LDH GENE RESPONSES TO HYPOXIA AND ANOXIA IN *ASTRONOTUS CRASSIPINIS*.

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EXTENDED ABSTRACT ONLY- DO NOT CITE

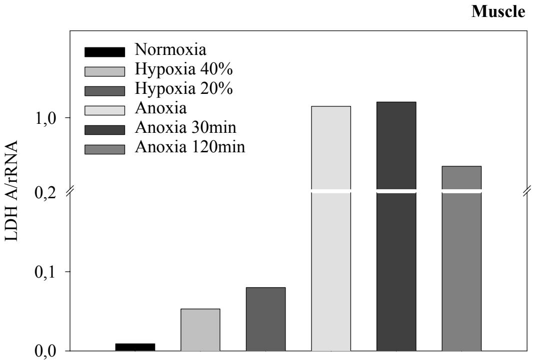
Adaptation to hypoxia depends on the modulation of physiological relevant genes. Low O_2 concentration induces the stabilisation of the hypoxia inducible factor (HIF1), which in turn, induces the transcription of the hypoxia inducible genes, such as erythropoietin, transferrin, vascular endothelial growth factor, phosphofructokinase and lactate dehydrogenese A (Hochchachka et al, 1997). Hypoxia induction of LDH-A mRNA and/or protein was observed in normal and tumour cells in culture, but *in vivo* studies that corroborate these observations are scarce. In this work we have aimed at the study of the modulation of LDH-A expression by hypoxic conditions in an amazonian cychlid, *Astronotus crassipinis*, which has shown to be extremely tolerant to low oxygen concentrations and even to anoxia, as its sibling species *Astronotus ocellatus* (Muusze et al., 1998).

Different-sized specimens of *Astronotus crassipinis* were exposed to hypoxia and anoxia conditions in a respirometer chamber. When the desired O_2 concentration was reached the animals were sacrificed, blood was taken, tissues were excised and immediately homogenised for RNA extraction. The animals were separated by size as: group A (10cm of fork length) and group B (15-20cm). Total RNA was extracted from white muscle, heart and liver, transferred to nylon filters and hybridised to *Astronotus ocellatus* LDH-A and human rRNA ^{32}P-labelled probes.

Lactate dehydrogenase is a key enzyme in the control of energy metabolism, catalysing the interconvertion of pyruvate to lactate and regulating the levels of these substrates in function of the oxygen availability. The LDH-A isoenzyme converts preferentially pyruvate to lactate under anaerobic conditions and is found predominantly in tissues normally exposed to physiological hypoxia as the skeleton muscle. On the other hand, LDH-B is more active in aerobic conditions, converting preferentially lactate to pyruvate in well oxygenated tissues, as the cardiac muscle (Coppes, 1992).

In our experiments we have found LDH-A expression in skeleton and cardiac muscle, but we could not detect LDH-A mRNA in the liver of these animals. The presence of LDH-A in the heart, a highly aerobic tissue, was already observed in hypoxia tolerant species such as *Astronotus crassipinis* and *Astronotus ocellatus*, which present an anaerobic preference (predominance of the A form) in the cardiac muscle (Almeida-Val et al., 1995).

When we analysed LDH-A mRNA expression in *A. crassipinis* exposed to experimental hypoxia we verified an increase in transcription of this gene in white muscle and heart of younger animals (10 cm of length). In the white muscle we observed a maximum of LDH-A expression, compared to the animals exposed to normoxia, after 30 min in anoxia. In the cardiac muscle the induction was more effective than that observed in white muscle: even with 20% of O_2 saturation we could observe an increase in LDH-A transcription and the expression is maintained increased after 2 hrs in anoxia (Fig. 1). We believe that the different induction patterns observed in these two tissues are due to the differences in the ability of these tissues to cope with hypoxia. While the white muscle survives hypoxia through a metabolic suppression, the cardiac muscle would keep a very low activity based on anaerobic glycolysis.



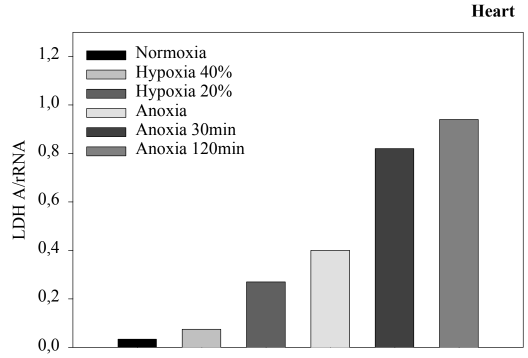


Figure 1. Expression of LDH-A in muscle and heart of young *Astronotus crassipinis* exposed to normoxia, hypoxia (40 and 20% O_2) and anoxia for 0, 30 and 120 min.

In the adults, 15-20 cm of length, we could not observe the relationship between hypoxia and increasing levels of LDH-A expression. Fig. 2 shows the comparative expression of LDH-A in the two groups, juveniles and adults, in normoxia and after 30 min in anoxia. We believe that adult animals might have developed other mechanisms of tolerance based on suppression of metabolic rates and do not depend on anaerobic activation to survive hypoxia episodes. Previous studies have shown that hypoxia tolerance in the sibling species *Astronotus ocellatus* is directly related to the increase in body mass. (Almeida-Val et al., 2000).

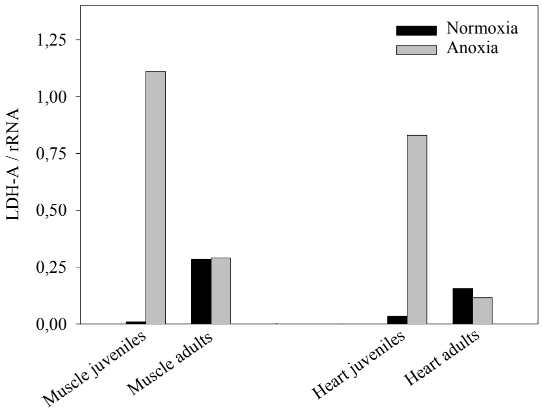


Figure 2. Comparison of LDH-A expression in white muscle and heart of young and adults *Astronotus crassipinis* under normoxia and after 30 min of anoxia.

Our results corroborate previous observations on the scaling factor in hypoxia tolerance and show that the activation of the anaerobic metabolism is regulated in different ways in different tissues, according to the energetic characteristics. We can also conclude that hypoxia tolerance in fishes of the *Astronotus* genera is based, at least in part, on gene regulation.

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