METABOLIC STRATEGIES OF *Hypostomus regani* (CASCUDO), A FRESHWATER TELEOST FISH UNDER EXTREME ENVIRONMENTAL HYPOXIA

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ABSTRACT

The present paper reports metabolic responses of *Hypostomus regani* (cascudo) under sever hypoxia. Glucose metabolism was studied through changes into metabolites as glucose, lactate, pyruvate and glycogen in plasma, liver, hart red muscle and white muscle. That teleost fish shows low hepatic glycogen store and higher levels of cardiac glycogen. Cory cycle may not be discarded at first sight in *H regani* but the role of alanine into lactate transport has to be studied. The main strategy of *H. regani* against hypoxia seems to be air breathing and oxygen exchange inside the digestive tube.
Key words: fish, *Hypostomus regani*, cascudo, metabolism, hypoxia.

RESUMO

Estratégias metabólicas de *Hypostomus regani* (cascudo), teleosteo de água doce, submetido a hipóxia ambiental extema.

Este trabalho relata as respostas metabólicas de *Hypostomus regani* quando submetido à hipóxia severa por 4,5 horas. O metabolismo da glicose foi estudado através de alguns de seus principais intermediários tais como a glicose, o piruvato, o glicogênio e o lactato. Este metabólitos foram determinados no plasma, fígado, coração e músculos vermelho e branco. Este teleósteo apresentou altos níveis de glicogênio cardíaco, maiores que o glicogênio hepático. O ciclo de Cori não foi descartado *a priori* nesta espécie, todavia o papel da alanina, e de amino transferases envolvidas no metabolismo deste aminoácido, deve ser estudado no sentido de se esclarecer seu possível envolvimento no transporte de lactato oriundo de processos fermentativos. A principal estratégia de *H. regani* contra a hipóxia ainda parece ser a deglutição de ar seguida da troca gasosa ao nível de trato digestivo.

Palavras chaves: peixe, *Hypostomus regani*, cascudo, metabolismo, hipóxia.

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INTRODUCTION

Seldom terrestrial vertebrates endure oxygen reduction. Such gas concentrations keep constant values around 21% (McFarland. *et al.*, 1993). However, in aquatic environments is different. Oxygen levels are usually lower in natural water bodies. Therefore, aquatic organisms, as fish, necessarily develop adaptive strategies to ensure the maximum oxygen diffusion from the outer medium to the organism. Among that, ram ventilation, counter current and cardio-respiratory adjustments have been reported in fish (McFarland, *et al* 1993).

Tropical freshwater living organisms are frequently exposed to variations of oxygen partial pressure (pO_2). Temperature changes and microbial metabolism are mainly responsible for such variations. Fish from such environments have to develop special strategies to keep ordinary metabolic demands. Among these, auxiliary respiratory structures as modification in swim bladder, mouth, stomach, intestine and skin as well as true lung and lip growth have been reported (Braum and Junk, 1982). Also some species have developed metabolic responses enabling them to survive under low oxygen concentrations (Moraes, *et al.* 1996). Pursuant to obligatory use or not of respiratory strategies, other than oxygen diffusion through the gills, fish may be classified in two groups: facultative and obligatory air breathing. Among the facultative air breathing fish *Hypostomus regani* is included. It comes to surface, under low oxygen tensions, to gulp air to stomach (Nikolsky, 1963; Withers, 1992).

Several studies concerning physiological and morphological adaptations have been done (Val, 1990; Rantin, 1993). However, biochemical changes on the metabolic routes under anaerobiosis have been reported and some kinds of responses are observed (Moraes, *et al* 1995; Moraes *et al* 1996). Usually, a drop into the levels of inspired oxygen results in a reduction of metabolic rate. This may engender biochemical preferences causing detours of metabolic profiles. Such changes lead to variations on concentration of the end point metabolites (Driedzic and Hochachka, 1978).

The present paper reports the behavior and the metabolic responses of *H. regani* under severe hypoxia. Biochemical profile was done through measurement of glucose, glycogen, pyruvate and lactate in a few tissues.

MATERIAL AND METHODS

Specimens of *H. regani* were kindly furnished by CEPTA/IBAMA Pirassununga-SP. It was employed eighteen animals weighing 193 ± 10 g gauging 25 ± 3 cm. The fish were kept in tanks of 4000 liters with flowing water and deprived of food two days before starting the experiments.

