups and were as follows: normoxia 9.1 ± 2.8, hypoxia 3.7 ± 1.4, hypoxia with atropine 15.7 ± 7.0, and hypoxia with atropine + 8SPT 16.3 ± 4.7 nmol·kg\(^{-1}\)·min\(^{-1}\).

ECF volume in the sculpin heart (Table 1) is high relative to spleen and white muscle but compares well with estimates from the toadfish \((~53\% \text{ of total tissue } H_2O) \) (8) and Pacific.
glucose uptake was 1.85 \( P \) (fish exposed to hypoxia without pharmacological treatment heart glucose uptake rates increased fivefold compared with served, percent glucose extraction in the hypoxia treatment (22). Although no statistically significant changes were observed, coronary glucose extraction in the in vitro perfused rat heart (23) tended to have higher glucose uptake levels in heart than fish (Fig. 3). Atropine-treated animals exposed to hypoxia and no significant changes were observed in hypoxia-exposed (8SPT) (15). Under normoxic conditions, cardiac glucose extraction was 0.034 \( P \) in short-horned sculpins. In that tissue, a significant 20% decline was nearly twice that of plasma under resting conditions. An identical pattern of high white muscle glucose content relative to muscle glucose content was significantly correlated to plasma glucose concentrations in all tissues except white muscle (Fig. 4). Heart intracellular glucose-to-plasma glucose ratios were significantly lower than 1 for all groups except hypoxia-treated fish (without pharmacological agents) (Table 2). Brain, gill, and spleen also exhibited intracellular-to-plasma glucose ratios significantly lower than 1 for all treatments. White muscle glucose content was significantly higher than plasma glucose levels under normoxic conditions. Intracellular glucose content was nearly twice that of plasma under resting conditions. An identical pattern of high white muscle glucose content relative to plasma is observed in the armored catfish Liposarcus parado- lis (25). Similar to the catfish, white muscle-to-plasma glucose ratios dropped to values not different from 1 in hypoxia-exposed short-horned sculpins.

G-6-P levels were similar in hypoxia-treated and atropine-treated fish (Fig. 5). Atropine + 8SPT treatment led to a significant decline in heart G-6-P compared with hypoxic fish without pharmacological treatment and a significant increase in gill G-6-P compared with that shown in normoxic fish. No changes in white muscle G-6-P were observed, but concentrations were high relative to muscle glucose levels and also compared with G-6-P content in other tissues.

A novel finding is that, in all of the tissues examined, the majority of the intracellular 2-DG remains unphosphorylated. The level of unphosphorylated 2-DG in heart, brain, gill, and spleen was between 65 and 80%; in white muscle, 90% of the 2-DG remained unphosphorylated (Fig. 5). The relative amount of free 2-DG was similar across treatments in all tissues except spleen. In that tissue, a significant 20% decline groups followed a similar pattern to that observed for cardiac glucose uptake: hypoxia treated = 0.033 ± 0.009%, atropine treated = 0.082 ± 0.042%, and atropine + 8SPT treated = 0.142 ± 0.047%.

The brain glucose uptake rates presented here are not corrected for ECF space and should be regarded as relative rates of glucose uptake across the blood-brain barrier. Brain glucose uptake occurred at 1.00 ± 0.23 nmol·ml tissue H₂O⁻¹·min⁻¹ in normoxic fish, and no changes were noted under hypoxia, regardless of pharmacological treatment (Fig. 3). Glucose uptake rates in the gill and spleen of fish held under normoxia were 0.45 ± 0.10 and 0.99 ± 0.23 nmol·ml tissue H₂O⁻¹·min⁻¹, respectively, and did not change in any treatment group. White muscle glucose uptake occurred at 0.51 ± 0.14 nmol·ml tissue H₂O⁻¹·min⁻¹ under normoxia, and no changes were observed among treatment groups.

Glucose, G-6-P, and percent unphosphorylated 2-DG. Ratios of intracellular glucose to plasma glucose were calculated based on glucose content in the tissue water corrected for the ECF marker \([^{14}C]\)LG. The latter correction was not performed in brain for reasons mentioned elsewhere. Intracellular glucose content was significantly correlated to plasma glucose concentrations in all tissues except white muscle (Fig. 4). Heart intracellular glucose-to-plasma glucose ratios were significantly lower than 1 for all groups except hypoxia-treated fish (without pharmacological agents) (Table 2). Brain, gill, and spleen also exhibited intracellular-to-plasma glucose ratios significantly lower than 1 for all treatments. White muscle glucose content was significantly higher than plasma glucose levels under normoxic conditions. Intracellular glucose content was nearly twice that of plasma under resting conditions. An identical pattern of high white muscle glucose content relative to plasma is observed in the armored catfish Liposarcus paradalisis (25). Similar to the catfish, white muscle-to-plasma glucose ratios dropped to values not different from 1 in hypoxia-exposed short-horned sculpins.

