Comparative Biochemistry and Physiology, Part A 154 (2009) 224–232

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

The relationship between O_2 chemoreceptors, cardio-respiratory reflex and hypoxia tolerance in the neotropical fish *Hoplias lacerdae*

Mariana A. Micheli-Campbell [a,c], Hamish A. Campbell [c], Ana L. Kalinin [a,b], Francisco T. Rantin [a,b,*]

[a] Department of Physiological Sciences, Federal University of São Carlos-UFSCar, 13565-905 São Carlos, São Paulo, Brazil
[b] National Institute of Science and Technology-Comparative Physiology (FAPESP/CNPq) São Paulo, Brazil
[c] School of Biological Sciences, The University of Queensland, St. Lucia, Brisbane, Queensland 4072, Australia

ARTICLE INFO

Article history:
Received 2 April 2009
Received in revised form 16 June 2009
Accepted 17 June 2009
Available online 25 June 2009

Keywords:
Cardiorespiratory control
Denervation
Fish
Phylogeny
NaCN
Characiformes

ABSTRACT

The localization, distribution and orientation of O_2 chemoreceptors associated with the control of cardio-respiratory responses were investigated in the neotropical, *Hoplias lacerdae*. Selective denervation of the cranial nerves (IX and X) was combined with chemical stimulation (NaCN) to characterize the gill O_2 chemoreceptors, and the fish were then exposed to gradual hypoxia to examine the extent of each cardio-respiratory response. Changes in heart rate (f_H) and ventilation amplitude (V_{amp}) were allied with chemoreceptors distributed on both internal and external surfaces of all gill arches, while ventilation rate (f_R) was allied to the O_2 chemoreceptors located only in the internal surface of the first gill arch. *H. lacerdae* exposed to gradual hypoxia produced a marked bradycardia (45%) and 50% increase in V_{amp}, but only a relatively small change in f_R (32%). Thus, the low f_R response yet high V_{amp} were in accord with the characterization of the O_2 chemoreceptors. Comparing these results from *H. lacerdae* with hypoxia-tolerant species revealed a relationship existent between general oxygenation of the individual species environment, its cardio-respiratory response to hypoxia and the characterization of O_2 chemoreceptors.

1. Introduction

For fish, the ability to sense and respond rapidly to changes in the oxygen content of the aquatic environment is critical. To facilitate this, fish possess a complex array of O_2 chemoreceptors within the orobranchial and parabranchial cavities, and on the internal or external surfaces of the gills, allowing detection of O_2 concentrations in both blood and water. The signal is transferred to the brain and cardio-respiratory responses are initialized (Smith and Jones, 1978; Lomholt and Johansen, 1979; Randall, 1982; McKenzie et al., 2000; Sundin et al., 2000; Milsom et al., 2002; Florindo et al., 2006). If hypoxic conditions are detected, a common response is to reduce heart rate (f_H), increasing the blood transit time through the gills, and increase respiration frequency (f_R) and/or ventilation amplitude (V_{amp}), to raise the volume of water traveling across the gill lamellae. The purpose of which is to maximize the effectiveness of oxygen transfer from the water across the gill surface and into the blood stream (Taylor et al., 1999; Campbell and Egginton, 2007). However, profound inter-species differences exist between teleosts in the extent of each of the cardio-respiratory responses (f_H, arterial blood pressure (Pa), f_R, V_{amp}), when subjected to hypoxic conditions, and this has been suggested to relate to variations in the location, orientation and distribution of O_2 chemoreceptors (Burleson and Smatresk, 1990b; McKenzie et al., 1991; Sundin et al., 1999, 2000; Milsom et al., 2002). For example, under hypoxic conditions, the tambaqui (*Colossoma macropomum*), a neotropical benthopelagic freshwater fish, shows a substantial bradycardia, with a 63% reduction in f_H, and an increase in f_R of 57%. The populations of O_2 chemoreceptor related to these variables are orientated in both internal and external gill surfaces, and also it has extrabranchial chemoreceptors allied to both of them (Sundin et al., 2000; Milsom et al., 2002; Florindo et al., 2006). In contrast, *Amia calva*, which is a temperate demersal freshwater fish, responds to hypoxia with a moderate bradycardia, with only a 15% reduction in f_H, and ~ 90% increase in f_R. This species has no gill O_2 chemoreceptors allied to the cardiac response, but has only populations of chemoreceptors orientated internally and externally on the gills for the ventilatory response (McKenzie et al., 1991). However, the genotypic and phenotypic variation between the species studied has made direct comparisons unclear.

