The Journal of Experimental Biology 200, 2871–2880 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JEB1092

ACUTE REGULATION OF GLUCOSE UPTAKE IN CARDIAC MUSCLE OF THE AMERICAN EEL *ANGUILLA ROSTRATA*

KENNETH J. RODNICK[1,*], JOHN R. BAILEY[2], JENNY L. WEST[2], ALISON RIDEOUT[2] AND WILLIAM R. DRIEDZIC[2]

[1]*Department of Biological Sciences, Idaho State University, Pocatello, ID 83209-8007, USA* and [2]*Department of Biology, Mount Allison University, Sackville, New Brunswick, Canada EOA 3CO*

Accepted 21 August 1997

Summary

We investigated the effects of anoxia and contractile activity on glucose uptake and the intracellular location of hexokinase in cardiac muscle of the American eel *Anguilla rostrata*. Uptake of 2-deoxyglucose (2-DG) by ventricle strips at 15 °C was increased by 45 % by anoxia and by 85 % by contractile activity over basal conditions. The anoxia- and contraction-induced increase in basal 2-DG uptake was inhibited completely by 25 μmol l^{-1} cytochalasin B, suggesting that facilitated glucose transporters are involved. Maximal activity of hexokinase in whole homogenates (approximately 10 μmol min^{-1} g^{-1} tissue) was 200 times higher than the maximal rate of 2-DG uptake measured *in vitro* (46 nmol min^{-1} g^{-1} tissue). Only 20–25 % of hexokinase activity was localized to the mitochondrial fraction, and this was not altered by perfusion of the hearts with anoxic media. It is therefore unlikely that anoxia-induced stimulation of 2-DG uptake is mediated by intracellular translocation of hexokinase. As in the case of mammalian muscle, glucose 6-phosphate is a potent inhibitor of hexokinase in eel cardiac muscle (IC_{50}=0.44 mmol l^{-1}). In summary, anoxia and contractile activity significantly increase 2-DG uptake in cardiac muscle of American eels, and glucose transport may be rate-limiting for glucose utilization. Increased utilization of glucose during anoxia or contractile activity may involve the recruitment of facilitative glucose transport proteins to the cell surface of myocytes or an increase in the intrinsic activity of glucose transporters already residing at the cell surface.

Key words: *Anguilla rostrata*, eel, glucose uptake, cardiac muscle, 2-deoxyglucose, anoxia, contractile activity, cytochalasin B, hexokinase.

Introduction

The heart of fishes, like all vertebrate hearts, has a continuous requirement for energy substrates for the production of ATP and the maintenance of contractile function. Under aerobic conditions, the energy metabolism of fish hearts, like that of mammalian hearts, is based on oxidative degradation of glucose and fatty acids. Tracer studies *in vivo* (West *et al.* 1993, 1994) and using isolated preparations (Lanctin *et al.* 1980; Driedzic and Hart, 1984; Sidell *et al.* 1984, 1995; West *et al.* 1993) have demonstrated that the fish heart uses glucose as a metabolic fuel under aerobic conditions. Glucose also enhances the performance of isolated hearts under anaerobic conditions (Driedzic, 1978; Driedzic *et al.* 1978; J. Bailey, K. J. Rodnick and W. R. Driedzic, in preparation). However, unlike the heart of mammals, the heart of most teleosts is part of a single-circuit low-pressure system and normally receives only partially oxygenated venous blood for metabolic support (Santer, 1985). Some active fishes, including the American eel (*Anguilla rostrata*), possess both a ventricular epicardial layer supplied by an arterial coronary circulation and a trabecular endocardium receiving venous blood *via* the ventricular lumen (Santer and Greer Walker, 1980; personal observations). Many fishes also live in water with a low oxygen content and display high levels of locomotory activity, both of which may further reduce the availability of oxygen to the heart. One potential strategy to cope with the lower oxygen availability and to maintain cardiac performance is an increased reliance on anaerobic glycolysis for ATP production. Currently, however, we have little insight into the physiological mechanisms by which fish cardiac muscle increases its rate of glucose uptake.

Several physiological stimuli, including anoxia, contractile activity, insulin and hyperosmolarity, increase glucose uptake in mammalian cardiac muscle (Neely and Morgan, 1974). However, it is not clear which metabolic step is rate-limiting for glucose utilization by mammalian straited muscle. Both the

*e-mail: rodnkenn@isu.edu

transport of glucose across the plasma membrane and its subsequent intracellular phosphorylation by hexokinase (HK) appear to be important (Berger *et al.* 1975; Manchester *et al.* 1994). The accepted mechanism of glucose uptake by mammalian cells is facilitated transport of glucose through the plasma membrane by one or more integral membrane proteins called glucose transporters or GLUTs (Pessin and Bell, 1992). The regulation of glucose transport in mammalian cardiac muscle is accomplished by reversibly translocating GLUT-4 from an intracellular compartment to the cell surface (Slot *et al.* 1991). Current evidence exists for GLUT-like proteins in fish erythrocytes (Tse and Young, 1990; Young *et al.* 1994; Soengas and Moon, 1995); however, nothing is known about GLUTs in the heart of fishes.

Like glucose transporters, HK is not confined to one cellular compartment. In mammalian tissues, including cardiac muscle, a significant fraction of HK is reversibly bound to the outer mitochondrial membrane (Gerbitz *et al.* 1996). Mitochondrially bound HK has a higher affinity for glucose and shows less susceptibility to product inhibition by glucose 6-phosphate (G-6-P) relative to the soluble form (Viitanen *et al.* 1984). Binding of HK to mitochondria would potentially allow anaerobic glycolysis to proceed during periods of anoxia. Although total HK activity correlates well with glucose flux rates in skeletal muscle of vertebrates (Crabtree and Newsholme, 1972), measured rates of glucose utilization in fish cardiac muscle (Lanctin *et al.* 1980; West *et al.* 1993, 1994) are dramatically lower than maximal activities of HK (Driedzic *et al.* 1987; Sidell *et al.* 1987; Bailey *et al.* 1991). Possible explanations for this discrepancy include *in vivo* effects of lowered oxygen tension on the functional compartmentation of HK in the cytosol and the concentration of putative inhibitors, such as G-6-P, on HK activity (England and Randle, 1967; Viitanen *et al.* 1984; Gerbitz *et al.* 1996). To date, nothing is known about the subcellular distribution of HK, during normoxia or anoxia, and feedback inhibition of HK by G-6-P in the cardiac muscle of fishes.

The purpose of this study was to increase our understanding of glucose metabolism and HK compartmentation in teleost cardiac muscle. Experiments were conducted on the hearts of American eels to address the following questions. (1) Does anoxia or contractile activity stimulate glucose uptake? (2) Does anoxia alter the subcellular distribution or activity of HK?

Materials and methods

Animals

Female American eels (*Anguilla rostrata* LeSueur) were purchased from a local dealer (South Shore Trading, Port Elgin, New Brunswick) and transported to Mount Allison University. They ranged in size between 233 and 666 g (mean ± S.E.M. 442±39 g, N=36). Animals were kept in flow-through holding tanks receiving well water (14±1 °C), under a constant photoperiod (12 h:12 h light:dark) and were not fed for 1–3 weeks before experiments. Just prior to experiments, eels were netted individually, removed from the holding tank, rendered torpid in a bucket of ice-water for 30 min, and doubly pithed. All studies were conducted during June and July of 1996.