Experimental protocol: Animals were set in three dark aquariums of 60 liters, equally distributed. Cork plaques were lay on the surface of the water to prevent air breathing. Oxygen partial pressure was manually controlled employing an oxymeter and a nitrogen bubbling gadget. The partial pressure of oxygen was 8 mg O_2/liter at 25°C (normoxia). The animals were kept under such conditions along 24 hours before the experiments. After this acclimatiza-

tion period a couple of aquarium called B and C were submitted to oxygen drop but the other (called A) was maintained under normoxia for control. The lower pressure of oxygen reached 0.7 ± 0.07mg O_2/liter. After such point, nitrogen bubbling was ceased and the animals from aquarium B and C were submitted to hypoxia along 3 and 4.5 hours respectively. After exposed to hypoxia the animals were anesthetized with MS222 and bled through caudal vein with heparinized syringes. Blood was chilled immediately after being collected. After bled, the animals were killed by a head blow and, heart, liver, red and white muscle were excised into three minutes. Tissues were transferred to liquid nitrogen and kept for subsequent analyses.

Biochemical analysis: Tissues were mechanically homogenized by a rotor Teflon pestle into 0.6N perchloric acid (PCA) under ice-bath. The supernatant from 5000 rpm was neutralized by 1:1 6N ($KHCO_3$-KOH) solution. That mixture was centrifuged at 8000 rpm for 10 minutes and the supernatant analyzed as tissue extract. Biochemical analyses of blood were done in the plasma after removing red blood cells by centrifugation at 5000 rpm for 5 minutes. Plasma was employed for Na^+ and K^+ determination by flame photometry. All centrifuging procedures were done at $4°C$.

Glucose was determined by DuBoie method (1960). Pyruvate was determined by dinitrophenilhydrazine (DNPH) in 0.1N HCl. Lactate was estimated by Harrower method (1972). Glycogen was determined after alkaline dissolution in 6N KOH at $100°C$ for three minutes. Glycogen was precipitated by ethanol and K_2SO_4. The precipitate was removed by centrifuging and re suspended into distilled water. Glycogen was estimated through glucose determination by DuBoie hydrolytic method (1960).

Hematological Parameters: Right after blood punction the samples were employed to estimate hematocrit, red blood cell count, total hemoglobin concentration and, oxyhemoglobin and deoxyhemoglobin estimation.

Statistic: All the results were statistically analyzed comparing the means by Student's Test at IC = 0.05 for different variances.

RESULTS

Severe hypoxia drives *H. regani* to changes on hematological parameters. Red blood cells and hematocrit increased as well as potassium concentration. Plasma sodium contents decreased. Total hemoglobin and deoxyhemoglobin increased but oxyhemoglobin dropped. Hematological results may be observed in Table I.

All results below are expressed in Figures 2, 3 and 4. White muscle shows mild decrease on glycogen contents followed by glucose enhance as well as lactate. In this tissue was observed a pyruvate decrease along hypoxia. Liver upheld constant lactate and pyruvate contents. Considering the decrease of glycogen contents and the slight increase of lactate concentrations, red muscle appears very active under the metabolic viewpoint, in spite of constant glucose levels. Similar responses were observed in cardiac muscle concerning glycogen, glucose and lactate. White muscles kept constant the glycogen level as well as glucose but lactate showed a slight increase. Decrease of pyruvate was noticed at the end of hypoxia. The metabolic responses observed for different

tissues were reflected into plasma by large increase of glucose or lactate concentration but none significant changes may be reported for pyruvate.

DISCUSSION

Biochemical responses observed in *H. regani* under hypoxia were very interesting. Several species hitherto studied have showed metabolic responses along six to eight hours. However, the present species seems to be death after four hours of hypoxia. Curiously, after discarded, several specimens started to breathe. Afterwards, such animals presented the stomach completely stuffed by air.

Hematological responses observed in *H. regani* are similar to such observed for several species (Fievet, 1987; Peterson, 1990; Swift; 1981). Hematocrit increase seems to occur as a response to hypoxia adapting the erythrocyte rate to improve oxygen transport (Swift, 1982; Fievet, 1987). Changes of plasma ionic pattern reflect variations into acid-base pattern of the animals.

Lactate increase was observed in white muscle. However, the content of such metabolite was not excessive as observed into the majority of studied species presenting the Pasteur Effect (Mauro, 1984; Milligan and Girard, 1993; Hochachka and Guppy, 1987). Glucolytic activity seems to be mild in white muscle and the short decrease of glycogen suggests the exogenous origin of glucose, probably explaining the increase into lactate at the first moments of hypoxia.

Likely the neoglycogeneses occurs in the liver of several fishes under hypoxia (Hochachka, 1980; Withers, 1992). This condition ought to explain the lactate recover and, due to the liver ability for holding the glucemia, the metabolic energy source would be kept to subsequent demands. The observed moderate increase into hepatic pyruvate levels ($P=0.09$), followed by constant lactate and striking glycogen-glucose decrease, suggest the role of the liver supporting glucemia in *H. regani* under hypoxia.

