Effects of anoxia exposure and aerobic recovery on metabolic enzyme activities in the freshwater turtle *Trachemys scripta elegans*

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Abstract: The effects of anoxic submergence (20 h at 7°C in nitrogen-bubbled water) and subsequent aerobic recovery (24 h at 7°C) on the maximal activities of 21 metabolic enzymes were assessed in liver, kidney, heart, brain, and red and white skeletal muscle of an anoxia-tolerant freshwater turtle, the red-eared slider, *Trachemys scripta elegans*. Anoxia exposure affected the activities of only a few enzymes; for example, it reduced the activity of phosphofructokinase in liver and brain, hexokinase in kidney, glycerol-3-phosphate dehydrogenase and glutamate-oxaloacetate transaminase in heart, glutamate dehydrogenase and serine dehydratase in brain, and 3-hydroxyacyl-CoA dehydrogenase in red muscle. During aerobic recovery, activities of most of these enzymes rebounded and activities of 10 others that were not affected by anoxia rose during recovery. Anoxia-induced changes in selected enzymes appear to meet very specific needs such as glycolytic-rate depression, regulation of glycolytic versus gluconeogenic flux in liver, or alterations in amino acid neurotransmitter levels in brain. Overall, the data demonstrate that the enzymatic make-up of turtle organs undergoes very few changes during anoxia exposure and recovery, which shows that the constitutive activities of enzymes are well designed to meet the metabolic demands of anoxic excursions.

Résumé : Nous avons évalué les effets d'une submersion anoxique (20 h à 7°C, dans de l'eau où barbotait de l'azote) et de la récupération aérobie subséquente (24 h à 7°C) sur l'activité maximale de 21 enzymes métaboliques dans le foie, les reins, le coeur, le cerveau, ainsi que dans les muscles squelettiques blanc et rouge d'une tortue d'eau douce tolérant l'anoxie, *Trachemys scripta elegans*. L'exposition aux conditions anoxiques affecte l'activité de seulement quelques enzymes : par exemple, celle de la phosphofructokinase est réduite dans le foie et dans le cerveau, celle de l'hexokinase dans le rein, celle de la glycérol-3-phosphate déshydrogénase et de la glutamate-oxaloacétate transaminase dans le coeur, celle de la glutamate-déshydrogénase et de la sérine déshydratase dans le cerveau, enfin celle de la 3-hydroxyacyl-CoA déshydrogénase dans le muscle rouge. Durant la récupération en conditions aérobies, l'activité de la plupart de ces enzymes reprend et l'activité de 10 autres enzymes non affectées par l'anoxie augmente. Les changements dans l'activité de certaines enzymes particulières générés par l'exposition à l'anoxie semblent répondre à des besoins spécifiques comme, par exemple, la diminution du taux de glycolyse, la régulation du flux glycolytique par rapport au flux gluconéogénique dans le foie, ou des modifications des concentrations des acides aminés neurotransmetteurs dans le cerveau. Dans l'ensemble, ces données démontrent que le complexe enzymatique des organes de la tortue subit peu de changements au cours d'une exposition à l'anoxie et de la période de récupération subséquente, ce qui veut dire que les activités constitutives des enzymes sont aptes à répondre aux exigences métaboliques pendant les excursions anoxiques.

[Traduit par la Rédaction]

Introduction

Tolerance of oxygen deprivation varies widely among vertebrate species, ranging from the mere minutes that the brains of most mammals can endure without injury to the extraordinary ability of some turtles to survive as long as 3 months when submerged in oxygen-depleted cold water (Ultsch 1985). Freshwater turtles of the genera *Trachemys* and *Chrysemys* are frequently cited as the best facultative anaerobes among vertebrates. Their tolerance serves both breath-hold diving and winter hibernation under water (Ultsch 1989). Although some extrapulmonary gas exchange

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occurs in submerged turtles, and turtles survive considerably longer in oxygenated versus deoxygenated water (Ultsch and Jackson 1982; Ultsch 1985), their capacity for long-term anaerobiosis is remarkable.