Incubation medium and conditions

The basic medium used in these studies was a modification of a standard Ringer's solution for freshwater teleosts (Hoar and Hickman, 1975) consisting of 111 mmol l^{-1} NaCl, 2.0 mmol l^{-1} KCl, 2.0 mmol l^{-1} $CaCl_2$, 10 mmol l^{-1} $MgSO_4$, 5 mmol l^{-1} NaH_2PO_4 and 10 mmol l^{-1} $NaHCO_3$, pH 7.8 at 20 °C. Extra bicarbonate was added to increase the long-term buffering capacity of the medium. In some preparations, 5 mmol l^{-1} D-glucose was added as an exogenous fuel source. This concentration of glucose was within the physiological range reported for this species (Cornish and Moon, 1985). Media were pregassed with either 0.5 % CO_2:99.5 % O_2 (oxygenated) or 0.5 % CO_2:99.5 % N_2 (anoxic). Although we use the term 'anoxic' to describe one of our experimental conditions for ventricle strips, trace levels of O_2 (0.5–1.3 kPa) were routinely present in the medium during incubations. The oxygen tension in the incubation and perfusion media was measured using a Strathkelvin 1302 microcathode oxygen electrode connected to a Strathkelvin model 781 oxygen meter.

Preparation of ventricle strips

After cutting carefully through the pericardium, the entire heart was excised, and the ventricle was dissected free of the atrium, bulbus arteriosus, connective tissue and visible fat. The ventricle was bisected, rinsed in ice-cold oxygenated medium (minus glucose) to remove intraluminal blood, blotted and weighed. Four strips (1–1.5 mm diameter, weighing approximately 25 mg) were cut from each ventricle by slicing parallel to the long axis of the ventricle with a single-edged razor blade. To permit recovery from the effects of splitting and to reduce intrinsic contractile activity, ventricle strips were preincubated for 60 min in stoppered 25 ml Erlenmeyer flasks containing 2 ml of basic medium supplemented with 5 mmol l^{-1} D-glucose, 35 mmol l^{-1} sorbitol and 0.1 % bovine serum albumin (BSA) (Cohn Fraction V, essentially fatty acid free, Sigma Chemical Co.). Flasks were gassed continuously with either 99.5 % O_2:0.5 % CO_2 for basal and contracting ventricle strips or 99.5 % N_2:0.5 % CO_2 for anoxic strips. All flasks were incubated at 15 °C in a reciprocating incubator (Haake, model SWB 20) at 70 cycles min^{-1} throughout the experiment. Osmolarity was kept constant in all incubations by varying the concentration of D-sorbitol, such that the sum of D-glucose, sorbitol and 2-deoxyglucose (2-DG) concentrations was 40 mosmol l^{-1}. Sorbitol was also used as a marker of the extracellular space because of its similar diffusion characteristics to those of glucose; however, unlike glucose, it does not penetrate the myocyte (Morgan *et al.* 1961). The water content of ventricle strips was determined by drying tissue to a constant mass in an oven set at 80 °C. Water content (ml g^{-1}) was calculated as follows: [(wet mass minus dry mass)/wet mass]×100.

Contractile activity

A separate set of experiments was conducted to examine the

effects of defined contractile activity on 2-DG uptake in cardiac muscle. Each ventricle strip was mounted in a thermostatically controlled (15 °C) bath and connected to an isometric force transducer (Harvard, model 60-2994) interfaced with a strip chart recorder (Biotronix, model BL-882). Basic medium (20 ml), supplemented with 5 mmol l^{-1} glucose and 35 mmol l^{-1} sorbitol, was added to each bath and oxygenated continuously with 99.5 % O_2:0.5 % CO_2. After adjusting strip length for maximum force production, strips were stimulated at 36 contractions min^{-1} for 10 min *via* field stimulation to produce maximum contractility using a Grass S9 square-wave stimulator (0.2 ms rectangular pulses). This contraction frequency was within the range of values reported for unanaesthetized, chronically cannulated European eels (*Anguilla anguilla*) (Peyraud-Waitzenegger *et al.* 1980).

Measurement of 2-DG uptake and extracellular space

2-DG, an analogue of D-glucose, is transported into myocytes and phosphorylated by the same mechanisms as glucose. Thus, 2-DG uptake is defined as glucose transport and intracellular phosphorylation by HK. Phosphorylation serves to trap 2-DG inside the myocyte as 2-deoxyglucose 6-phosphate (2-DG-6-P), making it possible to determine rates of sugar uptake over extended periods. 2-DG uptake was measured by a modification of the method described for mammalian skeletal muscle (Hansen *et al.* 1994). Following a 1 h preincubation, and in some cases an additional 10 min of prescribed contractile activity, ventricle strips were rinsed for 10 min in 2 ml of basic medium containing 40 mmol l^{-1} sorbitol and 0.1 % BSA to remove glucose from the extracellular space. Preliminary time course experiments demonstrated that it takes approximately 10 min for sorbitol to reach equilibrium in the extracellular space of ventricle strips, and 2-DG uptake is linear between 10 and 30 min under basal conditions (Fig. 1). The final incubation lasted for 20 min in 1.5 ml of medium containing 2 mmol l^{-1} sodium pyruvate, 5 mmol l^{-1} 2-deoxy-D-[2-^{3}H]glucose (3.7×10^4 Bq ml^{-1}, New England Nuclear) and 33 mmol l^{-1} D-[U-^{14}C]sorbitol (1.1×10^4 Bq ml^{-1}, New England Nuclear). To terminate experiments, ventricle strips were blotted briefly on filter paper moistened with ice-cold medium, freeze-clamped and stored at –80 °C. Muscles were weighed and processed by incubation in 1 ml of boiling water for 10 min and centrifugation at 1000 ***g*** for 15 min at 4 °C. Duplicate samples (200 µl) of muscle extracts and diluted (1:10) incubation medium were placed in scintillation vials containing 7 ml of CytoScint cocktail (ICN Biomedicals) and counted in a Wallac liquid scintillation counter with channels preset for simultaneous counting of ^{3}H and ^{14}C. Thus, ^{3}H counts were corrected for spillover of ^{14}C. Extracellular space (ml g^{-1} muscle) and intracellular concentration of 2-DG (µmol ml^{-1} intracellular water) were determined as described previously for mammalian skeletal muscle (Hansen *et al.* 1994). Cytochalasin B (25 µmol l^{-1}), an inhibitor of facilitative glucose transport proteins, was included in some incubations to assess whether glucose uptake is specific for a glucose transporter in teleost cardiac muscle. Cytochalasin additions came from a 10 mmol l^{-1} stock solution containing absolute ethanol.

