Comparative Biochemistry and Physiology, Part A 166 (2013) 17–25

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Branchial O_2 chemoreceptors in Nile tilapia *Oreochromis niloticus*: Control of cardiorespiratory function in response to hypoxia

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ARTICLE INFO

Article history:
Received 8 March 2013
Received in revised form 24 April 2013
Accepted 29 April 2013
Available online 4 May 2013

Keywords:
Gill chemoreceptors
Hypoxia
Hypoxic bradycardia
Oreochromis niloticus
Ventilation

ABSTRACT

This study examined the distribution and orientation of gill O_2 chemoreceptors in *Oreochromis niloticus* and their role in cardiorespiratory responses to graded hypoxia. Intact fish, and a group with the first gill arch excised (operated), were submitted to graded hypoxia and their cardiorespiratory responses (oxygen uptake - $\dot{V}O_2$, breathing frequency - f_R, ventilatory stroke volume - V_T, gill ventilation - $\dot{V}_G$, O_2 extraction from the ventilatory current - EO_2, and heart rate - f_H) were compared. Their responses to bolus injections of NaCN into the bloodstream (internal) or ventilatory water stream (external) were also determined. The $\dot{V}O_2$ of operated fish was significantly lower at the deepest levels of hypoxia. Neither reflex bradycardia nor ventilatory responses were completely abolished by bilateral excision of the first gill arch. EO_2 of the operated group was consistently lower than the intact group. The responses to internal and external NaCN included transient decreases in f_H and increases in f_R and V_{amp} (ventilation amplitude). These cardiorespiratory responses were attenuated but not abolished in the operated group, indicating that chemoreceptors are not restricted to the first gill arch, and are sensitive to oxygen levels in both blood and water.

1. Introduction

Aquatic environments frequently exhibit daily fluctuations in dissolved O_2 concentrations. Such variations can sometimes result in severe O_2 depletions, known as environmental hypoxia, in particular in tropical and subtropical regions (Randall et al., 1997). Cardiorespiratory responses to environmental hypoxia have been documented for a considerable number of fish species. Hypoxia usually induces increases in breathing frequency (f_R) and ventilatory stroke volume (V_T) as well as a reduction in heart rate (f_H). Hyperventilation and bradycardia are reflex responses that serve 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). They are controlled by reference to O_2 chemoreceptors sensitive to O_2 partial pressures of the inspired water (P_IO_2), or arterial blood (PaO_2), or both (Burleson et al., 1992; Burleson and Milsom, 1993; Sundin et al., 1999, 2000; Leite et al., 2007; Micheli-Campbell et al., 2009; Lopes et al., 2010). Afferent nerves then relay the information from chemoreceptors to the cardiac and respiratory centers in the central nervous system. This elicits reflex homeostatic cardiorespiratory responses, via efferent inputs to the ventilatory muscles and heart (Gilmour and Perry, 2007).

The precise anatomical locations and distributions of O_2-sensitive chemoreceptors in fishes are not completely established, but physiological and pharmacological evidence consistently indicate the gills (including the pseudobranch in those species that possess this structure) and the oro-branchial cavity as the structures housing these receptors (Laurent and Rouzeau, 1972; Randall and Jones, 1973; Butler et al., 1977; Daxboeck and Holeton, 1978; Smith and Davies, 1984; Smatresk et al., 1986; Burleson and Smatresk, 1990; McKenzie et al., 1991; Burleson and Milsom, 1993; Sundin et al., 1999; Milsom, 2002; Florindo et al., 2006). The receptors located in the oro-branchial cavity are innervated by branches of the cranial nerve V (trigeminal) and/or VII (facial). Those in the pseudobranch are also innervated by the cranial nerve VII, while those on the gill arches are innervated by branches of the IX (glossopharyngeal) and/or X (vagus) (Perry and Reid, 2002). Histological and neurophysiological evidence suggest that O_2 chemoreception in the gills may arise from neuro-epithelial cells containing vesicular serotonin (Jonz and Nurse, 2003; Jonz et al., 2004; Coolidge et al., 2008; Porteus et al., 2012; Zachar and Jonz, 2012). However, the distribution of O_2 chemoreceptors can vary considerably among different gill

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http://dx.doi.org/10.1016/j.cbpa.2013.04.024

arches and among species (Burleson and Milsom, 1993; Sundin et al., 2000). Furthermore, there is also evidence of O_2-chemosensitive sites outside the branchial apparatus (Butler et al., 1977; Barret and Taylor, 1984). The location, distribution, orientation and function of the gill chemoreceptors in the fish species studied to date have recently been reviewed by Milsom (2012).

The innervation, location and orientation of the O_2 chemoreceptors triggering reflex responses to hypoxia have also been determined based on cardiorespiratory responses to different O_2 levels and/or to NaCN, which is a potent stimulant of O_2 receptor activity (Burleson et al., 1992). These reflexes can be modified or eliminated by sectioning nerves to the gills and oro-branchial cavity (Burleson and Milsom, 1993; Sundin et al., 1999, 2000; Milsom, 2002; Florindo et al., 2006; Leite et al., 2007; Micheli-Campbell et al., 2009) or by the excision of gill arches (Jia and Burggren, 1997; Perry and Reid, 2002).