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![Fig. 2. Plasma glucose concentrations for sculpins exposed to normoxia and to hypoxia in the absence of drugs and in the presence of atropine or atropine + 8-p-(sulfophenyl)theophylline (8SPT) (n = 6). Inset: plasma lactate levels (nmol/l) at the beginning and end of the experimental treatment. Dotted lines indicate initiation of hypoxia.](http://ajpregu.physiology.org/)
Fig. 3. Tissue glucose uptake rates for short-horned sculpins after various treatment conditions. O2, normoxia; Hyp, hypoxia without pharmacological agents; Atro, atropine-treated animals; 8SPT, atropine + 8SPT-treated animals. Solid lines denote treatment groups exposed to hypoxia (n = 6). W., muscle, white muscle.

in the proportion of unphosphorylated 2-DG was noted in hypoxia-exposed sculpins without pharmacological treatment.

Plasma and tissue lactate content. Plasma lactate concentrations were lower under normoxic conditions (0.016–0.182 mmol/l) in all treatment groups (Fig. 2). Plasma lactate increased significantly in hypoxia-treated and atropine + 8SPT-treated fish exposed to 2.0 mg/l DO2 for 4 h, but no increase was observed in fish treated only with atropine. Fish treated with atropine + 8SPT had significantly higher lactate levels in brain, gill, and spleen tissue than fish exposed to hypoxia without pharmacological treatment (Fig. 5). Equivalent differences were absent in heart and white muscle; however, lactate levels in these tissues were significantly higher than those observed for normoxic control fish.

Heart and brain adenosine content. Heart adenosine content in normoxia-held fish was 12.04 ± 3.88 nmol/g (n = 6), which is in the upper range of values reported for this species (24). Adenosine levels did not vary significantly during hypoxia, regardless of pharmacological treatment (data not shown). Brain adenosine content was 92.69 ± 9.62 nmol/g (n = 6) in normoxia-held sculpins. These values were higher than those measured previously (24) but were within the range of normal levels for this species. No statistically significant changes were noted in brain adenosine content; however, atropine-treated fish tended to have lower adenosine levels than hypoxic fish that did not receive atropine (68.38 ± 8.27 vs. 109.36 ± 15.40 nmol/g; P = 0.066).

DISCUSSION

This work is the first to characterize cardiovascular parameters in conjunction with in vivo glucose uptake rates in a fish. We present new data regarding the influence of hypoxia on tissue glucose utilization and the impact that hypoxic bradycardia has on exogenous glucose use. Short-horned sculpin have circulating glucose levels at least 10-fold lower than most fish and mammals, which limits the availability of fuel for anaerobic metabolism. In this context, we provide new information on the characteristics and control of glucose utilization in an animal with naturally low blood glucose levels.

Glucose uptake under normoxia: sculpin vs. trout. The rate of glucose uptake in the short-horned sculpin heart is about one-half of that observed in brown trout (5) and rainbow trout hearts (44). The relative efficiency of glucose transport mechanisms may be an important factor in determining the rate of tissue glucose utilization. Heart glucose extraction for the rainbow trout can be calculated with a Q˙ of 20 ml·kg⁻¹·min⁻¹ (17), a cardiosomatic index of 0.095% (11), a plasma glucose concentration of 3.5 mmol/l, and a glucose uptake rate of 5.31 nmol·g⁻¹·min⁻¹ (44). From these assumptions, glucose extraction in the rainbow trout heart is only 0.0072%, nearly fivefold less than the sculpin heart. A lower fi increases the residence time of blood in the lumen of the heart, potentially allowing a greater opportunity for glucose to diffuse into the myocardium. Alternatively, sculpin may have a higher density of GLUT proteins at the sarcolemmal membrane or GLUTs with a higher affinity for glucose than those found in the trout heart.

White muscle glucose uptake rates in the sculpin are only slightly lower than those reported for the brown trout (5); however, the rates of glucose uptake in the brain, gill, and

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Values are means ± SE, O2, animals exposed to normoxia; Untr, untreated animals exposed to hypoxia; Atro, atropine-treated animals exposed to hypoxia; 8SPT, atropine + 8-(sulfophenyl)theophylline (8SPT)-treated animals exposed to hypoxia. †Tissue H2O glucose-to-plasma glucose ratio, not corrected for extracellular H2O. *Significant difference from hypothesized equilibrium value of 1.

This work is the first to characterize cardiovascular parameters in conjunction with in vivo glucose uptake rates in a fish. We present new data regarding the influence of hypoxia on tissue glucose utilization and the impact that hypoxic bradycardia has on exogenous glucose use. Short-horned sculpin have circulating glucose levels at least 10-fold lower than most fish and mammals, which limits the availability of fuel for anaerobic metabolism. In this context, we provide new information on the characteristics and control of glucose utilization in an animal with naturally low blood glucose levels.