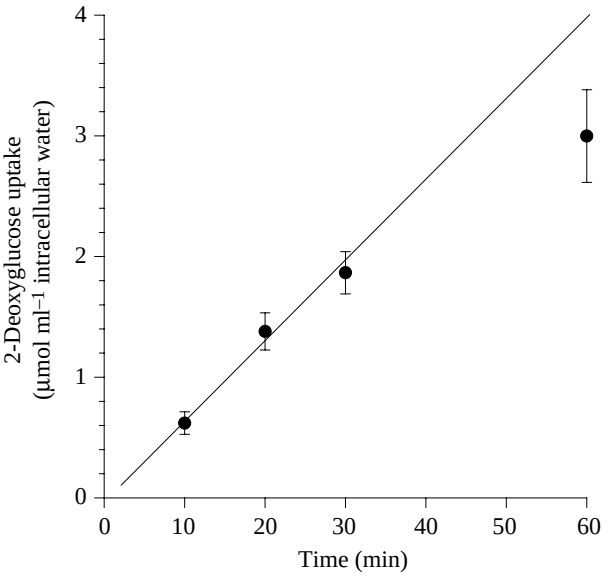

Fig. 1. Time course of 2-deoxyglucose (2-DG) uptake in eel ventricle strips incubated with 5 mmol^{-1} 2-DG. Ventricle strips were incubated at 15 °C for 60 min in a Ringer's solution containing 5 mmol l^{-1} D-glucose, 35 mmol l^{-1} sorbitol and 0.1 % bovine serum albumin (BSA). Strips were rinsed for 10 min and then incubated in medium containing 5 mmol^{-1} 2-deoxy-D-[2-^{3}H]glucose, 33 mmol l^{-1} D-[U-^{14}C]sorbitol, 2 mmol l^{-1} pyruvate and 0.1 % BSA to assay uptake of 2-DG for the indicated times. Values are means ± S.E.M. for four strips per point. The line is a regression line through the points up to 30 min. 2-DG uptake was linear between 10 and 30 min. y=0.52x+0.179; r^2=0.967.

Subcellular fractionation of HK activity in perfused ventricles under normoxic and anoxic conditions

Entire hearts were excised and rinsed in ice-cold medium. Hearts (atria, ventricle and bulbus arteriosus) were then mounted in a water-jacketed perfusion apparatus (Bailey and Driedzic, 1989) controlled at 15 °C. Both normoxic and anoxic hearts were perfused initially for 10 min with normoxic medium (gassed with 0.5 % CO_2:99.5 % air) at 25 ml min^{-1} kg^{-1} eel to rinse them of intraluminal blood. Hearts were then perfused for an additional 20 min with either normoxic or anoxic medium (gassed with 0.5 % CO_2:99.5 % N_2), which defined our experimental treatments. The oxygen content of the anoxic perfusion medium was always below the detectable limits of our electrode (Strathkelvin 1302 Microcathode). The atria and bulbus arteriosus were removed from the ventricle, which was weighed and homogenized in a ground-glass Tenbroeck tissue grinder (Kontes, Vineland, NJ, USA) with nine volumes of ice-cold extraction buffer containing 5 mmol l^{-1} KH_2PO_4, 5 mmol l^{-1} K_2HPO_4 (pH 7.8 at 20 °C), 0.5 mmol l^{-1} EDTA and 250 mmol l^{-1} sucrose. All subsequent procedures were carried out at 4 °C. A small volume of crude homogenate was kept for assays of HK and monoamine oxidase activity; the remainder was centrifuged at

600 ***g*** for 10 min to remove cell debris. The supernatant was centrifuged at 9000 ***g*** for 10 min to pellet mitochondria. The resulting supernatant was kept for analysis, whereas the pellet was washed with extraction buffer, recentrifuged at 9000 ***g*** for 10 min, and resuspended in 1 ml of extraction buffer. This crude mitochondrial fraction was divided into two equal samples of 500 µl and treated either with 500 µl of extraction buffer or with 500 µl of extraction buffer containing 1 mol l^{-1} KCl to solubilize bound HK. Both fractions were centrifuged at 9000 ***g*** for 10 min, and the supernatants were kept for testing. The resulting pellets were resuspended in approximately 300 µl of extraction buffer containing 0.1 % BSA and used immediately for biochemical assays.

Hexokinase (HK, EC 2.7.1.1) activity was measured spectrophotometrically according to the methods of Sidell *et al.* (1987). The assay medium consisted of 75 mmol l^{-1} Tris (pH 7.4 at 20 °C), 7.5 mmol l^{-1} $MgCl_2$, 0.8 mmol l^{-1} EDTA, 1.5 mmol l^{-1} KCl, 0.4 mmol l^{-1} $NADP^+$, 2.5 mmol l^{-1} ATP, 10 mmol l^{-1} creatine phosphate, 0.9 i.u. ml^{-1} creatine phosphokinase, 0.7 i.u. ml^{-1} glucose-6-phosphate dehydrogenase and 1.0 mmol l^{-1} D-glucose. The reaction was initiated by the addition of D-glucose and carried out at 15 °C in a final assay volume of 2 ml. Blank trials did not include D-glucose. Reduction of $NADP^+$ to NADPH was followed at 340 nm for 5 min. HK activity is reported as µmol min^{-1} g^{-1} tissue.

To assess the specific association of HK with the outer membrane of mitochondria, we also measured monoamine oxidase (MAO, EC 1.4.3.4) activity in subcellular fractions. MAO is a specific marker for the outer membrane of mitochondria, and the activity of this enzyme was assayed using a modification of the methods of Szutowicz *et al.* (1984). The assay medium consisted of 100 mmol l^{-1} sodium phosphate (pH 7.4), 10 mmol l^{-1} tyramine and 5 mmol l^{-1} NaN_3. The final assay volume, including a 25 µl sample, was 550 µl, and blank trials (minus tyramine) were run for each sample. Following the addition of samples, glass reaction tubes (13 mm×100 mm) were incubated in a shaking water bath at 37 °C for 60 min. Reactions were stopped with 0.5 ml of H_2O_2-measuring solution, which consisted of 0.5 mol l^{-1} phosphate citrate buffer (pH 4.0), 1.8 mmol l^{-1} 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 5 units of horseradish peroxidase. After waiting 15 s, 0.25 ml of a solution containing 0.75 mol l^{-1} HCl, and 5 % sodium dodecyl sulphate was added to stabilize the colour and to remove turbidity caused by subcellular particles. The coloured product was measured at 414 nm. Blank values were subtracted from samples, and the number of nmoles of H_2O_2 produced was determined using a standard curve. MAO activity is expressed as nmol H_2O_2 min^{-1} g^{-1} tissue.

i.u. definitions

Creatine phosphokinase: 1 unit will transfer 1 µmole of phosphate from phosphocreatine to ADP per minute at pH 7.5, 30 °C; glucose-6-phosphate dehydrogenase: 1 unit will oxidise 1.0 µmole of D-glucose-6-phosphate to 6-phospho-D-gluconate per minute in the presence of NADP at pH 7.4 at 25 °C; horseradish peroxidase: 1 unit will oxidise 1 µmole of 2,2′-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) per minute at 25 °C, pH 5.0.

