IMEDIATE CHANGES ON METABOLIC PARAMETERS OF THE FRESHWATER TELEOST FISH *Piaractus mesopotamicus* (PACU) UNDER SEVERE HYPOXIA.

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ABSTRACT

Day in day out the most tropical freshwater fish have to cope with environmental hypoxia, one of the commonest problems to solve. Such fact request adaptive mechanisms either behavioral, physiological or biochemical. Among that, biochemical responses have arisen in several species under different ways. The present paper reports some changes in the carbohydrate metabolism of pacu, *Piaractus mesopotamicus* submitted to severe hypoxia. The role of the liver is proposed for lactate recovery and glucose supply. The red muscle seems to release lactate toward plasma and, even under hypoxia, low anaerobiosisis observed in such tissue. Cori cycle is suggested as a probable mechanism employed by *P. mesopotamicus* as a biochemical strategy against hypoxia.

Key words: fish, *Piaractus mesopotamicus*, metabolic parameters, hypoxia

RESUMO

Alteração nos parâmetros metabólicos de teleósteo de água doce *Piaractus mesopotamicus* (pacu) sumetido a hipóxia severa.

Talvez a hipóxia seja o problema mais freqüente a ser enfrentado pelos peixes tropicais de água doce. Este fato permite prever adaptações, quer comportamentais quer fisiológicas ou bioquímicas. Neste trabalho são apresentados aspectos gerais do metabolismo de carboidratos de pacu *Piaractus mesopotamicus* em resposta a um quadro de hipóxia aguda ambiental. Sugerimos ainda o papel do fígado na recuperação do lactato produzido e no fornecimento de glicose, a liberação de lactato para o plasma pelo músculo vermelho além de baixa anaerobiose neste tecido, mesmo sob hipóxia. Também é proposto o ciclo de Cori como estratégia metabólica de resistência à hipóxia nesta espécie.

Palavras chaves: peixes, *P. mesopotamicus*, parâmetros metabólicos, hipóxia

INTRODUCTION

Aquatic environment is under frequent changes submitting the living organisms to such conditions and claiming to constant adaptations. Among several environmental parameters, the partial pressure of oxygen is usually changing into a large number of water bodies. Such phenomenon, consequent on biological activities, compels the organisms to respond adaptively. Responses to hypoxia are different and the tolerance to it is associated to environmental oxygen availability (Kramer *et al.*, 1978; Junk *et al.*, 1983; Almeida-Val and Val, 1990).

Among several responses to hypoxia the behavioral is perhaps the most frequent one. Low oxygen tensions drive the animals to look for better conditions and escaping is the first choice. Moreover, breathing mechanisms, which evolved to optimization of dissolved oxygen uptake, are ordinarily observed. Several species have developed interesting strategies for surviving under low oxygen levels such as gas exchange through intestine and stomach, as observed in cascudo (*Hypostomus regani*), and the lip growth in tambaqui (*Colossoma macropomum*) as reported by Brawn and Junk (1982) and Junk *et al*, (1983). As well, body shape and mouth position are indicators concerning to environmental oxygen availability. Nevertheless, molecular mechanisms, hidden to naked eyes, are many times employed by several species. Biochemical responses may occur as the capital strategy against hypoxia in many fish. Species as goldfish (*Carassius auratus*) (Shoubridge and Hochachka, 1981), rosbora and carp (*Ciprinus carpio*) (Hochachka, 1961) have developed a striking capacity to anaerobiosis under high levels of hypoxia.

The present paper aimed to assess alterations in carbohydrate metabolism of pacu (*Piaractus mesopotamicus*) under severe hypoxia. This species presents a considerable resistance to hypoxia conditions but lip growth is its most known strategy so far described. Carbohydrate metabolism intermediates were the main compounds under consideration and this approach was done through quantification of them.

MATERIAL AND METHODS

Experimental protocol: Fish weighting 90 ± 5 g were caught from fish culture ponds at CEPTA-IBAMA Pirassununga and transferred to tanks in the lab. The external piped water from local reservoir filled the tanks running at constant flow and preventing the animals of metabolic waste. Experiments started after fifteen days under acclimatization in the laboratory conditions. Twenty-four young fishes were arranged in four groups of six and distributed in 60 liters glass containers of dark walls to prevent external stress. Control group was constantly aerated and kept under normoxia and the experimental groups were kept under hypoxia of 0.5 mg of oxygen per litre over 2, 4 and 6 hours. The water temperature was around 26±1 °C.

Blood and tissue collection: After hypoxia, the blood was drawn by puncture of the caudal vein with heparinised syringes and the animals were killed by a head blow and immediately dissected. Liver, white and red muscle

were excised and kept under -70°C. The whole operation lasted no more than three minutes.