Glucose uptake under normoxia: sculpin vs. trout. The rate of glucose uptake in the short-horned sculpin heart is about one-half of that observed in brown trout (5) and rainbow trout hearts (44). The relative efficiency of glucose transport mechanisms may be an important factor in determining the rate of tissue glucose utilization. Heart glucose extraction for the rainbow trout can be calculated with a Q˙ of 20 ml·kg⁻¹·min⁻¹ (17), a cardiosomatic index of 0.095% (11), a plasma glucose concentration of 3.5 mmol/l, and a glucose uptake rate of 5.31 nmol·g⁻¹·min⁻¹ (44). From these assumptions, glucose extraction in the rainbow trout heart is only 0.0072%, nearly fivefold less than the sculpin heart. A lower fi increases the residence time of blood in the lumen of the heart, potentially allowing a greater opportunity for glucose to diffuse into the myocardium. Alternatively, sculpin may have a higher density of GLUT proteins at the sarcolemmal membrane or GLUTs with a higher affinity for glucose than those found in the trout heart.

White muscle glucose uptake rates in the sculpin are only slightly lower than those reported for the brown trout (5); however, the rates of glucose uptake in the brain, gill, and
Fig. 5. Glucose, glucose-6-phosphate (G-6-P), the proportion of unphosphorylated intracellular 2-deoxyglucose (2-DG), and lactate content in tissues of the short-horned sculpin. O2, normoxia; Hyp, hypoxia without pharmacological agents; Atro, atropine-treated fish; 8SPT, atropine + 8SPT-treated fish (n = 6 unless noted in parentheses). Solid lines denote treatment groups exposed to hypoxia.
spleen of sculpin are roughly 20- to 50-fold slower than rates for the trout. In the sculpin, these tissues may have substantially lower energy demands and/or may utilize alternative metabolic fuels such as lipids or amino acids.

**Impact of hypoxia and atropine on heart performance and glucose metabolism.** In the short-horned sculpin, hypoxia exposure alone did not increase tissue glucose uptake or glucose turnover rates, indicating that exogenous glucose use did not change. A similar observation of sustained glucose turnover is evident in hypoxic rainbow trout (13). The observed bradycardia, in addition to the lack of tissue lactate accumulation, suggests that this species reduces energy demand when exposed to hypoxia. The majority of the remaining ATP demand is likely met by aerobic metabolism, with elevations in plasma lactate possibly indicating a mild activation of anaerobic metabolism.

Increasing \( f_1 \) and blocking adenosine receptors with atropine + 8SPT treatment during hypoxia increased heart glucose uptake by fivefold. These are the first data illustrating the potential of the fish heart to significantly increase glucose utilization in vivo. Similar increases in percent glucose extraction were also observed, suggesting that increased uptake is not simply due to increased circulatory glucose delivery. Heart lactate did not increase in atropine- or atropine + 8SPT-treated fish, suggesting that most of the additional glucose taken up by the heart in these fish is catabolized aerobically. These data provide evidence that a decrease in \( f_1 \) and an activation of adenosine receptors in response to hypoxia may result in a depression in cardiac energy demand.

Vertebrate brains primarily utilize exogenous glucose for energy metabolism (37), and survival depends on the maintenance of adequate levels of circulating glucose. Because the short-horned sculpin has extremely low glucose levels, it may rely on other fuels for neural energy production. Lactate oxidation can occur at levels comparable to glucose oxidation in the rainbow trout brain (38). Given that levels of circulating glucose and lactate can be similar in resting sculpin, it is possible that plasma lactate is an important fuel in the brain of this species.

**Rate-limiting steps and the control of tissue glucose utilization.** Intracellular glucose-to-plasma glucose ratios of <1 indicate that HK activity proceeds faster than glucose uptake. Glucose uptake across the cell membrane is therefore the rate-limiting step in glucose utilization in the heart, brain, gill, and spleen of the sculpin. In fish hearts, maximal in vitro HK activity greatly exceeds that required to entirely support aerobic metabolism (12) and likely operates at only a fraction of its maximal capacity in vivo (10). In the eel heart, G-6-P is a potent inhibitor of HK (35); at the levels of G-6-P observed in the sculpin heart (~1 \( \mu \text{mol/ml} \)), HK would be operating at <20% of its maximal capacity. Subtle changes in HK activity have the potential to control the rate of glucose uptake without changing the absolute capacity for transport at the cell membrane. Decreases in HK activity will diminish the glucose gradient across the cell membrane and could decrease glucose uptake. Conversely, the importance of facilitated glucose transport in controlling the rate of glucose utilization will increase under conditions where HK activity is augmented.