Inhibition of HK activity by G-6-P and 2-DG-6-P

We determined the degree of non-competitive inhibition of HK activity by G-6-P and 2-DG-6-P using a modification of the methods of Crane and Sols (1954). Whole ventricles were excised, minced with scissors and homogenized in Tenbroeck tissue grinders with nine volumes of ice-cold extraction buffer containing 5 mmol l^{-1} KH_2PO_4, 5 mmol l^{-1} K_2HPO_4 (pH 7.8 at 20 °C), 0.5 mmol l^{-1} EDTA and 250 mmol l^{-1} sucrose. The homogenate was centrifuged at 600 ***g*** for 10 min at 4 °C, and a sample of supernatant was added to an assay mixture consisting of 2.5 mmol l^{-1} ATP, 5.0 mmol l^{-1} $MgCl_2$, 0.003 % Bromophenol Blue (pH 7.8), 10 mmol l^{-1} D-glucose and various concentrations of G-6-P (0, 100, 200 or 500 µmol l^{-1}, 1, 2 or 5 mmol l^{-1}) or 2-DG-6-P (0, 200 or 500 µmol l^{-1}, 1, 2, 5 or 10 mmol l^{-1}). Reactions was thermostatically controlled at 15 °C and followed for 5 min at 615 nm. Kinetic analyses of the HK inhibitors G-6-P and 2-DG-6-P were conducted using the Michaelis–Menten saturation model. Half-maximal inhibition concentrations (IC_{50}) for G-6-P and 2-DG-6-P were derived from linear Eadie–Hofstee plots.

Statistical analysis

Unless noted otherwise, values are presented as means ± S.E.M. Statistical analyses of 2-DG uptake measurements and HK assays were performed using one-way analysis of variance (ANOVA) followed by Duncan's *post hoc* test. $P \leqslant 0.05$ was considered to be statistically significant.

Results

Physical characteristics of the eel cardiac ventricle

Ventricles were pyramid-shaped and weighed on average 6.2 mg g^{-1} body mass, which equates to a relative ventricle mass of 0.062±0.002 % (N=36). Each ventricle strip contained both epicardial and endocardial layers, plus a very small amount of epicardial fat. The water content of ventricle strips from four hearts averaged 0.80 ml g^{-1} wet tissue, and we used this value for calculations of intracellular water content and 2-DG uptake rates. The sorbitol space of ventricle strips was 0.28–0.36 ml g^{-1} muscle and, by difference, the intracellular water was 0.44–0.52 ml g^{-1} muscle. These values agree favourably with a previous studies of perfused rat hearts (Morgan *et al.* 1961) and isolated frog muscle (Holloszy and Narahara, 1965).

Stimulation of 2-DG uptake by anoxia and contractile activity in ventricle strips

Changes in 2-DG uptake in ventricle strips induced by

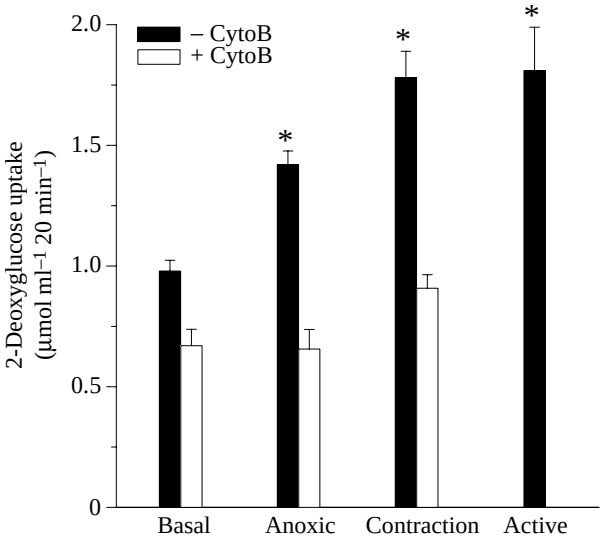

Fig. 2. Effects of anoxia, contractile activity and cytochalasin B on 2-deoxyglucose (2-DG) uptake in eel ventricle strips. Ventricle strips were incubated at 15 °C under oxygenated (basal) or anoxic conditions for 60 min in a Ringer's solution for freshwater teleosts, supplemented with 5 mmol^{-1} D-glucose, 35 mmol l^{-1} sorbitol and 0.1 % bovine serum albumin (BSA), followed by a 10 min rinse in glucose-free Ringer containing 40 mmol l^{-1} sorbitol and 0.1 % BSA. Strips were then incubated for 20 min in medium containing 5 mmol l^{-1} 2-deoxy-D-[2-^{3}H]glucose, 33 mmol l^{-1} D-[U^{14}C]sorbitol, 2 mmol l^{-1} pyruvate and 0.1 % BSA to assay 2-DG uptake. In some experiments, cytochalasin B (CytoB) (25 μmol l^{-1}) was present throughout the initial incubation, rinse and measurement of 2-DG uptake. Ventricle strips that were electrically stimulated to contract at 36 contractions min^{-1} for 10 min are grouped in the 'Contraction' category. Strips that contracted spontaneously throughout the incubation period (130 min) are labelled 'Active'. Each bar represents means ± S.E.M. for 5–10 muscles. *Significantly different from basal values (P<0.01).

anoxia, contractile activity or cytochalasin B are shown in Fig. 2. On average, anoxia stimulated glucose transport by 45 % and prescribed contractile activity (36 contractions min^{-1} for 10 min) stimulated glucose transport by 81 % over basal measurements (P<0.01). There was no diminution of force production, alteration in time to peak tension or change in time to 50 % relaxation during stimulation periods prior to measurement of 2-DG uptake. In some experiments, non-stimulated ventricle strips exhibited spontaneous contractions for the duration of incubation (130 min), and these strips also had significantly higher rates of 2-DG uptake (83 % greater) than non-contracting control strips (Fig. 2). It is noteworthy that stimulation of 2-DG uptake by contractile activity was identical whether induced by brief (10 min) electrical stimuli at a defined frequency (36 contractions min^{-1}) or by intrinsic activity over a prolonged period (130 min). Incubation of ventricle strips with cytochalasin B (25 μmol l^{-1}) partially inhibited 2-DG uptake under basal conditions and resulted in complete inhibition of the responses to physiological stimuli. Specifically, cytochalasin B inhibited all of the stimulation of 2-DG uptake induced by anoxia and contractile activity. Doubling the concentration of cytochalasin B in the incubation medium to 50 μmol l^{-1} did not increase the inhibition of basal or stimulated ventricle strips (data not shown).

Anoxia does not alter the subcellular distribution or activity of HK in the eel heart

Total HK activity was identical in eel hearts perfused with anoxic and normoxic medium (Table 1). No significant differences were detected in total enzyme recovery (91 % and 87 %) or in the distribution of HK activity following subcellular fractionation by differential centrifugation. Standardized ratios of soluble (9000 ***g*** supernatant) to particulate (9000 ***g*** pellet) enzyme activities were approximately 70:30 for both groups of eel hearts, suggesting that most of the HK activity was cytosolic. Washing the mitochondrial pellet with 0.5 mol l^{-1} KCl did reduce HK activity in this fraction, presumably by solubilization of bound HK. Average recoveries of HK activity in the KCl-washed 9000 ***g*** pellet were 23 % for normoxic and 36 % for anoxic hearts. These values were not significantly different and are consistent with a stable enzyme complex which does not

Table 1. *Effects of anoxia on subcellular distribution of hexokinase (HK) and monoamine oxidase (MAO) activity in perfused eel hearts*

	Anoxic				Normoxic			
Step	HK	Recovery (%)	MAO	Recovery (%)	HK	Recovery (%)	MAO	Recovery (%)
Total homogenate	10.1±0.8	100	33.8±3.3	100	10.6±2.2	100	44.7±6.1	100
600 ***g*** pellet	1.5±0.2	15	7.8±1.4	23	1.7±0.2	16	9.9±3.1	22
9000 ***g*** supernatant	5.5±0.6	54	30.4±4.4	90	5.0±0.4	47	35.7±2.6	80
9000 ***g*** pellet	2.2±0.4	22	7.1±1.9	21	2.5±0.2	24	8.2±0.6	18
9000 ***g*** pellet (KCl wash)	0.5±0.1		6.0±2.1		0.9±0.2		7.1±1.4	

Anoxic and normoxic hearts were perfused initially for 10 min with normoxic medium to rinse the heart of intraluminal blood. Hearts were then perfused for an additional 20 min with either normoxic or anoxic medium.