One main conclusion that can be drawn from all of these studies is that it is impossible to establish a unifying pattern for the location, distribution pattern and orientation of O_2 chemoreceptors in fishes. For example, phylogenetically closely related species, such as the characids pacu, *Piaractus mesopotamicus*, and tambaqui, *Colossoma macropomum* (Sundin et al., 2000; Leite et al., 2007), or species in the same genus such as the erythrinids traíra, *Hoplias malabaricus*, and trairão, *Hoplias lacerdae* (Sundin et al., 1999; Micheli-Campbell et al., 2009), all exhibit different O_2 chemoreceptor locations, distributions and orientation patterns.

Nile tilapia, *Oreochromis niloticus* (Cichlidae), is known for its tolerance of environmental factors such as temperature and pH, and low levels of dissolved oxygen (Chervinski, 1982; Fernandes and Rantin, 1986a, 1986b, 1987, 1989; Lague et al., 2012). Although the cardiorespiratory responses by this species to hypoxia have been extensively studied (Fernandes and Rantin, 1987, 1994; Kalinin et al., 1999; Speers-Roesch et al., 2010), the mechanisms of oxygen sensing and the distribution of chemoreceptors mediating reflex responses still remain to be elucidated. The present study aimed, therefore, to provide insight into the distribution and orientation of the branchial O_2 chemoreceptors involved in reflex hypoxic responses in this species. In particular, to test whether chemoreceptors are restricted to the first pair of gill arches, or are distributed over all gill arches, the first gill arch was excised bilaterally, as described by Perry and Reid (2002), and cardioventilatory responses to hypoxia and NaCN compared to those of intact fish.

2. Material and methods

2.1. Collection and maintence

Specimens of *O. niloticus* (Mb = 220–260g) were obtained from Santa Cândida fish farm, Santa Cruz da Conceição, São Paulo State, Brazil. In the laboratory fish were acclimated for 40 days prior the experimentation in 1000 l tanks supplied with recirculated and filtered normoxic water (~140 mmHg) at 25 ± 1 °C. During this period fish were fed *ad libitum* with commercial pellets (22% protein diet), but food was withdrawn 24 h prior to experimentation.

2.2. Surgical procedures

Fish were anesthetized by immersion in a benzocaine solution (0.1 g l^{-1}) until righting responses were lost. They were then transferred to a surgical table where their gills were irrigated with a vigorously aerated benzocaine solution at 0.05 g l^{-1}.

A flared polyethylene cannulae (PE 90) was placed in the buccal cavity through a hole drilled in the dorsal palate, and secured with a cuff. Subsequently, using the same technique, the borders of both opercula of some individuals (those to be exposed to hypoxia, Hyp) were also cannulated with PE-70. The buccal catheter was used to monitor buccal pressure variations to determine f_R. The same catheter served to inject NaCN solution into the ventilatory water stream (NaCN group). In the Hyp group, the buccal catheter was used to collect water samples to measure the oxygen partial pressure (PO_2) of inspired water (P_iO_2), while the opercular catheter was used to siphon water samples to measure PO_2 of the expired water (P_eO_2).

Fish were all fitted with ECG electrodes to record f_H, as described by Glass et al. (1991). One electrode (+) was inserted and sutured in a ventral position between the gills and the heart, and a second (−) in a ventrocaudal position close to the pelvic fins. This preparation results in ECG recordings equivalent to the bipolar lead I of standard electrocardiography.

The caudal vein was cannulated (PE 50) (Axelsson and Fritsche, 1994) for intravenous injections of saline and cyanide in the NaCN group. The intrabuccal and intravenous injections of NaCN were used selectively to stimulate external and internal O_2 chemoreceptors, respectively. To validate that the vein, and not the artery, was cannulated, the catheter was connected to a pressure transducer probe (Deltran® Pressure Transducer, Utah Medical Products, Inc., USA) of a pressure digital amplifier (AVS Projetos, São Carlos, SP, Brazil).

2.2.1. Excision of the first gill arch

In studies conducted with *H. malabaricus* (Sundin et al., 1999), *H. lacerdae* (Micheli-Campbell et al., 2009), jeju, *Hoplerythrinus unitaeniatus* (Lopes et al., 2010), *C. macropomum* (Sundin et al., 2000) and *P. mesopotamicus* (Leite et al., 2007) the cranial nerves (IX to the first gill arch and X for the first and other arches) could be assessed by a small incision made in the epithelium at the dorsal end of the gill arches where they meet the roof of the opercular cavity. In the *O. niloticus*, these cranial nerves cannot be assessed without damaging the ventilatory muscles.

Fish were anesthetized and placed on an operating table as described in 2.2. The first gill arch on either side was ligated dorsally and ventrally with two hemostatic clamps and then removed with scissors (Perry and Reid, 2002). Subsequently, the stumps were cauterized to prevent bleeding and treated with an antifungal emulsion (Trok - ketoconazole and betamethasone dipropionate) and antibiotic spray (Rifampin 10 mg ml^{-1}) to prevent infections. The stumps had completely scarred over at 72 h after surgery. The fish were then allowed to recover a further 24 h (96 h in total), after which catheters and ECG electrodes were implanted, and fish were allowed to recover a further 12 h before experiments began. Nile tilapia does not possess a pseudobranch.