Tissue and blood preparation: Capillary tubes were filled with blood for hematocrit determination and the remaining was centrifuged at 3000 rpm. Plasma was used for Na^+ and K^+ measurement after appropriate dilution and the remaining volume was submitted to protein removal by addition of 80% trichloracetic acid (TCA). After centrifuging at 5000 rpm the supernatant (free protein extract) was neutralized by 6N (KOH-$KHCO_3$ 1:1 v/v) and kept frozen for further analysis of glucose, pyruvate and lactate.

Tissues were mechanically disrupted by a Teflon pestle motor driver under ice cold bath for 1 minute. The final tissue concentration was 100 mg of wet tissue per ml of 80% TCA. Following, the homogenates were centrifuged and the supernatant neutralized as described above for the plasma samples. The free protein extracts were used for estimating glucose, pyruvate and lactate. For glycogen determinations about 150 mg of muscle, or 50 mg of liver, were quickly weighed and immediately transferred to 1.0 ml of 6N KOH under boiling water bath for 1 minute. After complete dissolution of tissue, the samples were cooled, an appropriate aliquot was transferred to a clean test tube and 3.0 ml of 90% ethanol plus 0.1 ml of K_2SO_4 saturated solution were added. The cloudy precipitate was removed by low speed centrifugation and re-suspended in distilled water. Glycogen was estimated after hydrolysis followed by quantification of glucose contents (DuBoie 1960) and expressed in µmols of glucosyl-glucose and glucose determination.

Chemical determination of metabolites: Lactate was determined according to Harrower's colorimetric method at 540 nm adjusted to experimental conditions (Harrower, 1972). Glucose was colorimetricaly estimated at 420 nm by phenol sulfuric acid (DuBoie, 1960). Pyruvate was colorimetricaly determined adjusting free protein extract samples to 1,0 ml of 1% phenilhydrazine solution in 0.1N HCl and adding 1N Na OH to complete 3.0ml. Absorbance was measured at 640 nm. Sodium concentrations were determined by flame photometry.

Statistic analysis: All data were analyzed by T-Student Test for comparison of means at different variances and $CI = 0.95$.

RESULTS

Different patterns of metabolic responses of the analyzed tissues were observed in *P. mesopotamicus* under severe hypoxia. Hepatic and red muscle glycogen stores remained practically constant along six hours of hypoxia exposure while white muscle revealed significant decrease (Table I).

Along hypoxia glucose increased in liver and decreased in red muscle. However, white muscle and plasma revealed a sharp increase at first, followed by drop to the initial levels (Fig. 1).

Lactate increased sharply in white muscle. However, red muscle presented a different pattern. The first four hours showed significant decrease turning to original values after six hours (Fig. 2). Liver and plasma revealed similar patterns. At the beginning of hypoxia it was observed significant enhancement of lactate contents returning to initial values after six hours of hypoxia (Fig. 2).

Pyruvate contents were not determined in red muscle. However, the pyruvate values observed in the other three tissues were almost unchanged (Table I). Lactate/pyruvate ratios revealed significant metabolic changes in liver, white muscle and plasma (Table II).

Hematocrit values increased significantly while plasma Na^+ contents dropped after six hours under hypoxia. Plasma K^+ contents remained constant (Table I).

DISCUSSION

Metabolic adaptations against hypoxia, in fish, belong to a large body of possible responses. However, we are observing that species showing morphological, behavioural or physiological changes against hypoxia fit under a large group which usually show metabolic shifts much more as an immediate response than a metabolic strategy. Pacu, for instance, is well known by the lip growth under low levels of oxygen. Other species as *Colossoma macropomum* and *Brycon cephalus* display similar response. However, no reports have been done, so far, concerning to metabolic profiles in pacu under hypoxia.

Hepatic tissue of *P. mesopotamicus* showed significant increase on glucose level. Such fact, followed by decrease on lactate/pyruvate ratio, suggests neoglucogenesis along six hours of hypoxia. Liver glycogen store remained constant along this period. This points out to lactate recovery through hepatic synthesis of glucose.

White muscle is usually adapted to fermentation. The sharp glucose increase observed in that tissue, followed by five folds lactate increase, suggests that hypoxia affects deeply the white muscle metabolism enforcing glucose catabolism through anaerobiosis. The glucose catabolism expends glycogen as carbohydrate source. This explains the significant glycogen decrease in white muscle. However, plasma seems to be very important for buffering such system. Initial increase of glucemia and lactemia, followed by recovery of initial values, suggests reciprocal involvement of liver and white muscle, at the first moment, driving to equilibrium. First, hyper glucemia may be due to hepatic glucose release as well as hyper lactemia due to white muscle lactate release. Both tissues seem to play in opposite ways. Therefore, glucose source for white muscle is also supposed to be exogenous.

Red muscle ought to contribute to hyper lactemia but not so intensively. Smooth decrease on glucose contents associated to lactate profile suggests low anaerobic glucose catabolism in this tissue.

Comparing the responses between *P. mesopotamicus* and *Hoplias malabaricus* it is possible to observe different metabolic patterns (Moraes, *et al* 1996). While *H. malabaricus* shows metabolic strategies against hypoxia, storing white muscle glycogen, probably originated of protein metabolism, *P. mesopotamicus* seems to make use of a special pathway, Cory cycle, to recycle lactate.