HK activity in total homogenates and subcellular fractions is expressed as μmol min^{-1}g^{-1} tissue.

MAO activity is expressed as nmol H_2O_2 min^{-1} g^{-1} tissue.

Values are means ± S.E.M. of 5–6 ventricles.

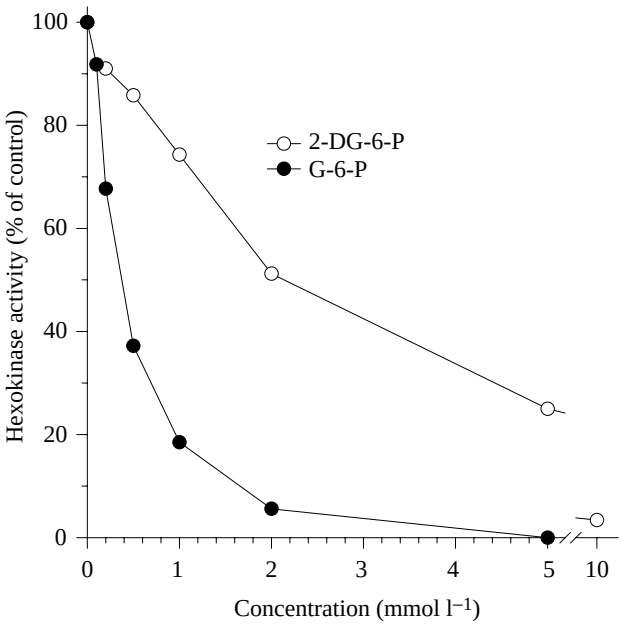

Fig. 3. Inhibition of hexokinase activity in eel ventricle homogenates by glucose 6-phosphate (G-6-P) and 2-deoxyglucose 6-phosphate (2-DG-6-P). Glucose concentration was 10 mmol l^{-1}. Activity was measured spectrophotometrically at 15 °C and is expressed as a percentage of the activity in the absence of added G-6-P or 2-DG-6-P. Values represent means of duplicate measurements from two animals.

change with oxygen availability. Although quite variable, MAO activities in whole homogenates did not differ between anoxic and normoxic hearts. In addition, recovery values for MAO in the mitochondrial fraction were the same (approximately 20 %), providing evidence that subcellular fractionation was not affected by the oxygen content of the perfusion medium. Thus, it appears that exposure of the eel heart to anoxia for 20 min does not affect the distribution of HK between the soluble and mitochondrial compartments in cardiac myocytes.

Effects of 2-DG, G-6-P and 2-DG-6-P on HK activity

Maximal activities of HK with D-glucose or 2-DG at concentrations of 10 mmol l^{-1} were essentially identical in homogenates of eel cardiac muscle (data not shown). The effects of increasing concentrations of G-6-P and 2-DG-6-P on HK activity are illustrated in Fig. 3. A progressive inhibition of HK activity with increasing concentrations of both compounds was observed, with G-6-P being a stronger inhibitor of HK than 2-DG-6-P. At a common concentration of 1.0 mmol l^{-1}, HK activity was reduced to approximately 20 % of the control value by G-6-P and to 75 % of the control value by 2-DG-6-P. The IC_{50} for G-6-P was 0.44 mmol l^{-1} and that for 2-DG-6-P was 2.0 mmol l^{-1}. These findings promote G-6-P as a potential physiological inhibitor of HK and confer caution that 2-DG-6-P inhibition of HK activity may be significant during prolonged measurements of 2-DG uptake in teleost cardiac muscle.

Discussion

Stimulation of 2-DG uptake in eel cardiac muscle by anoxia and contractile activity

Glucose uptake in the mammalian heart is increased by anoxia and contractile activity (Neely and Morgan, 1974). However, rates of uptake of glucose or glucose analogues are not comparable between mammalian and teleost cardiac muscle, as should be expected because of the much lower energy requirement of teleost tissue. The intrinsic rate of contraction of the rat heart *in vivo* is 300–400 beats min^{-1}, or 200–240 beats min^{-1} during perfusion, and systolic blood pressures (13.3–16.0 kPa) are much higher than values for the European eel (37 beats min^{-1} and 5.1 kPa respectively) (Peyraud-Waitznegger and Soulier, 1989). Accordingly, the rate of glucose uptake of the perfused rat heart (approximately 0.5–1.9 μmol min^{-1} g^{-1}, Morgan *et al.* 1961) and rat heart *in vivo* (approximately 0.4–1.0 μmol min^{-1} g^{-1}, James *et al.* 1985) are almost two orders of magnitude faster than the corresponding values for 2-DG in isolated eel ventricle strips (25–46 nmol min^{-1} g^{-1}, present study). Given that we did not determine a lumped constant to correct for differences between tissue uptake of glucose and 2-DG, we can only estimate glucose utilization rates of eel cardiac muscle under different physiological conditions. If we assume that the lumped constant of 0.40 for trout (*Oncorhynchus mykiss*) ventricle strips (West *et al.* 1993) applies equally to eel ventricle strips, corrected rates of 2-DG uptake (62–115 nmol glucose min^{-1} g^{-1}) are still dramatically slower than mammalian values. Notably, our results are consistent with *in vitro* rates of glucose uptake in isolated hearts of rainbow trout (30–120 nmol min^{-1} g^{-1}, West *et al.* 1993), but higher than *in vivo* rates of glucose utilization of hearts of carp (*Cyprinus carpio*) (10–36 nmol min^{-1} g^{-1}, West *et al.* 1994) and rainbow trout (approximately 5 nmol min^{-1} g^{-1}, West *et al.* 1993).

On the basis of previous studies, we can also estimate the contribution of glucose to cardiac energy production in American eel under aerobic and anoxic conditions. The ATP turnover required to support aerobic eel hearts performing physiological levels of work at 15 °C has been estimated at 3.1 μmol min^{-1} g^{-1} tissue (Bailey *et al.* 1991). Given an ATP yield from glucose of 38 molecules under aerobic conditions, calculated rates of glucose uptake in the current study (115 nmol min^{-1} g^{-1}) could yield up to 4.4 μmol ATP min^{-1} g^{-1}. Given this agreement of ATP values, we believe that energy metabolism under normoxia could be supported to a large extent by glucose and that glucose uptake is probably the rate-controlling step for glucose utilization in eel cardiac muscle. Anoxia presents a significant challenge to maintain tissue energy balance, and glycolysis could theoretically protect myocardial performance. Another consideration with ectothermic hearts, including that of the eel, is the possibility that contractility may be maintained under anoxia, albeit at lower levels of force (Hartmund and Gesser, 1996). Although we are not aware of any studies showing anoxia-induced metabolic depression in eels, this response

occurs in goldfish (*Carassius auratus*) (lowering metabolic rate to 30 % of normoxic values, van Waversveld *et al.* 1989) and may apply to the current study. If the heart in the intact animal responds in the same manner as the isolated heart, then energy demand will be decreased under anoxia. It is still unlikely, however, that glucose alone could support the cardiac energy metabolism of the American eel under anoxia. Maximum anaerobic ATP production from glucose would only be approximately $0.18\,\mu mol\,ATP\,min^{-1}\,g^{-1}$ ($88\,nmol\,glucose\,min^{-1}\,g^{-1} \times 2ATP/glucose$), which is less than 10 % of values for normoxic hearts performing physiological levels of work (Bailey *et al.* 1991). Thus, even with significant reductions in work rates, it is likely that a combination of exogenous glucose and endogenous glycogen support the energy requirements of the anoxic eel heart.