Heart and red muscle of *H. regani* showed similar metabolic response under hypoxia except by glycogen hydrolysis ten times greater in heart. Decrease of cardiac glycogen may be due to high glucolytic activity in such tissue. In spite of lactate levels reach significant enhance in red muscle and heart it seems to be not explained by the high glycogen hydrolytic rate observed. The metabolism of both tissues may explain the high lactemia observed in *H regani*. Otherwise, it was reported a relatively short metabolic response in heart of rainbow trout under hypoxia (Dunn and Hochachka 1986).

Glycogen store from liver are the top source of glucose (Hochachka and Somero, 1984; Van den Thillart *et al*, 1980; Van Waarde *et al* 1983) and its decrease, followed by plasma glucose increase, suggest the role of the liver holding normal glucemia. The plasma glucose increase observed in *H. regani*, under hypoxia, may be explained in a like fashion. Blood is the carrier of glucose to peripheral tissues. White muscle arises as the first consumer of exogenous glucose. However, in regard to heart and red muscle remain doubts whether the main glucose source is the internal glycogen or the plasma glucose. Glycogen ought to be the glucose source in such tissues of *H. regani* under hypoxia.

High lactate plasma levels observed after four hours under hypoxia points out to wide mobilization of such intermediate and high fermentative activity. Low resistance to hypoxia is compatible to low levels of hepatic glycogen observed in *H. regani*. However, the very high glycogen store in cardiac fibers of *H. regani* is too puzzling. They are higher than hepatic contents, which may be indicative of great ability to glucose consumption in such tissue.

The strategy of Cori cycle may not be discarded at first glance, but the significant increase of plasma pyruvate may reflect amino transferase activity. This fact permits to suppose upon the role of alanine into the transport of lactate from fermentative processes.

In spite of several responses arisen from the present work several questions are yet remaining. For example, the energetic potential of ATP and phosphocreatine as well as the lactate concentration into cerebral tissue should permit to surmise about a possible metabolic rest under hypoxia. Other point is: -Which is the biochemical responses of *H regani* under moderate hypoxia? The biochemical responses observed in the present work claim to knowing about the lipid and protein metabolism under such conditions. The difficulties faced to keep alive the specimens under hypoxia certainly hamper attaining that response. Although, up to now we can suppose that *H. regani* seems to employ behavioral strategies, as getting the air on the surface and exchange oxygen into stomach, rather than biochemical one.

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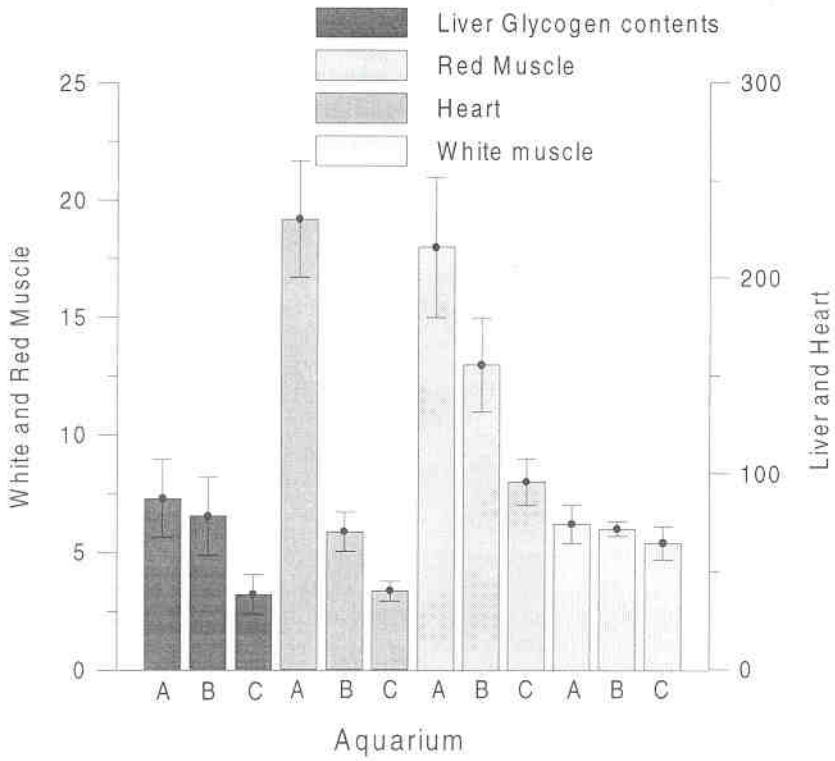WITHERS, P.C. *Comparative Animal Physiology.* Souders College Publishing, v. 15; p. 42-67, 1992.