In general, warm tropical freshwaters have low dissolved oxygen (Alekseev et al., 1984) and, consequently, many fish species are hypoxic tolerant (Randall et al., 1981; Val and Almeida-Val, 1995; Graham, 1997; Reid et al., 2005). A number of detailed physiological studies have been undertaken on South American freshwater fishes from within the Order Characiformes. These have mainly examined the graded response to hypoxia under selected denervation, and together, provide a general

* Corresponding author. Department of Physiological Sciences, Federal University of São Carlos-UFSCar, 13565-905 São Carlos, São Paulo, Brazil. Tel.: +55 16 3351 8314; fax: +55 16 3351 8401.
E-mail address: ftrantin@power.ufscar.br (F.T. Rantin).

1095-6433/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2009.06.014

picture of the location and relative roles of O_2 chemosensitive areas involved in cardio-respiratory reflexes for this group of fishes (Sundin et al., 1999, 2000; Lopes, 2003; Leite et al., 2007). However, all species studied so far inhabit low oxygenated environments (*Hoplias malabaricus*, *Piaractus mesopotamicus* and *C. macropomum*), and are hypoxic tolerant. Therefore, it is inconclusive whether or not the observed inter-species differences in the distribution and location of O_2 chemoreceptors occur due to environmental adaptation or a genotypic trait of the Order Characiformes in South America.

Comparing closely related species of similar genotype, but inhabiting very different niches in terms of oxygen availability, offers the opportunity to study the link between environmental conditions and the ecotype/phenotypic traits of an organism. While this does not solve the problem of whether an observable trait is the result of an evolutionary strategy to accommodate environmental heterogeneity in the strict adaptionist sense (as opposed to, for example, genetic drift, indirect selection, selection ± adaptation, etc.), it does help to simplify the examination of genotypic constraint (Campbell et al., 2008). There is a member of the Order Characiformes, in the Erythrinidae family, *Hoplias lacerdae*, which does not have pseudobranch, cannot aerially respire and inhabits highly oxygenated environments (Godoy, 1975). Comparative studies have found significant differences between *H. lacerdae* and its close relative *H. malabaricus*. This species is considered to be highly hypoxic tolerant because of its lower critical oxygen tensions ($P_cO_2 = 20$ mm Hg) compared to *H. lacerdae* ($P_cO_2 = 35$ mm Hg; Rantin et al., 1992, 1993). Additionally, *H. lacerdae* has a 43% lower P_cO_2, 88% smaller mass-specific respiratory surface area, a higher metabolic rate, and lower anaerobic capacity than *H. malabaricus* (Rantin and Johansen, 1984; Rantin et al., 1992, 1993; Fernandes et al., 1994). A histological comparative study of the gills of *H. malabaricus* and *H. lacerdae* found no difference in the abundance and distribution of neuroepithelial cells (Coolidge et al., 2008), suggesting genotypic similarity. Therefore, while this study accurately determined the exact location and concentration of putative O_2 sensing cells within the gills, it did not illustrate which cardio-respiratory response they were associated with.

Thus, the present study aimed to indirectly investigate the location, orientation and distribution of O_2 chemoreceptors related to both cardiovascular and respiratory responses in *H. lacerdae*. We used similar methodologies of selective denervation of cranial nerves and stimulation of the gill O_2 chemoreceptors with NaCN, as previously used on phylogenetically related hypoxic tolerant Characiformes species (Sundin et al., 1999, 2000; Lopes, 2003; Florindo et al., 2006; Leite et al., 2007). The extent to which each cardio-respiratory variable response to hypoxia has been shown to differ between *H. lacerdae* and its hypoxia tolerant congeneric cousin *H. malabaricus*, and therefore, we hypothesized that the location and distribution of O_2 chemoreceptors involved in specific cardio-respiratory responses would also differ from these species, accordingly.

2. Materials and methods

2.1. Animals

Adult *H. lacerdae* (Characiformes, Erythrinidae) (402.0 ± 206.7 g, $n = 38$) were obtained from Furnas Hydroelectric Power Plant fish culture station and Cascata fish farm, Minas Gerais State, Brazil. The fish were transported to the Federal University of São Carlos, São Paulo, Brazil, and held in 1000-L tanks, filled with filtered and dechlorinated water, and maintained at 25 ± 1 °C. Fish were fed *ad libitum* once weekly with the usual prey fish that *H. lacerdae* may encounter in the wild.