Our findings are the first to demonstrate stimulatory effects of anoxia and contractile activity on 2-DG uptake in teleost cardiac muscle. Unlike studies *in vivo*, we have eliminated the potential confounding influences of variable blood flow (substrate delivery) and substrate concentration. We have also corrected for 2-DG present in the extracellular space. Thus, increases in 2-DG uptake cannot be attributed to haemodynamic effects or to differences in extracellular substrate concentration. Given that the sizes of the eel ventricle strips were also similar, we cannot explain increases in 2-DG uptake by shorter diffusion distances. It is more likely that increases in 2-DG uptake were brought about by recruitment of regulatable glucose transport proteins from an intracellular compartment to the plasma membrane of myocytes or by increases in the intrinsic activity of glucose transporters that are present at the cell surface.

Inhibition of 2-DG uptake by cytochalasin B

Incubation of eel ventricle strips with cytochalasin B inhibited 2-DG uptake under basal and stimulated conditions. The characteristic of glucose transport being inhibited by cytochalasin B correlates with the presence of facilitative glucose transport proteins in the plasma membrane of mammalian tissues (Silverman, 1991) and agrees with studies of erythrocytes from the American eel (Soengas and Moon, 1995), common eel (*Anguilla japonica*) (Tse and Young, 1990), Pacific hagfish (*Eptatretus stouti*) (Young *et al.* 1994) and giant muscle fibres of the barnacle (*Balanus nubilus*) (Carruthers, 1983). In mammalian glucose transporters, cytochalasin B is known to bind the eleventh membrane-spanning α-helix of each GLUT isoform with different binding constants (Silverman, 1991). Given that anoxia- and contraction-induced increases in basal 2-DG uptake were inhibited completely by $25\,\mu mol\,l^{-1}$ cytochalasin B in eel ventricle strips (Fig. 2), glucose uptake under stimulated conditions appears to be mediated by glucose transporter proteins. However, a significant component of 2-DG uptake during basal conditions (68 %) was insensitive to inhibition by cytochalasin B. A similar finding has been demonstrated in the giant muscle fibres of the barnacle (Carruthers, 1983) and to a lesser degree in isolated rat skeletal muscle (Henriksen and Holloszy, 1991). Insensitivity of 2-DG uptake to cytochalasin B may be due to membrane damage during the isolation of the ventricle strips and may reflect a non-transporter-mediated process. It is also possible that eel cardiac muscle may contain a glucose transporter isoform with reduced affinity for cytochalasin B.

Compartmentation of HK in eel cardiac muscle does not vary in response to anoxia

The present study has demonstrated that HK binds to mitochondria in eel ventricle; however, the distribution of this enzyme does not change with oxygen availability. In mammalian skeletal muscle, mitochondrially bound HK has a higher affinity for glucose and is less susceptible to inhibition by glucose 6-phosphate (G-6-P) relative to the soluble form (Viitanen *et al.* 1984). It was therefore reasonable to hypothesize that enhanced binding of HK to mitochondria would allow anaerobic glycolysis to proceed during periods of anoxia in fish muscle. We perfused eel hearts in the presence or absence of oxygen, rapidly homogenized the tissue in the presence or absence of oxygen, and isolated mitochondrial fractions using differential centrifugation. Given that total HK activity, enzyme recoveries (MAO and HK) and the distribution of HK and MAO activity following subcellular fractionation did not differ in eel hearts perfused with anoxic and normoxic media, we feel justified in comparing HK distribution between these treatments. The proportions of soluble and particulate enzyme activity were approximately 70:30 for both groups of eel hearts, and this value is in close agreement with a previous report for rat heart (England and Randle, 1967). After exposure of the eel heart to anoxic conditions for 20 min, we were unable to demonstrate reversible association of HK with the outer mitochondrial membrane. This stable association may be related to close coupling between HK activity (ADP generation) and oxidative phosphorylation (ATP production) and the important coordination of cytoplasmic glycolysis and mitochondrial metabolism of glucose during aerobic conditions (BeltrandelRio and Wilson, 1992). In support of a stable association between HK and mitochondria during increased metabolic need, Brooks and Storey (1988) have shown no change in binding of HK to subcellular particles of cardiac muscle following forced exercise in rainbow trout. Thus, on the basis of just two studies in fishes, it remains to be determined whether a reversible association of HK with mitochondria occurs in the cardiac muscle of teleosts. More work is also needed to define the overall importance of subcellular HK compartmentation to glucose metabolism in fish hearts.

HK activity and inhibition by G-6-P

In mammalian cardiac muscle, maximum rates of aerobic carbohydrate utilization are correlated with total tissue activity of HK (Crabtree and Newsholme, 1972), and the rate-limiting step for glucose utilization may be glucose phosphorylation (Manchester *et al.* 1994). Our analysis of enzymic data and *in*

vitro measurements of 2-DG uptake under stimulated conditions provides compelling evidence that the maximal activity of HK is not an accurate predictor of glucose utilization rates in eel cardiac muscle (aerobic, anoxic or after contractile activity). Specifically, the upper limit of measured rates of 2-DG uptake (46 nmol min^{-1} g^{-1} tissue) and the calculated rates of glucose utilization (115 nmol min^{-1} g^{-1}) approximate to just 1 % of maximal HK activity (approximately 10 μmol min^{-1} g^{-1}). Previous studies have shown that the eel heart, like the heart of most teleosts, displays extremely high levels of HK (Driedzic *et al.* 1987; Sidell *et al.* 1987; Bailey *et al.* 1991). Yet, given much lower rates of ATP turnover and glucose utilization than in mammalian hearts, no one has come up with a good explanation of why there is an excess capacity to phosphorylate glucose in the hearts of fishes.