The molecular mechanisms supporting tolerance of anoxia by turtles have been well studied. These include high levels of fermentative fuels (glycogen) in all organs, the ability to buffer high lactic acid loads from anaerobic glycolysis (and to store lactate in the shell), and metabolic-rate depression (Clark and Miller 1973; Storey 1996; Lutz and Storey 1997; Jackson et al. 2000). By lowering the metabolic rate during anoxia to a value only 10–20% of the aerobic rate at the same temperature (Herbert and Jackson 1985), animals reduce ATP expenditure to a level that can be supported over the long term by glycolytic ATP output and gain a 5- to 10-fold extension of the time that endogenous fuels can support survival. Metabolic-rate depression is achieved via a coordinated reduction in the rates of most or all metabolic processes. For example, channel arrest in brain strongly reduces the net rates of ion movements across membranes (via ion channels and ATP-dependent pumps) while largely maintaining membrane potential difference (Bickler et al. 2001), whereas rates of protein synthesis are reduced by >90% in hepatocytes (Land and Hochachka 1994). Biochemical mechanisms that contribute to metabolic arrest include post-translational modification of selected enzymes and functional proteins, changes in protein/enzyme aggregation or binding to subcellular structures, allosteric controls, and changes in the amounts of selected proteins as a result of altered gene expression or altered rates of protein biosynthesis or degradation (Storey and Storey 1990; Storey 2000). Studies that analyzed protein synthesis patterns in vivo, mRNA translation patterns in vitro, and differential gene expression have all indicated that organ-specific changes in the amounts or types of selected proteins occur under anoxic conditions (Brooks and Storey 1993; Douglas et al. 1994; Cai and Storey 1996; Willmore et al. 2001).

Another approach to identifying metabolic adjustments that support anoxia survival is to assess changes in the maximal activities of enzymes in response to the stress. By analyzing enzymes in multiple pathways of intermediary metabolism, an overall picture can be developed of the pathways and types of cellular functions that are enhanced or suppressed in order to provide a coordinated metabolic response to low oxygen levels. The present study measures the effects of 20 h of anoxia exposure and subsequent 24-h aerobic recovery on the maximal activities of 21 metabolic enzymes in six organs of the red-eared slider, *Trachemys scripta elegans*. These include enzymes of carbohydrate, fatty acid, adenylate, and amino acid metabolism. Anoxia-induced changes in enzyme activities indicate altered flux potential in selected pathways during anoxic submergence.

Materials and methods

Chemicals and animals

Chemicals were purchased from Sigma Chemical Company (St. Louis, Mo., U.S.A.) or Boehringer Mannheim Corporation (Montreal, Quebec). Adult red-eared sliders were obtained from Wards Natural Science, Mississauga, Ontario, in February and maintained in large tanks of dechlorinated water at 7°C for 3 weeks prior to experimentation. Turtles were fed ad libitum on a diet of trout pellets, lettuce, and eggshells. For experimentation, turtles were carried to the laboratory in buckets of pool water, which were placed in an incubator at 7°C. Aerobic control turtles were sampled from this condition within 1 h. For anoxic submergence, groups of 3 turtles were transferred to 40-L containers filled with water that had been previously bubbled with 100% nitrogen gas for 10 h; wire mesh was fitted about 10 cm below the water surface to prevent turtles from surfacing. Bubbling with nitrogen gas was continued over the 20-h anoxia exposure and the container was held in the 7°C incubator. After anoxia exposure, one group of turtles was sampled immediately, while turtles in a second group were transferred to buckets of aerated water with no obstruction to surfacing and allowed to recover from anoxia exposure for 24 h at 7°C. All animals were killed by decapitation and tissues were immediately removed, frozen in liquid nitrogen, and transferred to –70°C for storage until use. Animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care and all experimental procedures had the prior approval of the Carleton University Animal Care Committee.

Preparation of tissue extracts for enzyme assay

Frozen tissue samples were quickly weighed and homogenized 1:5 (w/v) in ice-cold buffer containing protein kinase and protein phosphatase inhibitors (50 mM imidazole, pH 7.5, 10% v/v glycerol, 30 mM 2-mercaptoethanol, 100 mM NaF, 5 mM EDTA, 5 mM EGTA, a few crystals of phenylmethylsulfonyl fluoride) with a Diamed Pro 200 homogenizer set to full power. Homogenates were centrifuged in a Heraeus Biofuge 15 at 20 000 × *g* for 25 min and the supernatant was collected and stored on ice. Extracts for the assay of carnitine palmitoyltransferase (CPT) and carnitine octanoyltransferase (COT) were prepared as above, except that the pH of the extraction buffer was 8.1 and NaF and 2-mercaptoethanol were omitted.