Thus, there were four experimental groups, Hyp-ctr (intact fish exposed to hypoxia); NaCN-ctr (intact fish exposed to intrabuccal and intravenous injections of NaCN); Hyp-opr (fish with the first gill arches excised and submitted to hypoxia), and NaCN-opr (fish with the first gill arches excised and subject to intrabuccal and intravenous injections of NaCN).

2.3. Cardiorespiratory responses to hypoxia

After 12 h recovery from catheter and ECG surgery, fish were individually placed inside a flow-through respirometer (Rantin et al., 1992; Kalinin et al., 1999) positioned in an experimental tank. The following cardiorespiratory variables were measured: oxygen uptake ($\dot{V}O_2$) gill ventilation ($\dot{V}_G$), V_T, f_R, oxygen extraction from the ventilatory current (EO_2), and f_H.

Oxygen uptake ($\dot{V}O_2$ - mlO_2 kg^{-1} h^{-1}) was measured as described by Rantin et al. (1992). The O_2 tensions of ingoing ($P_{in}O_2$) and outgoing ($P_{out}O_2$) water were continuously monitored by siphoning water via PE catheters through an O_2 electrode (FAC 001-O_2; FAC Instruments, São Carlos, SP, Brazil) connected to a FAC 204A O_2 Analyzer. The water flow through the respirometer (V_R - mlH_2O min^{-1}) was adjusted according to the fish size and the difference between the $P_{in}O_2$ and $P_{out}O_2$ which, in this case, was set at approximately 15 mmHg. This procedure is essential to avoid reflex swimming activity and to ensure

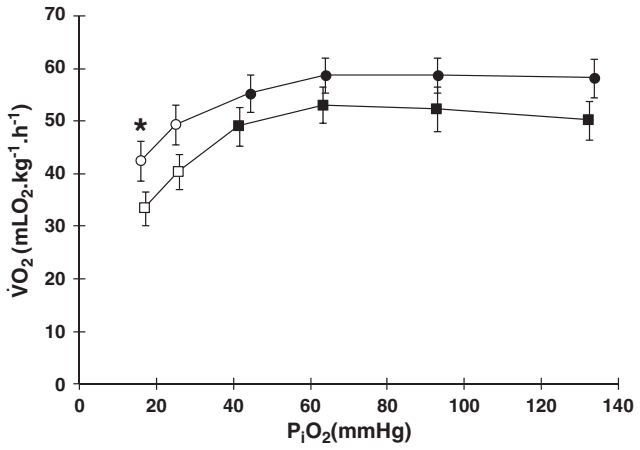

Fig. 1. The effect of graded hypoxia on oxygen uptake ($\dot{V}O_2$ - mlO$_2$ kg^{-1} h^{-1}) of *Oreochromis niloticus*. Control group - ● (n = 10), operated group - ■ (n = 10). Open symbols indicate values that are significantly different from initial (normoxic) values. * indicates values that are significantly different in relation to the control group ($P < 0.05$). Points are mean ± S.E.M.

known oxygen levels in the respirometer during graded hypoxia (Steffensen, 1989). $\dot{V}O_2$ was calculated as:

$$\dot{V}O_2 = (P_{in}O_2 - P_{out}O_2) \cdot \alpha \cdot V_R / Wt$$

where: α is the solubility coefficient for O_2 in water and Wt is the body mass (kg).

Total gill ventilation ($\dot{V}_G$- mlH$_2$O kg^{-1} min^{-1}) was measured as described by Hughes (1973). Permanently implanted catheters allowed continuous measurement of inspired (P_iO_2 - buccal catheter) and expired (P_eO_2 - opercular catheter) water O_2 tensions. $\dot{V}_G$ was calculated according to Hughes and Saunders (1970):

$$\dot{V}_G = V_R[(P_{in}O_2 - P_{out}O_2)/(P_iO_2 - P_eO_2)]/Wt$$

The breathing frequency (f_R - breaths min^{-1}) was measured from buccal pressure variations. The buccal catheter was connected to a pressure transducer (MLT0380/D Reusable BP Transducer - ADInstruments) which was coupled to an amplifier and connected to a data-acquisition system (Quad Bridge Amp ADInstruments model: ML224).

The ventilatory tidal volume (V_T - mlH$_2$O kg^{-1} breath^{-1}) was calculated by dividing total gill ventilation by breathing frequency ($\dot{V}_G/f_R$).

The oxygen extraction from the ventilatory current (EO_2 - %) was estimated according to the following equation (Dejours, 1981):

$$EO_2 = 100.(P_iO_2 - P_eO_2)/P_iO_2$$

The heart rate (f_H - bpm) was measured by connecting the ECG electrode to an amplifier (Animal Bio Amp - ADInstruments) and recorded on a PC using a data acquisition system (Power Lab - ADInstruments). The f_H was calculated by counting the QRS complex of the ECG, and expressed in bpm.

The Hyp groups were subjected to the following $P_{in}O_2$ tensions: 140 mmHg (normoxia), 100, 70, 50, 30 and 20 mmHg for 40 min at each tension. The f_R and f_H were monitored continuously and the other variables ($\dot{V}O_2$, $\dot{V}_G$, V_T and EO_2) were calculated based on the measurements ($P_{in}O_2$, $P_{out}O_2$, P_iO_2, P_eO_2) obtained in the last 3 min of each tension. Normoxia was maintained by bubbling the water with air and the different hypoxic levels by bubbling the water with N_2 at controlled rates.