Thirty years ago, England and Randle (1967) proposed that inhibition or activation of HK in rat heart occurs by alterations in the intracellular concentration of G-6-P. The inhibitory constant (K_i) for G-6-P ranged from 0.16 to 0.33 mmol l^{-1} and, under physiological conditions, it was deemed possible to inhibit the catalytic potential of HK to just 20 % of maximum (England and Randle, 1967). We have now demonstrated that HK in eel hearts can also be inhibited by G-6-P. It is noteworthy that the IC_{50} for G-6-P in eel ventricle (0.44 mmol l^{-1}) is comparable to K_i values reported for rat heart. Previous studies have measured the concentration of G-6-P in fish hearts during a transition from aerobic to anoxic conditions. Under aerobic conditions, G-6-P levels in the heart of goldfish (*Carassius auratus*) were 0.20 μmol g^{-1} wet mass (Shoubridge and Hochachka, 1983). If we assume that the intracellular water content of the teleost ventricle is approximately 0.5 ml g^{-1} wet mass (present study) and that the volume density of mitochondria is 15 %, the cytosolic concentration of G-6-P would approach 0.5 mmol l^{-1} and exceed the IC_{50} of HK of eel hearts. Thus, in the teleost heart, inhibition of HK by G-6-P during aerobic conditions may be of physiological relevance. In addition, African lungfish (*Protopterus aethiopicus*) (Dunn *et al.* 1983), Atlantic hagfish (*Myxine glutinosa*) (Hansen and Sidell, 1983) and goldfish (Shoubridge and Hochachka, 1983) all demonstrate a significant decrease in cardiac G-6-P concentration under oxygen-limiting conditions, suggesting that HK may be an important site of metabolic control during anoxia. However, it is possible that HK in fish hearts does not play a regulatory role in glucose utilization as it does in the mammalian heart. Although HK in hearts of both mammals and fishes is inhibited by G-6-P, it is unlikely that this modulator can reduce HK activity to levels approaching measured rates of glucose uptake (present study) and ATP production (Bailey *et al.* 1991) in the eel heart. It is also now clear that HK can associate with mitochondria in fish hearts; however, once again, we doubt that compartmentation of HK can reduce total enzyme activity sufficiently to promote glucose phosphorylation as a rate-controlling step in cardiac glucose utilization. It appears more likely that glucose transport across the plasma membrane rather than intracellular phosphorylation of glucose is rate-limiting for glucose utilization in the eel heart.

Precautions with the use of 2-DG in fish cardiac muscle to measure glucose uptake

2-DG is phosphorylated by HK and trapped in the myocyte as 2-DG-6-P. The results from mammalian skeletal muscle demonstrate that 2-DG uptake accurately reflects glucose transport activity (Hansen *et al.* 1994); however, there may be problems with the use of substrate levels (millimolar) of 2-DG in incubated cardiac muscle. In mammalian heart (Chen and Gueron, 1992), but not skeletal muscle (Hansen *et al.* 1994) or brain (Crane and Sols, 1954), 2-DG-P can inhibit HK at similar concentrations to G-6-P. As a consequence, high rates of 2-DG uptake or extended incubation periods may cause accumulation of 2-DG-6-P to levels that inhibit HK, resulting in underestimates of 2-DG uptake rates. We chose a measurement time of 20 min because 2-DG uptake was linear between 10 and 30 min; however, it is still possible that we could have underestimated glucose uptake rates. Our estimate of the inhibitory constant of 2-DG for HK in eel ventricle (2.0 $mmol^{-1}$) is similar to that noted by Chen and Gueron (1992) in bovine heart (1.4 mmol l^{-1}). Together, these observations demonstrate that trapped 2-DG-P in fish or mammalian cardiac muscle could reduce the rate of phosphorylation of sugars by HK. We therefore recommend that considerable care must be exercised when using substrate levels of 2-DG to assess glucose uptake rates in fish cardiac muscle *in vitro*. At the same time, it is unlikely that the same precaution applies to studies in which radiolabelled 2-DG is used in trace quantities (nanomolar). Finally, because our research focused on comparative measurements of 2-DG uptake under controlled conditions, we feel confident that they reflect true differences due to physiological stimuli.

This work was supported by grants from the Natural Sciences and Research Engineering Council of Canada (W.R.D.), New Brunswick Heart and Stroke Foundation (W.R.D.) and Faculty Research Committee Grant 744 from Idaho State University (K.J.R.).

References

BAILEY, J. R. AND DRIEDZIC, W. R. (1989). Effects of acute temperature change on cardiac performance and oxygen consumption of a marine fish, the sea raven (*Hemitripterus americanus*). *Physiol. Zool.* **62**, 1089–1101.

BAILEY, J., SEPHTON, D. AND DRIEDZIC, W. R. (1991). Impact of an acute temperature change on performance and metabolism of pickerel (*Esox niger*) and eel (*Anguilla rostrata*) hearts. *Physiol. Zool.* **64**, 697–716.

BELTRANDELRIO, H. AND WILSON, J. E. (1992). Coordinated regulation of cerebral glycolytic and oxidative metabolism, mediated by mitochondrial bound hexokinase dependent on intramitochondrially generated ATP. *Archs Biochem. Biophys.* **296**, 667–677.

BERGER, M., HAGG, S. AND RUDERMAN, N. B. (1975). Glucose metabolism in perfused skeletal muscle. Interaction of insulin and exercise on glucose uptake. *Biochem. J.* **146**, 231–238.

BROOKS, S. P. J. AND STOREY, K. B. (1988). Subcellular enzyme binding in glycolytic control: *in vivo* studies with fish muscle. *Am. J. Physiol.* **255**, R289–R294.

CARRUTHERS, A. (1983). Sugar transport in giant barnacle muscle fibers. *J. Physiol., Lond.* **336**, 377–396.

CHEN, W. AND GUERON, M. (1992). The inhibition of bovine heart hexokinase by 2-deoxy-D-glucose-6-phosphate: characterization by ^{31}P NMR and metabolic implications. *Biochimie* **74**, 867–873.

CORNISH, I. AND MOON, T. W. (1985). Glucose and lactate kinetics in American eel *Anguilla rostrata*. *Am. J. Physiol.* **249**, R67–R72.

CRABTREE, B. AND NEWSHOLME, E. A. (1972). The activities of phosphorylase, hexokinase, phosphofructokinase, lactate dehydrogenase and glycerol-3-phosphate dehydrogenases in muscle from vertebrates and invertebrates. *Biochem. J.* **126**, 49–58.

CRANE, R. K. AND SOLS, A. (1954). The non-competitive inhibition of brain hexokinase by glucose-6-phosphate and related compounds. *J. biol. Chem* **210**, 597–606.

DRIEDZIC, W. R. (1978). Carbohydrate metabolism in the perfused dogfish heart. *Physiol. Zool.* **51**, 42–50.

DRIEDZIC, W. R. AND HART, T. (1984). Relationship between exogenous fuel availability and performance by teleost and elasmobranch hearts. *J. comp. Physiol.* B **154**, 593–599.

DRIEDZIC, W. R., PHLEGER, C. F., FIELDS, J. H. A. AND FRENCH, C. (1978). Alterations in energy metabolism associated with the transition from water to air breathing in fish. *Can. J. Zool.* **56**, 730–735.

DRIEDZIC, W. R., SIDELL, B. D., STOWE, D. AND BRANSCOMBE, (1987). Matching of cardiac energy demand to energy metabolism. *Am. J. Physiol.* **252**, R930–R937.

DUNN, J. F., HOCHACHKA, P. W., DAVISON, W. AND GUPPY, M. (1983). Metabolic adjustments to diving and recovery in the African lungfish. *Am. J. Physiol.* **245**, R651–R657.

ENGLAND, P. J. AND RANDLE, P. J. (1967). Effectors of rat-heart hexokinases and the control of rates of glucose phosphorylation in the perfused rat heart. *Biochem. J.* **105**, 907–920.