Determination of enzyme activity

All assays were performed at 22°C using a Dynatech MR5000 microplate reader with absorbance measured at 340 nm for most enzymes and at 412 nm for COT and CPT in a final volume of 0.25 mL plus the volume of enzyme extract. Optimal assay conditions were determined for each enzyme using extracts from liver (these conditions were also used for kidney, brain, and heart) and red muscle (also used for white muscle). Blanks, measured in the absence of the most specific substrate, were subtracted to yield net activity values. One unit is defined as the amount of enzyme that produces one micromole of product per minute and all activities are reported as units per gram wet mass of tissue. All coupling enzymes were desalted before use by centrifugation through a small column of Sephadex G25 (Helmerhorst and Stokes 1980). Assay conditions for enzymes were as in Cowan et al. (2000) except for selected modifications to substrate concentrations as follows: (*i*) for hexokinase (HK) and glucokinase (GK): 10 and 100 mM glucose, respectively; (*ii*) for phosphofructokinase (PFK): 2.5 mM ATP and 8 mM fructose-6-phosphate; (*iii*) for pyruvate kinase (PK): 6 mM phosphoenolpyruvate (PEP) and 2 mM ADP; (*iv*) for fructose-1,6-bisphosphatase (FBPase): 1.4 mM fructose-1,6-bisphosphate; (*v*) for malate dehydrogenase (MDH): 6 mM oxaloacetate; (*vi*) for creatine kinase (CK): 20 mM creatine phosphate; and (*vii*) for serine dehydratase (SDH): 200 mM L-serine. Assay conditions for glycerol-3-phosphate dehydrogenase (G3PDH) were 50 mM imidazole buffer, pH 8.1, 30 mM glycerol-3-phosphate, and 0.8 mM NAD.

Soluble-protein concentrations were determined using the Coomassie blue G-250 binding method with the BioRad Laboratories prepared reagent with bovine serum albumin as the standard. Spectrophotometric quantification was performed at 595 nm using

Table 1. Maximal activities (units/gram wet mass) of enzymes in six organs of turtles (*Trachemys scripta elegans*).

	Liver	Kidney	Heart	Brain	Red muscle	White muscle
Carbohydrate metabolism						
HK (GK in liver)	0.14 ± 0.015	0.62 ± 0.02*	1.50 ± 0.30	1.98 ± 0.13	0.078 ± 0.003	0.04 ± 0.006
PFK	0.68 ± 0.044*	0.64 ± 0.07	1.71 ± 0.41	3.58 ± 0.20*	3.32 ± 0.49	2.83 ± 0.50
ALD	0.84 ± 0.067	1.28 ± 0.33*	7.49 ± 1.02	14.9 ± 1.27*	25.4 ± 0.79	21.0 ± 1.05
PK	19.4 ± 0.59	33.0 ± 0.67	61.5 ± 2.86	70.6 ± 0.67	69.2 ± 6.02	57.7 ± 3.73
LDH	267 ± 12	369 ± 33.1	684 ± 116	555 ± 16.8	60.4 ± 1.10	69.2 ± 5.14
G6PDH	1.58 ± 0.07	1.18 ± 0.035	0.43 ± 0.07*	0.99 ± 0.04	0.12 ± 0.007	0.092 ± 0.015
6PGDH	0.62 ± 0.046	0.75 ± 0.06	0.34 ± 0.033*	0.44 ± 0.033	nd	nd
FBPase	0.77 ± 0.060*	0.71 ± 0.06	0.18 ± 0.036	0.19 ± 0.023	nd	nd
PEPCK	0.20 ± 0.018	0.21 ± 0.03	0.082 ± 0.037	0.11 ± 0.004	nd	nd
G3PDH	0.15 ± 0.018	0.11 ± 0.006	0.23 ± 0.04*	0.17 ± 0.014	0.087 ± 0.006	0.07 ± 0.01
MDH	37.9 ± 1.28	65.8 ± 5.39	84.0 ± 15.0	87.0 ± 1.77	16.5 ± 1.51	12.8 ± 0.98
Fatty acid metabolism						
HOAD	1.34 ± 0.09	1.24 ± 0.034	0.87 ± 0.19	0.54 ± 0.031	0.96 ± 0.11*	0.30 ± 0.06
CPT	5.16 ± 1.27	3.35 ± 1.79*	0.58 ± 0.47*	3.10 ± 1.66*	1.23 ± 0.15	0.35 ± 0.07
COT	1.01 ± 0.27	1.94 ± 0.46	2.11 ± 0.37	0.66 ± 0.20	3.17 ± 0.56	1.06 ± 0.21
Adenylate metabolism						
AK	6.22 ± 0.74	22.9 ± 1.28	28.8 ± 5.71	4.55 ± 1.24	19.7 ± 1.15	17.8 ± 3.4
CK	18.1 ± 1.29	45.3 ± 2.87	225 ± 25.9	97.7 ± 3.00	742 ± 2.18	649 ± 60.8
Amino acid metabolism						
GOT	19.8 ± 0.82*	15.3 ± 0.68	27.9 ± 3.74*	14.8 ± 0.18	12.9 ± 1.23*	2.71 ± 0.25
GPT	0.29 ± 0.06	0.33 ± 0.03	nd	0.075 ± 0.008	1.84 ± 0.27	2.01 ± 0.76*
GDH	23.0 ± 0.49	12.7 ± 1.21	0.78 ± 0.12	6.89 ± 1.26*	0.59 ± 0.15*	nd
SDH	0.65 ± 0.06	0.38 ± 0.04	0.10 ± 0.025*	0.55 ± 0.05*	0.871 ± 0.217	0.82 ± 0.16*