2.4. Cardiorespiratory responses to NaCN

To determine the orientation of the chemoreceptors, fish of both NaCN subgroups were placed individually into a perforated holding chamber and maintained under normoxic conditions for at least 24 h. The buccal catheter and ECG electrodes were then connected to the data acquisition system described above.

After this period, NaCN injections were performed in the following sequence: (i) 0.5 ml bolus intravenous injection of saline (0.9%); (ii) bolus intravenous injection of 0.5 ml of a 750 μg ml^{-1} NaCN solution in saline); (iii) 1.0 ml bolus of water into the bucal cavity, and (iv) bolus injection of 1.0 ml of a 750 μg ml^{-1} NaCN solution in water. After the NaCN injections, the cannulae were flushed with 0.3 ml of saline (internally) and 1 ml of water (externally) to ensure complete drug administration. These NaCN doses were established by pilot experiments.

The response variables measured were: f_R (breaths min^{-1}), ventilation amplitude (V_{amp} - mmHg) and f_H (bpm). These variables were measured as described for the group exposed to hypoxia and were monitored continuously.

The V_{amp} was measured as the average difference between the peak and the trough of each pressure oscillation during breathing, at each time interval post-injection.

The cardio-respiratory variables of these two groups were recorded 30 s before each injection, to determine the pre-injection values, and at intervals of 10 s in the first min and in the last 30 s of the 2nd, 3rd, 4th and 5th min after each injection.

2.5. Data analysis

Data are presented as means ± standard error (± S.E.M.). Significant differences were detected by two-way repeated measures analysis of variance. To compare the cardiorespiratory responses to normoxia at each hypoxic tension, a Dunnet test was employed. Tukey tests were used to detect significant differences between groups.

The Dunnet test was also used to detect significant differences in cardiorespiratory variables before and after NaCN injections. Tukey test was used to detect significant differences between control and operated group. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Responses to hypoxia

The Hyp-ctr group (intact fish submitted to hypoxia) showed a relatively constant $\dot{V}O_2$ (~58 mlO$_2$ kg^{-1} h^{-1}) during graded hypoxia. The Hyp-opr group (fish with the first gill arches excised and exposed to hypoxia) displayed a similar response pattern. When compared to the Hyp-control, the Hyp-opr showed significantly lower $\dot{V}O_2$ (21%) at the lowest P_iO_2 (17 mmHg) (Fig 1).

During graded hypoxia $\dot{V}_G$ increased significantly in both intact and operated groups, reaching a maximum at a P_iO_2 of 16 mmHg (~696% - control) and 17 mmHg (~455% - operated). Compared to the normoxic values, both groups showed significant increases in $\dot{V}_G$ starting from a P_iO_2 of 44 and 41 mmHg (Hyp-ctr and Hyp-opr, respectively - Fig. 2). At the lowest P_iO_2 the Hyp-opr fish displayed a significant lower $\dot{V}_G$ in comparison to the Hyp-ctr animals (Fig. 2).

In both intact and operated groups, the increases in $\dot{V}_G$ were achieved by larger increments in V_T compared to f_R. Significant differences in V_T were detected at a P_iO_2 of 25 and 16 mmHg (Hyp-ctr) and 25 and 17 mmHg (Hyp-opr) (Fig. 2).

In the Hyp-ctr group, f_R increased gradually from a P_iO_2 of 44 mmHg to 16 mmHg. Similarly, fish of the Hyp-opr group increased f_R gradually from a P_iO_2 of 63 mmHg to 26 mmHg, below which it remained nearly constant. The Hyp-opr fish had significant higher f_R