2.2. Surgical procedures

All fish were anaesthetized by submergence in an aqueous solution of benzocaine (100 mg L^{-1}) predissolved in 2 mL of 70% ethanol. Once the fish showed no reaction response to touch it was transferred to an operating table and the gills ventilated with an aerated dilute solution of benzocaine (50 mg L^{-1}). The fish was orientated to its dorsal surface and the left operculum was exposed. The third afferent branchial artery was punctured with a 23-G needle and a cannulae (PE 50 tubing tipped with a short segment of PE 10 tubing), filled with a solution of saline and heparin (NaCl 0.9%, 100 IU.mL^{-1} of heparin), was inserted and sutured in place. One-second cannulae (PE 160) was inserted into the buccal cavity through a 1-mm hole drilled between the nostrils and fixed at the palate by a flanged tube (*Methods in* Axelsson and Fritsche, 1994).

All fish underwent the cannulation procedure, after which; eight fish (Wt = 506.2 ± 298.2 g) had no further surgery, nine fish (Wt = 312.6 ± 118.7 g) had the cranial nerve (IX) sectioned (group IX), nine fish (Wt = 355.4 ± 187.9 g) had the IX and the pretrematic branch of cranial nerve X sectioned (group G1), and eight (Wt = 455.7 ± 204.5 g) fish had IX and all branches of cranial nerve X sectioned for all gill arches (group G4). To section the nerves a single incision (± 1 cm) was made in the dorsal epithelium of the operculum wall, and the nerves exposed by blunt dissection. All denervations were confirmed post mortem as the intact state of the cardiac and visceral branches of the cranial nerve X. To determine if the surgical procedure, minus the gill sectioning, had any effect on measured parameters, a sham group had the opercular epithelium incised and the cranial nerves to each gill arch exposed but not sectioned ($n = 4$; Wt = 380.1 ± 137.7 g).

After surgery, fresh aerated water was passed over the gills until the fish showed signs of autoventilation. They were then placed into individual cylindrical chambers (40 cm L × 20 cm D) housed within a large experimental tank (80 L). The cannulae exited from a hole on the upper surface of each chamber. A recovery period of 24 h was allowed before commencing the experiment.

2.3. Experimental protocol

The branchial artery and buccal cavity cannulae were connected via a pressure transducer (Utah Medical Products) to an amplifier (AECAD 0804-AVS, São Paulo). Arterial blood pressure (Pa), heart rate (f_H), respiratory rate (f_R) and ventilatory amplitude (V_{amp}) were recorded by a data acquisition interface DATAQ DI 154RS. To calculate values for V_{amp} all treatments were expressed as a relative percentage increase or decrease of the resting value. Total ventilation (V_{tot}) was calculated using the equation: $V_{totx} = (f_{Rx} * V_{ampx}) / (f_{RPre} * V_{ampPre}) * 100$ (see Florindo et al., 2006), wherein x = treatment and Pre = resting values.

After a period of 15 min of recordings under normal conditions, the fish received an internal (via branchial artery cannulae) injection of NaCN (0.25 mL of 500 and 1000 mg mL^{-1}) and cardio-respiratory variables were recorded for a further 30 min. Once the cardio-respiratory variables returned to preinjections values, a further 15 min of recordings were taken before an external (via buccal cavity cannulae) injection of NaCN (1 mL of 500 and 1000 mg mL^{-1}) was delivered, and cardio-respiratory variables recorded until preinjection values were attained. Branchial artery NaCN injections stimulated O_2 chemoreceptors located in the blood vessels, and the buccal cavity NaCN injections stimulated O_2 chemoreceptors at the primary epithelium of the gills. Each of these O_2 chemoreceptor populations will be referred to, throughout the text, as being either internally or externally orientated on the gill surfaces. After each injection the cannulae was cleaned with a new solution of saline (0.2 mL, internally) or +water (0.5 mL, externally) to ensure complete drug delivery. Saline injections (0.9% NaCl; internal) and water (external) were given in four pilot studies of the injection procedure and we found no significant mechanic effect of the injection procedure.

After a 2-h period each fish was then exposed to graded hypoxia. In a large header tank the O_2 tension was gradually reduced from normoxia (140 mm Hg) until 10 mm Hg by aerating with N_2. The water PO_2 circulating across the fish was continuously monitored (O_2 electrode FAC 001 and FAC 204A O_2 analyzer, FAC Instruments, São Carlos, Brazil)