Note: Values are given as the mean ± SEM, $n = 12$ for combined values from control, 20 h anoxic, and 24 h recovered turtles or, as indicated by an asterisk, $n = 4$ for control turtles in those cases where values for anoxia or recovery differed significantly from control values. Glucokinase (GK) was detected only in liver; hexokinase (HK) was in all other organs. nd, not detected. Other abbreviations are as follows: AK, adenylate kinase; ALD, aldolase; COT, carnitine octanoyltransferase; CPT, carnitine palmitoyltransferase; CK, creatine kinase; FBPase, fructose-1,6-bisphosphatase; G6PDH, glucose-6-phosphate dehydrogenase; GDH, glutamate dehydrogenase; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate-pyruvate transaminase; G3PDH, glycerol-3-phosphate dehydrogenase; HOAD, 3-hydroxyacyl-CoA dehydrogenase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; PEPCK, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; 6PGDH, 6-phosphogluconate dehydrogenase; PK, pyruvate kinase; SDH, serine dehydratase.

a Dynatech MR-5000 microplate reader to a final well volume of 310 mL.

Enzyme rates provided from the microplate reader were interpreted with the use of the microplate analysis program (Brooks 1994). All values were compared by one-way ANOVA followed by two-tailed Dunnett's or Student–Newman–Keuls tests.

Results

Enzyme maximal activities and soluble-protein content in organs

Activities of many enzymes were unchanged during anoxia exposure (20 h at 7°C) or aerobic recovery (24 h at 7°C) and the overall mean maximal activities of these, combining data from control, anoxic, and recovered samples ($n = 12$), are shown in Table 1 for the six organs analyzed. Table 1 also shows control values ($n = 4$) for those enzymes whose activities did change significantly as the result of anoxia or aerobic recovery. Soluble-protein content in tissue extracts did not change significantly during anoxia or aerobic recovery in any tissue. Mean concentrations were 118 ± 19.8, 111 ± 16.3, 69.3 ± 7.46, 91.0 ± 5.33, 73.1 ± 16.9, and 58.7 ± 14.4 mg/g wet mass (mean ± SEM) in liver, kidney, brain, heart, red muscle, and white muscle, respectively (values are pooled for all three conditions; $n = 12$).

Effects of anoxia and recovery

Maximal activities of selected enzymes changed during anoxia exposure and aerobic recovery in turtle organs. Effects on enzymes in liver and kidney are shown in Fig. 1. Anoxic submergence affected the activity of only one enzyme in liver. The activity of PFK, a major regulatory enzyme of glycolysis, dropped by 33% in liver during anoxia and remained low during aerobic recovery. Activities of liver FBPase and glutamate-oxaloacetate transaminase (GOT) were unchanged during anoxia exposure but rose during aerobic recovery, increasing by 60 and 28%, respectively, compared with controls.

In kidney, the activities of only three enzymes changed under the experimental conditions (Fig. 1). HK activity decreased by 40% during anoxia exposure, whereas CPT activity in liver increased by nearly 3-fold during anoxia, to 9.59 ± 0.68 units/g wet mass, but had decreased to 6.12 ± 0.43 units/g wet mass after 24 h of aerobic recovery. Aldolase (ALD) activity doubled in kidney of recovered turtles compared with anoxic animals.