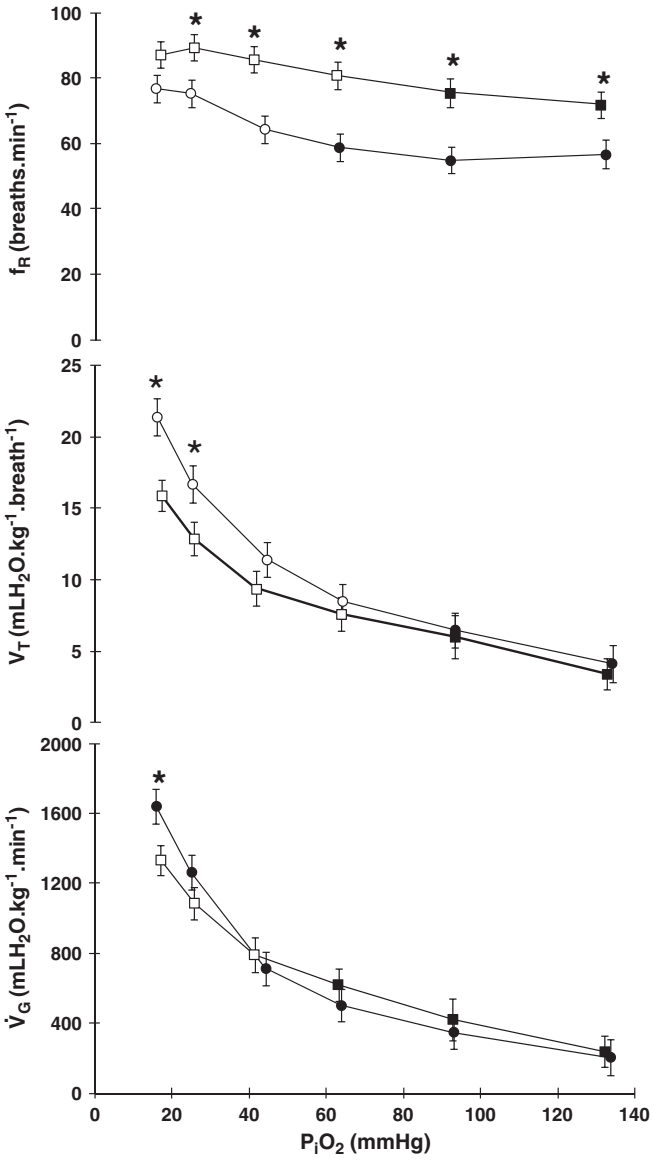

Fig. 2. The effect of graded hypoxia on total gill ventilation ($\dot{V}_G$ - mlH$_2$O kg^{-1} min^{-1}), breathing frequency (f_R - breaths min^{-1}) and tidal volume (V_T - mlH$_2$O kg^{-1} resp^{-1}) of *Oreochromis niloticus* during graded reduction of P_iO_2. Control group - ● (n = 10), operated group - ■ (n = 10). Open symbols indicate values that are significantly different from initial (normoxic) values. * indicates values that are significantly different in relation to the control group ($P < 0.05$). Values are mean ± S.E.M.

values (25 to 35%) than the Hyp-ctr, except at the most hypoxic P_iO_2 (Fig. 2).

The Hyp-ctr group maintained EO_2 constant as P_iO_2 was reduced from normoxia to 44 mmHg, below which a significant decrease was observed. The Hyp-opr group showed a similar pattern by maintaining a constant EO_2 (~60 to 65%) from normoxia to the most hypoxic P_iO_2. The Hyp-ctr group had significantly higher EO_2 than Hyp-opr in normoxia and at a P_iO_2 of 93 mmHg (Fig. 3).

The effects of graded hypoxia on f_H are shown in Fig. 4. The Hyp-ctr group had a constant f_H (~45 bpm) at all P_iO_2. The Hyp-opr fish displayed constant f_H from normoxia until a P_iO_2 of 41 mmHg. Below this oxygen tension the operated fish developed a significant bradycardia, with a reduction of about 26% in f_H at the most hypoxic tension (17 mmHg). In comparison to the control group, the Hyp-opr fish showed significantly higher f_H (~31%) from normoxia until a P_iO_2 of 41 mmHg. Below this tension both groups had practically the same f_H values.

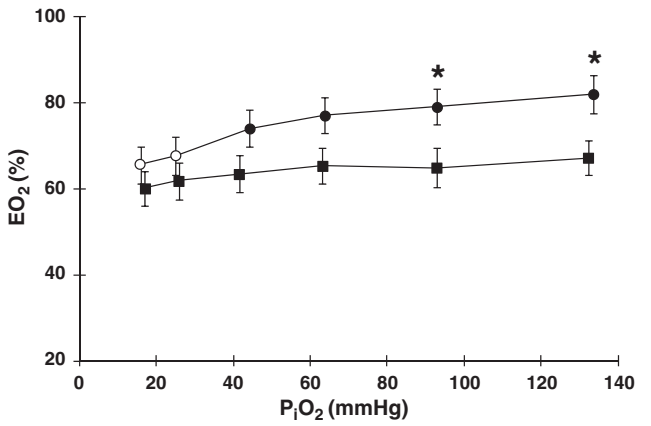

Fig. 3. The effect of graded hypoxia in the O_2 extraction from the ventilatory current (EO_2 - %) of *Oreochromis niloticus*. Control group - ● (n = 10), operated group - ■ (n = 10). Open symbols indicate values that are significantly different from initial (normoxic) values. * indicates values that are significantly different in relation to the control group ($P < 0.05$). Points are mean ± S.E.M.

3.2. Responses to NaCN

Both NaCN-ctr (intact fish exposed to NaCN injections) and NaCN-opr (fish with the first gill arches excised and exposed to NaCN injections) groups showed the same response pattern in relation to internal and external NaCN injections: increase of $\dot{V}_G$ resulting from increases in f_R and V_{amp}. Responses to internal injections lasted longer than to external ones in both groups. A 39% increase in f_R was detected in the NaCN-ctr group 50 s after the internal injection of NaCN. This variable returned to resting values about 5 min after injection (Fig. 5A). In the NaCN-opr group the f_R increased approximately 19% (40 s), in response to internal NaCN (Fig. 5B) returning to resting values in the 2nd min. The external NaCN injection provoked the largest increase (21%) in f_R just 10 s after the injection in the NaCN-ctr, returning to pre-injection values before the end of the 1st min (Fig. 5C). Within the same interval the NaCN-opr exhibited a 13% increase in f_R, returning to initial values in the 1st min post-injection (Fig. 5D).

In the NaCN-ctr group, V_{amp} increased 33% after internal injection of NaCN, and returned to pre-injection values after the 2nd min (Fig. 6A). In NaCN-opr group, V_{amp} increased approximately 18%, in response to internal NaCN, returning to resting values in the 2nd min (Fig. 6B).