GERBITZ, K. D., GEMPEL, K. AND BRDICZKA, D. (1996). Mitochondria and diabetes. Genetic, biochemical and clinical implications of the cellular energy circuit. *Diabetes* **45**, 113–126.

HANSEN, C. A. AND SIDELL, B. D. (1983). Atlantic hagfish cardiac muscle: metabolic basis of tolerance to anoxia. *Am. J. Physiol.* **244**, R356–R362.

HANSEN, P. A., GULVE, E. A. AND HOLLOSZY, J. O. (1994). Suitability of 2-deoxyglucose for *in vitro* measurement of glucose transport activity in skeletal muscle. *J. appl. Physiol.* **76**, 979–985.

HARTMUND, T. AND GESSER, H. (1996). Cardiac force and high-energy phosphates under metabolic inhibition in four ectothermic vertebrates. *Am. J. Physiol.* **271**, R946–R954.

HENRIKSEN, E. J. AND HOLLOSZY, J. O. (1991). Effect of diffusion distance on measurement of rat skeletal muscle glucose transport *in vitro*. *Acta physiol. scand.* **143**, 381–386.

HOAR, W. S. AND HICKMAN, C. P., JR (1975). *A Laboratory Companion for General and Comparative Physiology*, pp. 278–279. Englewood Cliffs, NJ: Prentice-Hall.

HOLLOSZY, J. O. AND NARAHARA, H. T. (1965). Studies of tissue permeability. X. Changes in permeability to 3-methyl-glucose associated with contraction of frog muscle. *J. biol. Chem.* **240**, 3493–3500.

JAMES, D. E., KRAEGEN, E. W. AND CHISHOLM, D. J. (1985). Effects of exercise training on *in vivo* insulin action in individual tissues of the rat. *J. clin. Invest.* **76**, 657–666.

LANCTIN, H. P., MCMORRAN, L. E. AND DRIEDZIC, W. R. (1980). Rates of glucose and lactate oxidation by perfused isolated trout (*Salvelinus fontinalis*) heart. *Can. J. Zool.* **58**, 1708–1711.

MANCHESTER, J., KONG, X., NERBONNE, J., LOWRY, O. H. AND LAWRENCE, J. C., JR (1994). Glucose transport and phosphorylation in single cardiac myocytes: rate-limiting steps in glucose utilization. *Am. J. Physiol.* **266**, E326–E333.

MORGAN, H. E., RANDLE, P. J. AND REGEN, D. M. (1961). Regulation of glucose uptake in muscle. I. The effects of insulin and anoxia on glucose transport and phosphorylation in the isolated, perfused heart of normal rats. *J. biol. Chem.* **236**, 253–261.

NEELY, J. R. AND MORGAN, H. E. (1974). Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. *A. Rev. Physiol.* **36**, 413–459.

PESSIN, J. E. AND BELL, G. I. (1992). Mammalian facilitative glucose transporter family: Structure and molecular regulation. *A. Rev. Physiol.* **54**, 911–930.

PEYRAUD-WAITZENEGGER, M., BARTHELEMY, L. AND PEYRAUD, C. (1980). Cardiovascular and ventilatory effects of catecholamines in unrestrained eels (*Anguilla anguilla*). *J. comp. Physiol.* **138**, 367–375.

PEYRAUD-WAITZENEGGER, M. AND SOULIER, P. (1989). Ventilatory and circulatory adjustments in the European eel (*Anguilla anguilla* L.) exposed to short-term hypoxia. *Exp. Biol.* **48**, 107–122.

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

SANTER, R. M. AND GREER WALKER, M. (1980). Morphological studies on the ventricle of teleost and elasmobranch hearts. *J. Zool., Lond.* **190**, 259–272.

SHOUBRIDGE, E. A. AND HOCHACHKA, P. W. (1983). The integration and control of metabolism in the anoxic goldfish. *Molec. Physiol.* **4**, 165–195.

SIDELL, B. D., CROCKETT, E. L. AND DRIEDZIC, W. R. (1995). Antarctic fish tissues preferentially catabolize monoenoic fatty acids. *J. exp. Zool.* **271**, 73–81.

SIDELL, B. D., DRIEDZIC, W. R., STOWE, D. B. AND JOHNSTON, I. A. (1987). Biochemical correlations of power development and metabolic fuel preferenda in fish hearts. *Physiol. Zool.* **60**, 221–232.

SIDELL, B. D., STOWE, D. B. AND HANSEN, C. A. (1984). Carbohydrate is the preferred metabolic fuel of the hagfish (*Myxine glutinosa*). *Physiol. Zool.* **57**, 266–273.

SILVERMAN, M. (1991). Structure and function of hexose transporters. *A. Rev. Biochem.* **60**, 757–794.

SLOT, J. W., GEUZE, H. J., GIGENGACK, S., JAMES, D. E. AND LIENHARD, G. E. (1991). Translocation of the glucose transporter GLUT 4 in cardiac myocytes of the rat. *Proc. natn. Acad. Sci. U.S.A.* **88**, 7815–7819.

SOENGAS, J. L. AND MOON, T. W. (1995). Uptake and metabolism of glucose, alanine and lactate by red blood cells of the American eel *Anguilla rostrata*. *J. exp. Biol.* **198**, 877–888.

SZUTOWICZ, A., KOBES, R. D. AND ORSULAK, P. J. (1984). Colorimetric assay for monoamine oxidase in tissues using peroxidase and 2,2′-azinodi(3-ethylbenzthiazoline-6-sulfonic acid) as a chromogen. *Analyt. Biochem.* **138**, 86–94.

TSE, C. M. AND YOUNG, J. D. (1990). Glucose transport in fish erythrocytes: Variable cytochalasin-B-sensitive hexose transport activity in the common eel (*Anguilla japonica*) and transport deficiency in the paddyfield eel (*Monopterus albus*) and rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **148**, 367–383.

VAN WAVERSVELD, J., ADDINK, A. D. F. AND VAN DEN THILLART, G. (1989). Simultaneous direct and indirect calorimetry on normoxic and anoxic goldfish. *J. exp. Biol.* **142**, 325–335.

VIITANEN, P. V., GEIGER, P. J., ERICKSON-VIITANEN, S. AND BESSMAN, S. P. (1984). Evidence of functional hexokinase compartmentation in rat skeletal muscle mitochondria. *J. biol. Chem*. **259**, 9679–9686.

WEST, T. G., ARTHUR, P. G., SUAREZ, R. K., DOLL, C. J. AND HOCHACHKA, P. W. (1993). *In vivo* utilization of glucose by heart and locomotory muscles of exercising rainbow trout (*Oncorhynchus mykiss*). *J. exp. Biol.* **177**, 63–79.

WEST, T. G., BRAUNER, C. J. AND HOCHACHKA, P. W. (1994). Muscle glucose utilization during sustained swimming in the carp (*Cyprinus carpio*). *Am. J. Physiol.* **267**, R1226–R1234.

YOUNG, J. D., YAO, S. Y.-M., TSE, C. M., DAVIES, A. AND BALDWIN, S. A. (1994). Functional and molecular characteristics of a primitive vertebrate glucose transporter: studies of glucose transport by erythrocytes from the Pacific hagfish (*Eptatretus stouti*). *J. exp. Biol.* **186**, 23–41.