ORGANIC PHOSPHATE, pH AND ION REGULATION IN NORMOXIC AND HYPOXIC RED BLOOD CELLS OF AMAZONIAN FISH FOLLOWING ADRENERGIC STIMULATION.

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Introduction

Catecholamines are released into the blood of many fish species during periods of stress. In the red blood cells (RBC) of several teleosts, catecholamines bind to β-adrenoreceptors and activate Na^+/H^+ exchange resulting in a suite of cellular events. These catecholamine induced changes ultimately lead to an increase in RBC volume, $[Na^+]$ and pH and a reduction in NTP levels (Nikinmaa, 1990). The adaptive significance of these effects is to secure HbO_2 transport during periods of oxygen stress by increasing HbO_2 affinity and O_2 transport capacity of the blood. While these effects are well characterized in many temperate fish species (Nikinmaa, 1990), relatively little is known about the diversity of this response among tropical fishes. A recent study indicated that the RBC adrenergic response is present in 2 of 4 species of characiformes but completely absent in 2 species of siluriformes (Val et al., 1998). In the present study, RBC

adrenergic responsiveness was investigated in 3 species of characiformes [pacu (*Mylossoma duriventris*), red bellied piranha (*Pygocentrus nattereri*) and tambaqui (*Colossoma macropomum*)] and 2 species of osteoglossiformes [arowana (*Osteoglossum bicirrhosum*) and pirarucu (*Arapaima gigas*)] to further investigate the ubiquity of this response.

Materials and Methods

Pacu, piranha and arowana were captured by gill net in the Rio Solimoes and experiments were conducted on board the INPA Research Vessel *Amanai* II moored approximately 20 km upstream from Manaus, Brazil. Tambaqui and pirarucu were purchased from a local fish farm and experiments on these species were conducted at INPA. The red blood cells of the respective teleosts were rinsed several times in ice cold 10 mM HEPES buffered Cortland saline and left in the fridge overnight. The following morning the red cells were rinsed again and resuspended in this saline and split into four aliquots. Two aliquots were maintained in normoxia (air saturated) and 2 aliquots were maintained in hypoxia (N_2) for 30 mins. A normoxic and a moderately hypoxic aliquot were each adrenergically stimulated (adrenaline = 10^{-5} M) and maintained in the same gas composition for 60 mins. The other normoxic and hypoxic aliquots were left for 60 mins as controls. The adrenaline-induced effects on the pH gradient across the red cell (pH_e-pH_i), haematocrit (Hct) and mean cell haemoglobin concentration (MCHC) as well as organic triphosphate (NTP) levels and plasma Na^+ and K^+ concentrations were investigated.

Results and Discussion

Two of the characins, pacu and tambaqui, exhibited adrenergically activated red cell Na^+/H^+ exchange as indicated by changes in plasma [Na^+], pH_e-pH_i, Hct and MCHC, that were more pronounced in hypoxia than normoxia. The response in tambaqui is consistent with that observed by Val et al., (1998). Adrenergic activation of red cell Na^+/H^+ exchange was not apparent in the red bellied piranha (consistent with that observed in the black piranha, Val et al., 1998) or in the osteoglossids (arowana and pirarucu). Interestingly, all species tested exhibited a significant adrenaline-induced reduction in plasma K^+ concentration that was more pronounced in hypoxia than normoxia which has also been observed in the blood of trout (Nielsen and Lykkeboe, 1992). The only fish species that exhibited a reduction in red cell NTP levels associated with adrenergic stimulation was pacu, however, the degree of change was modest (<10%) compared with that observed in salmonids. The lack of adrenergically

mediated changes in red cell NTP levels under a similar experimental protocol have also been reported for other Amazonian fishes, including characins and catfishes (Val et al., 1998).

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