Fig. 1. Changes in maximal activities of enzymes in turtle (*Trachemys scripta elegans*) liver and kidney during a 20-h anoxic submergence at 7°C and subsequent 24-h aerobic recovery at 7°C. Light-shaded bars represent the control, dark-shaded bars represent anoxia exposure, and solid bars represent aerobic recovery. Values are given as the mean ± SEM (n = 4). *a*, significantly different from the corresponding control value (one-way ANOVA with Student–Newman–Keuls test), $P < 0.05$; *b*, significantly different from the corresponding value in anoxia, $P < 0.05$.

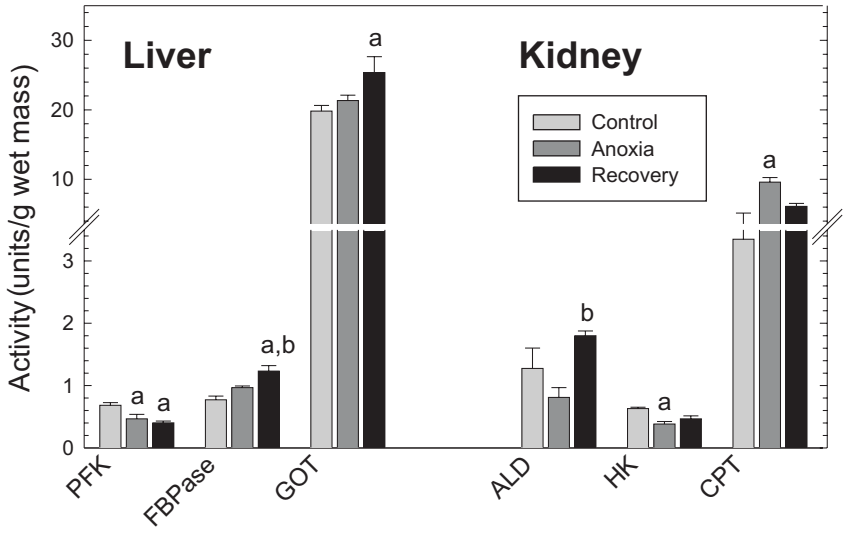


Fig. 2. Changes in maximal activities of enzymes in turtle heart over the course of anoxia and recovery. For details see Fig. 1.

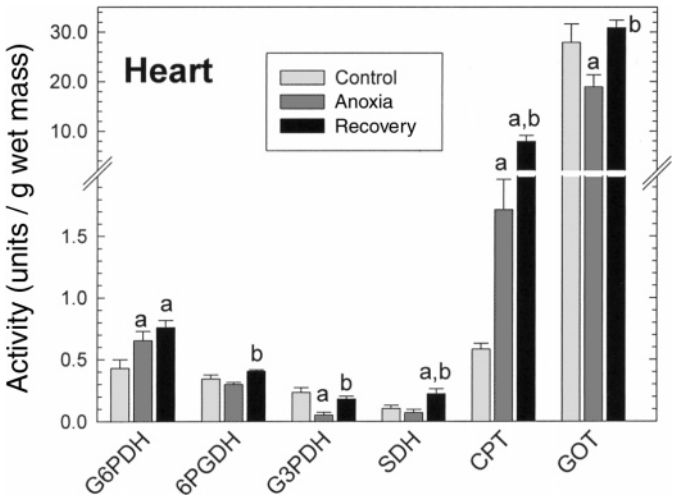


Fig. 3. Effects of anoxia exposure and recovery on enzyme activities in turtle brain. For details see Fig. 1.