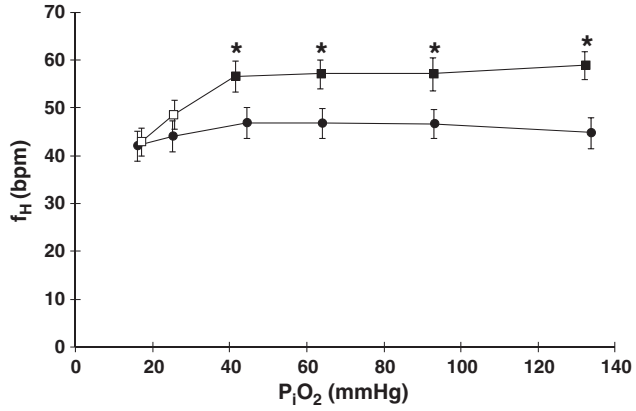

Fig. 4. The effect of graded hypoxia on heart rate (f_H - bpm) of *Oreochromis niloticus*. Control group - ● (n = 10), operated group - ■ (n = 10). Open symbols indicate values that are significantly different from initial (normoxic) values. * indicates values that are significantly different in relation to the control group ($P < 0.05$). Points are mean ± S.E.M.

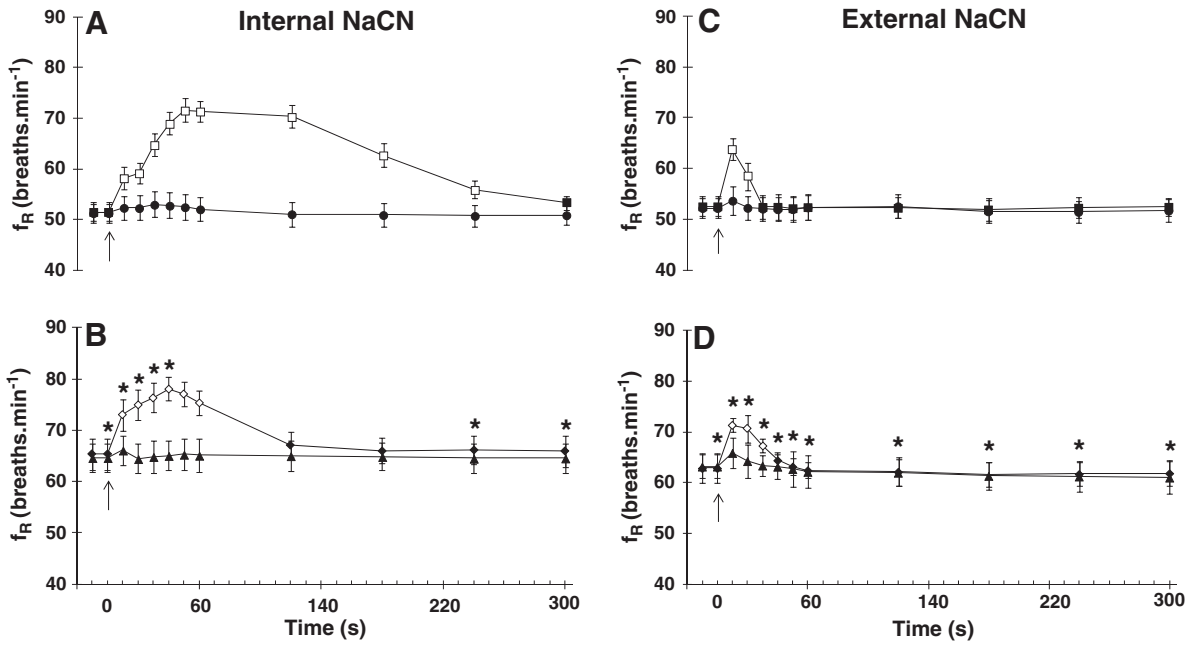

Fig. 5. Changes in breathing frequency (f_R - breaths min^{-1}) of *Oreochromis niloticus* following internal injections of saline (0.5 ml), NaCN (0.5 ml of 750 μg ml^{-1}) and external injections of H_2O (1.0 ml) and NaCN (1.0 ml of 750 μg ml^{-1}). **A** and **C** control (n = 10), **B** and **D** operated group (n = 10). The arrow indicates the moment of injection. Saline ●, ▲ and NaCN ■,◆, H_2O ●, ▲ and NaCN ■, ◆. Open symbols represent significant differences in relation to the initial values (pre-injection). * indicates values that are significantly different between groups. ($P < 0.05$). Points are mean ± S.E.M.

External NaCN injections provoked an increase of 34% in V_{amp} just 10 s after the injection in the NaCN-ctr, and quickly returned to resting values (Fig. 6C), while in NaCN-opr, V_{amp} increased by 38% at 10 s, returning to initial values within the 1st min post-injection (Fig. 6D).