Fig. 1. Tissue glycogen stores along 3 (B) and 4.5 (C) hours of severe hypoxia as explained in the text. The bars A correspond to controls under normoxia. Values are expressed in μmols of glucose per gram of wet tissue.
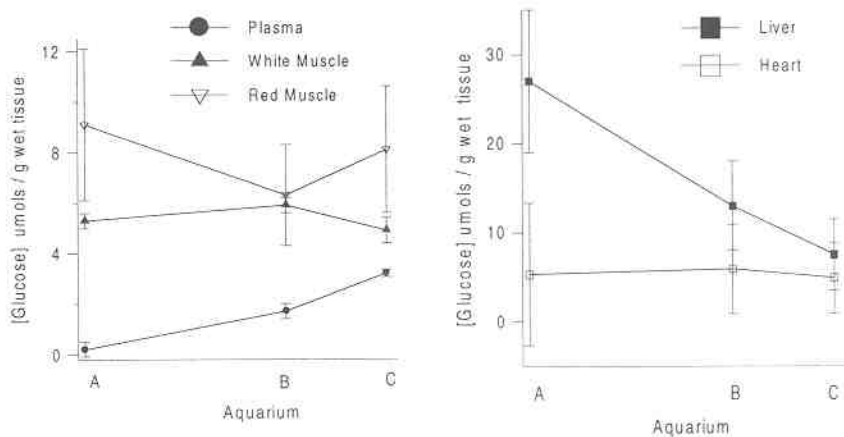
Fig. 2. Tissue glucose concentrations along 3 (B) and 4.5 (C) hours of severe hypoxia. Points A represent the control values under normoxia. Experimental conditions are described in Material and Methods.
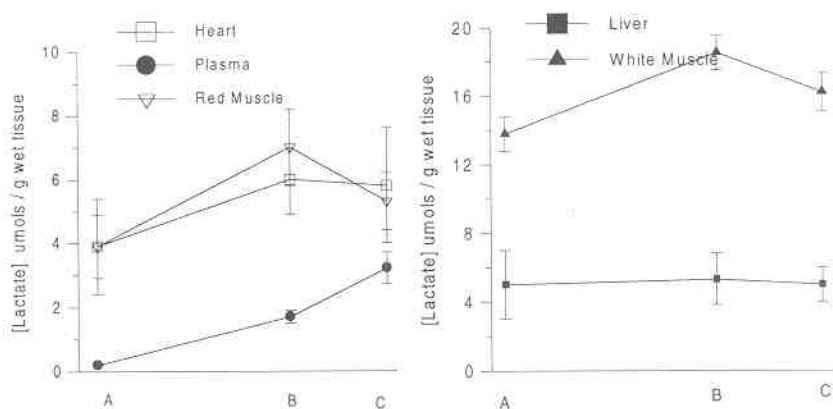

Fig. 3. Tissue lactate concentrations along 3 (B) and 4.5 (C) hours of severe hypoxia. Points A represent the control values under normoxia. Experimental conditions are described in Material and Methods.

Fig. 4. Tissue pyruvate concentrations along 3 (B) and 4.5 (C) hours of severe hypoxia. Points A represent the control values under normoxia. Experimental conditions are described in Material and Methods.
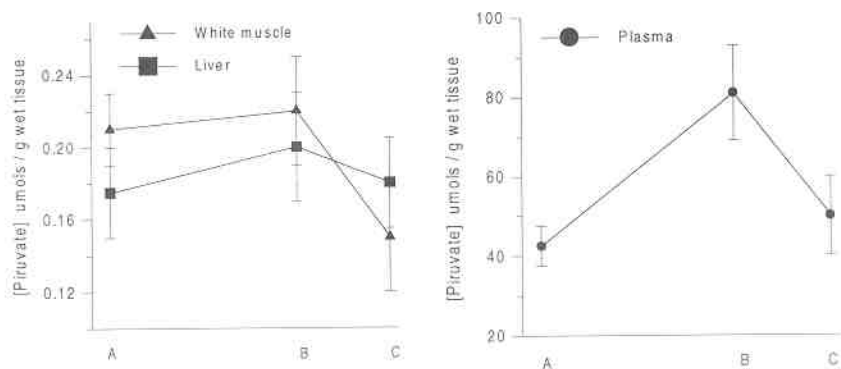
TABLE I - Hematological values observed in *H. regani* submitted to 3 (A) and 4.5 (B) hours of hypoxia compared to the control under normoxia (A). Plasma values of sodium and potassium are as well reported. Significant values are detached by (*) and were calculated at CI of 0.05 by comparison of two means through Student's T Test for different variances. References were Normoxia (A).

Physiological state	Hemat (%)	RBC $10^3 mm^{-3}$	Total Hb mg (%)	OxiHb (%)	DeoxHb (%)	Na mEq/l	K mEq/l
Normoxia (A)	21	0.62	7.5	78	23	138	4.2
Hypoxia (B)	28*	0.75*	9.0*	78*	25*	125*	4.8*
Hypoxia (C)	26*	0.61	9.1*	71*	27*	126*	4.9*