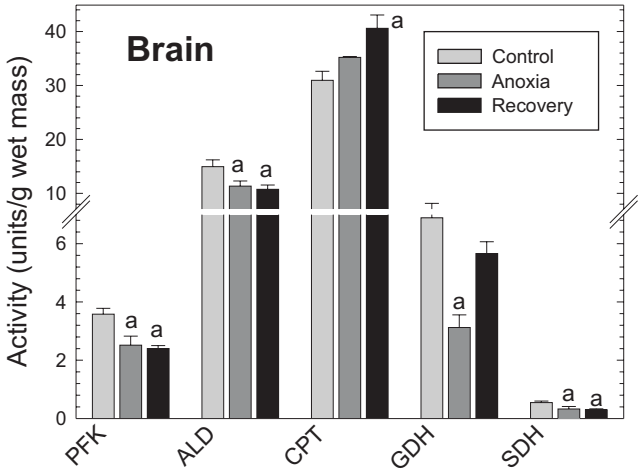
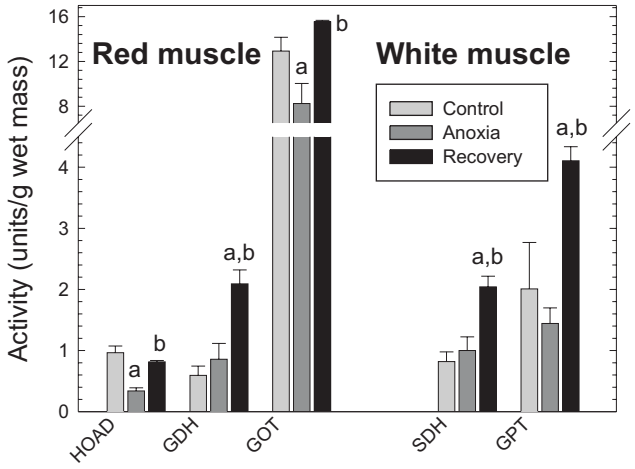


Figure 2 shows the effects of anoxia and aerobic recovery on enzyme maximal activities in turtle heart. Four enzymes showed significant changes in activity during anoxia. The activity of glucose-6-phosphate dehydrogenase (G6PDH) rose by 52% (and remained high during recovery) and CPT activity rose 3-fold in anoxia and then to 13.5-fold higher than controls during recovery. G3PDH and GOT activities decreased during anoxia by 78 and 32%, respectively, and both rebounded during aerobic recovery. Other enzymes changed only during recovery after anoxia exposure. SDH increased 2-fold compared with controls, whereas 6-phosphogluconate dehydrogenase (6PGDH) activity increased by 33% over the value in anoxia.

The effects of anoxia and aerobic recovery on enzyme activities in brain are shown in Fig. 3. Anoxia exposure reduced the activity of two glycolytic enzymes, PFK and ALD, by 30 and 24%, respectively. Glutamate dehydrogenase (GDH) and SDH also decreased significantly during anoxia to activities 43 and 60% of controls. PFK, ALD, and SDH activities remained low after 24 h of aerobic recovery and GDH activity rebounded, whereas CPT activity rose by 31% during recovery only.

Changes in enzyme activities in red and white skeletal muscle are shown in Fig. 4. Anoxia exposure resulted in a significant decrease in 3-hydroxyacyl-CoA dehydrogenase (HOAD) and GOT activities in red muscle, which fell to 35 and 64% of control values, respectively. Activities of both enzymes rebounded during aerobic recovery. The activity of glutamate dehydrogenase (GDH) in red muscle also rose during recovery by 3.5-fold. In white muscle, SDH and

Fig. 4. Effect of anoxia exposure and recovery on enzyme activities in turtle red and white muscle. For details see Fig. 1.



glutamate-pyruvate transaminase (GPT) activities also increased during aerobic recovery, by 2- and 2.5-fold, respectively.

Discussion

Overall, the present study illustrates that large-scale changes to the activities of enzymes of intermediary metabolism do not occur during anoxia exposure or aerobic recovery in anoxia-tolerant turtles. This indicates that the metabolic make-up of turtle organs is generally well designed, with constitutive activities of enzymes that can meet the metabolic demands of anoxic excursions whenever they occur. This is not unexpected, since turtles need to be prepared for submergence at any time and the energy-restricted anoxic state is not a time for large-scale protein synthesis in support of metabolic reorganization. Indeed, rates of protein synthesis by turtle hepatocytes were reduced by >90% under anoxic conditions, whereas rates in anoxic heart were only about one-third of values under aerobic conditions (Land and Hochachka 1994; Bailey and Driedzic 1996). Nevertheless, studies using ^{35}S to label new proteins synthesized in vivo or proteins produced in vitro via translation of mRNA isolated from anoxic animals showed that synthesis of selected proteins does occur during anoxia in turtle organs (Brooks and Storey 1993; Douglas et al. 1994). Furthermore, the anoxia-induced up-regulation of mitochondrially encoded genes associated with the electron transport system occurs in both turtle heart and brain in response to anoxic submergence at 5–7°C, suggesting increased synthesis of the protein products of these genes in anoxia (Cai and Storey 1996; Willmore et al. 2001).