Both internal and external NaCN injections also affected f_H (Fig. 7). Internal NaCN injection evoked pronounced bradycardia in both NaCN-ctr (56%) and NaCN-opr (46%) groups during the 1st min post-injection. In both groups, f_H returned to control levels after 4–5 min (Fig. 7A and 7B). In response to external NaCN, the NaCN-ctr group showed a brief but pronounced bradycardia (reduction of 80% in f_H during the first 10 s post-injection), then a significant tachycardia after about 2 min. This tachycardia persisted for the next 2 min and returned to initial

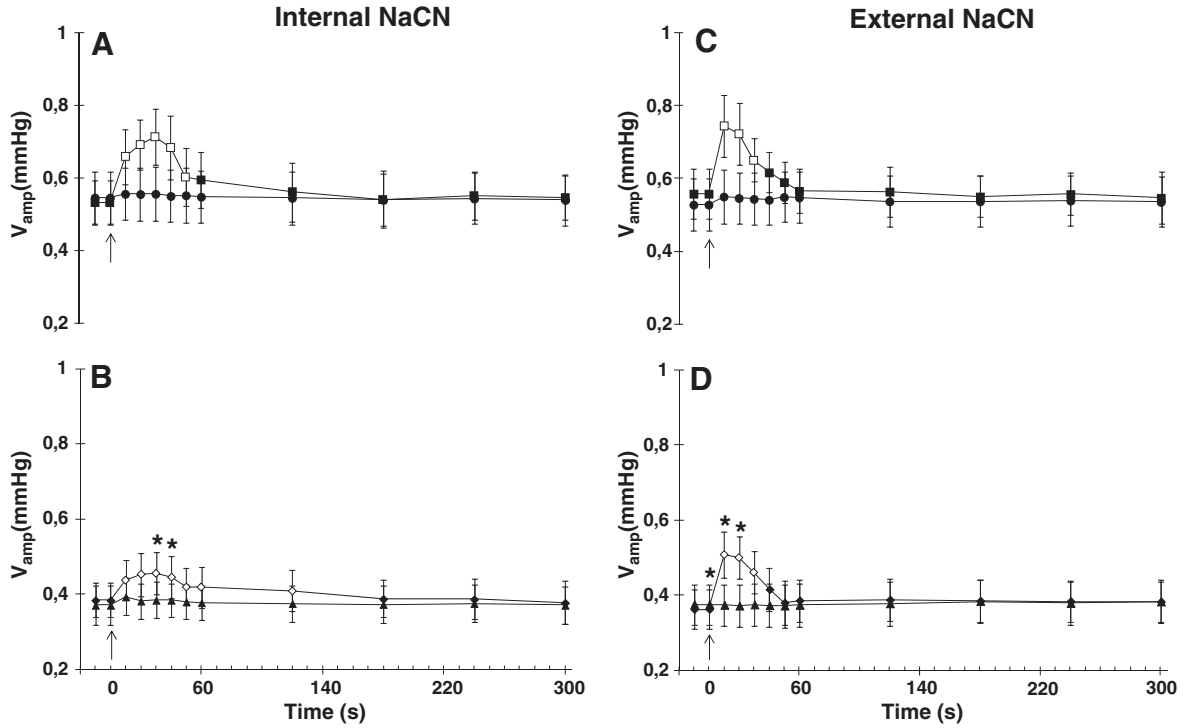

Fig. 6. Changes in ventilatory amplitude (V_{amp} - mmHg) of *Oreochromis niloticus* following internal injections of saline (●, ▲) NaCN (■, ◆), and external injections of H_2O (●, ▲) and NaCN (■, ◆). A and C control (n = 10), B and D operated group (n = 10). Open symbols represent significant differences in relation to the initial values (pre-injection). * indicates values that are significantly different between groups. ($P < 0.05$). Points are mean ± S.E.M.