The hematocrit increase may be reflecting the stress caused by hypoxia. Cathecolamines and other substances ought to explain this response and/or even prepare the organism to aggressive conditions. Decrease on plasma sodium concentration reflects possible ionic imbalance under the present ex-

perimental hypoxia. Maybe other metabolic systems are involved on responses here observed and are issue for future studies.

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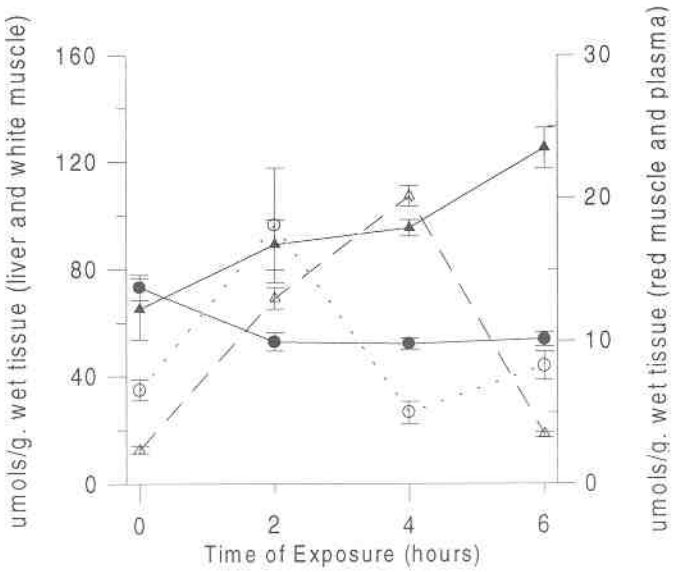

Fig. 1. Glucose contents in liver ▲—▲, white muscle Δ- -Δ, red muscle ●–● and plasma ○—○ of *P. mesopotamicus* submitted to severe hypoxia ([O_2] 0.5 mg/litre) during six hours.

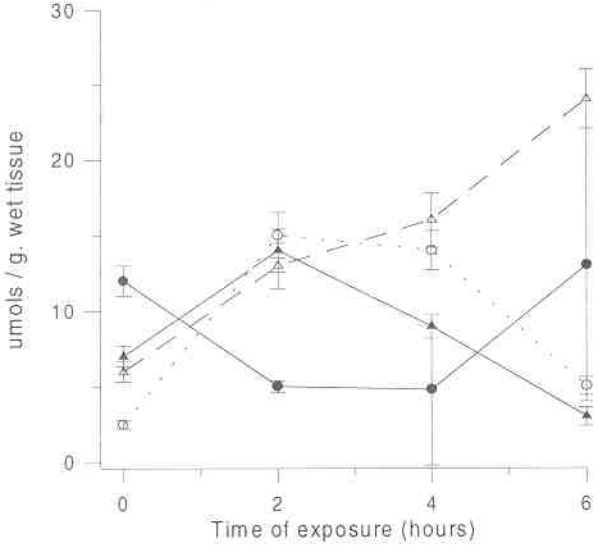

Fig. 2. Lactate contents in liver ▲—▲, white muscle Δ- -Δ, red muscle ●–● and plasma ○—○ of *P. mesopotamicus* submitted to severe hypoxia ([O_2] 0.5 mg/litre) during six hours.

TABLE I -Comparison of biochemical tissue parameters in *P. mesopotamicus* under environmental normoxia and hypoxia. Values are statistically significant higher (*) or different (**) at the level 0.05 when compared the means of 0h *versus* 6h by the T-Student test.

Tissue	Glycogen		Piruvate		Hematocrit		Na^+	
	Experimental time		Experimental time		Experimental time		Experimental time	
	0 h	6 h	0 h	6 h	0 h	6 h	0 h	6 h
Liver	672.13±29.12	859.32±32.21 *	118.50±8.68 **	79.58±4.12	---	---	---	---
White Muscle	4.01±0.38 **	2.24±0.05	104.08 ±3.51 **	87.06±5.02	---	---	---	---
Red muscle	14.69±2.43 **	8.13±0.32	---	---	---	---	---	---
Plasma	---	---	4.85±0.87	5.36±0.34	---	---	143.00 ± 3.89 **	89.33 ± 8.38
Whole blood	---	---	---	---	21.93±1.14	26.71±0.88 **	---	---

TABLE II - Tissue L/P ratio in *P. mesopotamicus* under environmental normoxia (0 hs) and hypoxia (6 hs). Values are statistically significant higher (*) at the level 0.05 when compared the means of 0h *versus* 6h by the T-Student test.

Tissue	(Lactate/Pyruvate) ratio	
	Experimental Time	
	0 h	6 h
Liver	0.054*	0.032
White muscle	0.11	0.28*
Plasma	0.09	0.198*