In the present study the maximal activities of 21 enzymes in six organs of adult turtles were surveyed. The data show only 13 instances where enzyme activities changed significantly during the 20-h anoxic submergence and, of these, only 3 involved significant increases in activity. CPT activity rose in kidney and heart by 3-fold in each case and G6PDH activity also increased in heart under anoxia. Notably, this contrasts with the responses of mammalian tissues to hypoxic/anoxic conditions, which include a rapid up-regulation of the genes for the most glycolytic enzymes in an attempt to meet energy demand by increasing glycolytic ATP production (Semenza et al. 1994; Firth et al. 1994). In turtles, by contrast, glycolytic enzymes are not induced under anoxia for two reasons: (1) constitutive activities of these enzymes are already high and (2) metabolic-rate depression reduces the demand for ATP produced by glycolysis. In the other 10 instances, in five organs (not white muscle) enzyme activities significantly decreased during anoxia exposure. This included decreases in activities of enzymes of glycolysis (HK in kidney, PFK in liver and brain, ALD in brain) and amino acid metabolism (GOT in heart and red muscle, GDH and SDH in brain), as well as G3PDH in heart and HOAD in red muscle.

Other enzymes responded to aerobic recovery from anoxia and, in most cases, activities increased during recovery. In 5 cases these represented reversals of activities that were reduced during anoxia, whereas 10 involved new increases in enzyme activities. Five enzymes that were reduced during anoxia remained low over the 24-h aerobic recovery. These patterns of reduced enzyme activities during anoxia and increased activities during aerobic recovery are consistent with a lower biosynthetic capacity in the anoxic state and a renewal of protein synthesis when oxygen returns. However, the many instances of unchanged enzyme activities in anoxia suggest that, in general, rates of protein synthesis and protein degradation are suppressed in a coordinated fashion as part of the overall metabolic-rate depression induced by anoxia exposure.

Against a background of largely unchanged enzyme activities, the specific enzymes affected by anoxia exposure gain more significance. In vitro determination of enzyme maximal activities has long been employed to provide insight into metabolic flux potential in vivo and to suggest possible rate-limiting and regulatory loci (Newsholme and Crabtree 1986). The utility of enzyme-activity measurements is valid for two reasons: (1) changes in enzyme levels can significantly affect flux through metabolic pathways and (2) enzyme (protein) synthesis is ATP-expensive, so those changes that do occur are likely of importance to organismal survival of stress.

In liver, anoxia exposure suppressed the maximal activity of PFK, one of the regulatory enzymes of glycolysis (Fig. 1). Such control of PFK in liver can have two functions: (1) when glycogenolysis is activated, PFK inhibition helps to divert hexose phosphates into the production of glucose for export, and (2) under gluconeogenic conditions, PFK inhibition facilitates the synthesis of hexose phosphates from substrates such as lactate, alanine, or glycerol-3-phosphate. The first function is undoubtedly the cause of the anoxia-induced effect on liver PFK, as liver glycogen is the primary source of the blood glucose that is used as an anaerobic fuel by other tissues. Anoxic inhibition at the PFK locus in turtle liver was also indicated from the pattern of changes in substrate and product levels of the enzyme (Kelly and Storey 1988). However, it is the second function that probably keeps PFK low during aerobic recovery, whereas the activity of FBPase, which reverses the PFK reaction, rises significantly in liver during recovery to facilitate gluconeogenesis from lactate. Such oppositely directed re-

sponses by PFK and FBPase are well known in vertebrate liver metabolism as one of the main controls on glycolysis versus gluconeogenesis and changes in the ratio of PFK:FBPase activities in turtle liver (0.88 in control, 0.48 in anoxia, and 0.33 in aerobic recovery) support this interpretation. However, the mechanism of PFK- and FBPase-activity changes over anoxia/recovery in liver may not be a change in enzyme amount but a change in protein phosphorylation state. Both enzymes in vertebrate liver are controlled by reversible phosphorylation that has opposite effects on their activities (inhibiting PFK, activating FBPase). A previous study of PFK in turtle organs noted anoxia-induced changes in the kinetic properties and maximal activity (a 40% reduction) of PFK in liver that were consistent with a less active enzyme in anoxia, probably resulting from anoxia-induced phosphorylation (Brooks and Storey 1989). The only other significant change in turtle liver enzyme activities during anoxia/recovery was a 28% rise in GOT during aerobic recovery that may be related to the enzyme's role in amino acid metabolism or the malate–aspartate shuttle; both would be suppressed in anoxia and reactivated during recovery.