An activation of HK may be the mechanism responsible for the increased rate of heart glucose uptake in fish treated with atropine + 8SPT during hypoxia. In these fish, higher heart glucose uptake rates were associated with a decline in G-6-P content and a lower intracellular-to-plasma glucose ratio. Decreasing G-6-P levels through an activation of glycolysis would release inhibition on HK, subsequently decreasing intracellular glucose content and enhancing inward-facilitated diffusion.

The present findings regarding the potential importance of HK in controlling the rate of glucose utilization are in contrast with earlier interpretations based on isolated heart preparations. In the sculpin, a significant proportion of the 2-DG taken up by tissues in vivo is unphosphorylated, but essentially all of the 2-DG taken up by the in vitro fish heart is phosphorylated. The latter observation has led to the conclusion that glucose utilization is controlled primarily by uptake mechanisms (7, 35). The reasons underlying the differences between in vitro American eel and Atlantic cod heart preparations and in vivo data are unclear; however, data from the latter studies (Ref. 5 and the present study) suggest that the importance of facilitated uptake in the regulation of glucose utilization has been overestimated.

**Glucose management in white muscle.** The observation of high white muscle glucose content relative to plasma levels in both sculpin and L. pardalis (25), coupled with the observation that intracellular glucose does not correlate with plasma glucose, provides further evidence that the regulation of glucose utilization in this tissue is unique. The observation of high G-6-P content supports the possibility that glucose accumulated in the white muscle is derived from glycogen or gluconeogenic precursors. The synthesis of glucose from glycogen requires a phosphoglucomutase to convert glucose-1-phosphate from glycogenolysis to G-6-P and the subsequent action of glucose-6-phosphatase to convert the latter to glucose. Phosphoglucomutase activity is likely to be ubiquitously present in fish white muscle (27) and glucose-6-phosphatase activity is detectable in muscles of other teleosts (29, 36). Intracellular lactate can be converted to glycogen in fish white muscle (27, 32), rendering this the likely carbon source for glycogen synthesis under normoxia.

The low rates of white muscle glucose uptake observed in the sculpin do not support the possibility of active glucose transport mechanisms in this tissue. There is evidence for sodium-dependent glucose transport in frog skeletal muscle (23) and several other fish tissues (20, 34); therefore, its potential role in glucose accumulation cannot be ignored. Identifying the mechanisms underlying white muscle glucose handling will be important to the understanding of glucose regulation in teleost fish.

**Adenosine.** Sculpin exposed to hypoxia show no change in cardiac adenosine levels (Ref. 24 and the present study). We previously attributed this to the sculpin’s ability to depress energy requirements under hypoxia to maintain ATP levels. Because adenosine levels are associated with cardiac energy demand in mammals (3), we hypothesized that increasing energy demand by eliminating the bradycardia would result in proportional increases in adenosine content. The data demonstrate, however, that even when \( f_1 \) is increased threefold during hypoxia adenosine does not accumulate. Lactate accumulation was also absent in heart tissue from atropine-treated fish, suggesting that oxidative phosphorylation was adequate to maintain energy production in this tissue, even after 4 h at 2.0 mg/l DO\(_2\).
Data on the cardiovascular parameters of fish treated with the adenosine A₁- and A₂-receptor blocker 8SPT suggest that adenosine has no influence on contractility in the sculpin heart, at least in the presence of atropine. This is not surprising in light of the paucity of heart adenosine accumulation. Clearly, the characteristics of adenosine metabolism in fish hearts are quite different from those of mammalian hearts. On the basis of numerous studies, it is probable that adenosine is a potentially important regulator of cardiovascular function in fish, particularly in the gill vasculature (e.g., Refs. 30, 41). The observation that even severe hypoxia does not trigger ATP depletion (11) or adenosine accumulation in a number of species (24, 25) emphasizes the need to reevaluate adenosine’s role in the fish heart.

Tissue lactate levels were consistently higher in hypoxic animals treated with atropine and 8SPT, supporting previous evidence that adenosine receptors have a depressive effect on anaerobic metabolism in fish (2). In atropine + 8SPT-treated fish, increased glucose uptake rates were only evident in heart, suggesting that lactate production may be preferentially fuelled by glycogen in the other tissues examined. The depressive effects of adenosine on anaerobic metabolism may negate the need for increased tissue glucose uptake, therefore sparing limited glucose reserves and prolonging hypoxia survival.

In conclusion, short-horned sculpins maintain tissue glucose uptake rates and may reduce myocardial energy demand during hypoxia through a decrease in $J_M$. Although uptake limits glucose utilization in most tissues, HK activity may control the rate of glucose uptake by altering the cellular glucose gradient. We present evidence that white muscle accumulates intracellular glucose under normal conditions, possibly using glycogen as a glucogenic precursor. Adenosine receptors do not influence cardiovascular parameters in atropine-treated fish, but they may depress anaerobic metabolism and cardiac glucose uptake during hypoxia exposure.

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