In kidney, HK activity was reduced during anoxia by 40% (Fig. 1); this could contribute to metabolic-rate depression by suppressing the entry of exogenous glucose into glycolysis. ALD also showed a trend towards reduced activity during anoxia, with a substantial rebound during aerobic recovery. Although not a regulatory enzyme, ALD is one of three low-activity glycolytic enzymes in kidney (HK, PFK, ALD), and changes in ALD also suggest a pattern of metabolic-rate suppression in anoxia and reactivation in recovery. CPT activity rose 3-fold during anoxia in kidney, although the functional significance of this increase is not apparent, since the role of CPT is to translocate fatty acids into the mitochondria for use in beta-oxidation. CPT activity also increased in turtle heart during anoxia (by 3-fold) and recovery (by 13-fold) and in brain during recovery (by 33%) (Figs. 2, 3). Elevated CPT activity during aerobic recovery could support a renewed reliance on lipid oxidation to fuel energy metabolism and biosynthesis when aerobic conditions return.

Enzyme changes in heart during anoxia included reduced activities of both G3PDH and GOT (Fig. 2). Both enzymes have multiple roles in metabolism but a function that they share is redox regulation. Both are components of shuttle systems that move cytoplasmic reducing equivalents into the mitochondria. Cytoplasmic G3PDH uses NADH to synthesize glycerol-3-phosphate, which moves into the mitochondria, where it is oxidized by FAD-linked glycerol-3-oxidase, with release of electrons into the electron transport system. The malate–aspartate shuttle, which is composed of cytosolic and mitochondrial isoforms of MDH and GOT, also carries out this function. Both shuttles would be halted by the interruption of the electron transport system when oxygen is depleted and reduced activities of these enzymes in anoxia may contribute to a reorganization of redox regulation for anaerobic survival. Sephton and Driedzic (1996) reported no change in brain HK, PK, lactate dehydrogenase (LDH), and HOAD over the course of 16 weeks of submergence in normoxic water at 3°C, which concurs with our findings for the shorter anoxia stress.

Anoxia exposure in brain reduced the activities of two glycolytic enzymes (PFK and ALD) and two enzymes of amino acid metabolism (GDH and SDH) (Fig. 3). The response by glycolytic enzymes may contribute to glycolytic-rate suppression, whereas changes in GDH and SDH may be linked with neurotransmitter metabolism. One negative effect of oxygen deprivation on mammalian brain is the release of high levels of the excitatory amino acids glutamate and aspartate into the extracellular fluid (Young et al. 1993). Turtles do not show this; in fact, both extracellular glutamate (Young et al. 1993) and total brain glutamate (Nilsson et al. 1990) decreased significantly during anoxia, whereas levels of inhibitory amino acids (g-aminobutyric acid, glycine, taurine, alanine) increased in anoxic turtle brain (Nilsson et al. 1990). Although GDH participates in both glutamate synthesis and catabolism, the 57% reduction in GDH activity in turtle brain in anoxia could reduce the rate of glutamate production, leading to lower glutamate levels. Elevated levels of glycine during anoxia may be the result of de novo synthesis but would also be favoured by blocking the two routes of glycine catabolism. In one route, glycine is first converted to serine and then SDH catalyzes conversion to pyruvate, and this is followed by pyruvate oxidation. In the other, glycine is directly cleaved (producing CO_2, NH_4^+, and a methylene group) by the mitochondrial NAD-dependent glycine-cleavage enzyme. Both routes would be suppressed in anoxic turtle brain (SDH activity is reduced by 40%; mitochondrial NAD availability is low) and this could promote sustained or elevated glycine levels.

Anoxia/recovery had few effects on enzyme activities in skeletal muscles and, except for HOAD, all enzymes affected were involved with amino acid metabolism (Fig. 4). The activity of HOAD was strongly reduced under anoxia in red muscle, falling to just 35% of the control value but rebounding again during aerobic recovery. Under aerobic conditions, red muscle relies on fatty acid oxidation for a high percentage of its energy needs. The inability to oxidize lipids during anoxia could account for suppressed activity of HOAD and perhaps other enzymes of fatty acid catabolism during anaerobiosis. Anoxic suppression of GOT in red muscle might have the same function as postulated above for heart or, taken together with the increases in other enzymes (GDH in red muscle and SDH and GPT in white muscle) during recovery, these might imply an overall increase in amino acid catabolism during recovery from anoxia.

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