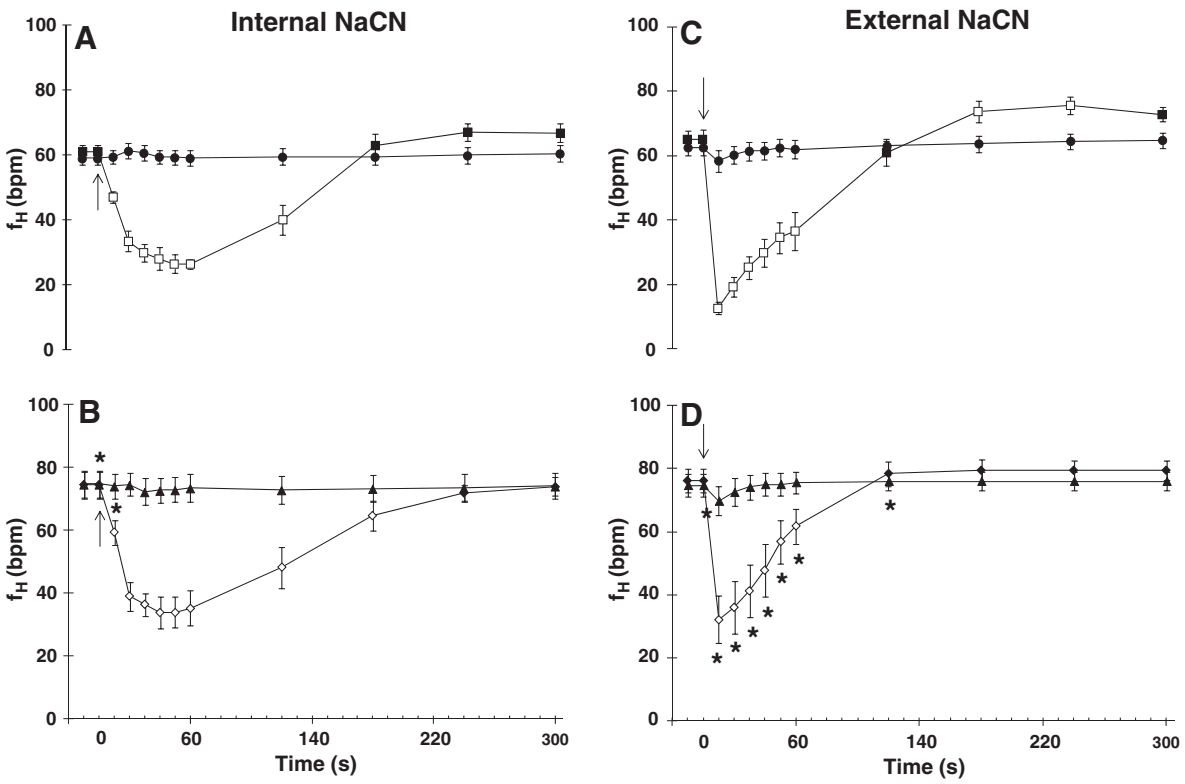

Fig. 7. Changes in heart rate (f_H - bpm) of *Oreochromis niloticus* following internal injections of saline (●, ▲) NaCN (■, ◆), and external injections of H_2O (●, ▲) and NaCN (■, ◆). A and C control (n = 10), B and D operated group (n = 10). Open symbols represent significant differences in relation to the initial values (pre-injection). * indicates values that are significantly different between groups. ($P < 0.05$). Points are mean ± S.E.M.

values after 5 min (Fig. 7C). A similar bradycardia was observed in the NaCN-opr group, but was less pronounced (reduction of 57% in f_H) than in the control group (reduction of 80% in f_H) and there was no subsequent tachycardia (7D). The cardiorespiratory variables were significantly attenuated after the NaCN injections.

4. Discussion

4.1. Oxygen uptake

Both Hyp-ctr and Hyp-opr groups displayed practically the same $\dot{V}O_2$ values in response to graded hypoxia, except at the lowest P_iO_2 (16 mmHg and 17 mmHg, respectively). Similar results were observed by Duthie and Hughes (1987) in rainbow trout *Oncorhynchus mykiss* with the 1st or 2nd gill arches cauterized. Significant differences in the $\dot{V}O_2$ of intact and operated *O. mykiss* were detected only when the fish were forced to swim. Moreover, in the operated group the $\dot{V}O_{2\ max}$ (maximum $\dot{V}O_2$ attained at the highest swimming speed) was lower than that of the intact group. According to these authors, the reduction of functional gill surface area led to a proportional decrease in $\dot{V}O_{2\ max}$, indicating that the fish were unable to make compensatory adjustments in cardiac performance to enhance their $\dot{V}O_2$. Under these conditions of maximum aerobic demand, all available gill area would appear to be fully perfused to ensure O_2 supply to the tissues. This highlights the necessity of the entire gill surface area when the recruitment of a larger number of secondary lamellae is required (Duthie and Hughes, 1987), such as in severe hypoxia.

The similar $\dot{V}O_2$ values during graded hypoxia in Hyp-ctr and Hyp-opr groups, except at the lowest P_iO_2, also indicate that removal of the first gill arch did not compromise the ability of the tilapia to regulate metabolism through adjustments of $\dot{V}_G$ and gill perfusion.

4.2. Ventilatory responses

Most fish species studied to date are able to maintain a constant $\dot{V}O_2$ in response to graded hypoxia, until a critical O_2 tension ($P_{crit}O_2$), by raising $\dot{V}_G$ (Randall et al., 1997). This is achieved by increases in both f_R and V_T (Randall, 1982). Hypoxia tolerant species, such as *O. niloticus*, normally increase $\dot{V}_G$ through a more profound increase in V_T as compared to f_R. This could be a strategy to lower energy costs of the homeostatic response, assuming that conservation of a constant velocity of muscular contraction is energy saving, whereas a higher frequency of contraction is limited by work against higher internal blood viscosity and high viscosity of water (Johansen et al., 1967; Rantin et al., 1992). This strategy was observed in fish of both experimental groups. The patterns of change in V_T and f_R are similar to previous studies on this species, reported by Fernandes and Rantin (1987), Martins et al. (2011) and Thomaz et al. (2009).

Increases in V_T were recorded in both experimental groups. This response differs from that observed by Perry and Reid (2002) in *O. mykiss* exposed to hypercarbia, where the increase in V_T was completely abolished following first gill arch excision. Denervation of the first gill arch attenuated the increases in both V_T and f_R in *P. mesopotamicus* exposed to graded hypoxia (Leite et al., 2007). A similar response was also found in the bowfin, *Amia calva*, but only after total gill denervation and excision of the pseudobranch (Mckenzie et al., 1991). The location and orientation of the chemoreceptors involved in reflex cardiorespiratory responses to hypoxia are extremely variable among species of water and air-breathing fishes, with no unifying pattern (Milsom, 2012). In *O. mykiss*, ablation of the 1st gill arch abolished reflex bradycardia and the increase in V_{amp} in response to increased water CO_2 levels, hypercarbia (Perry and Reid, 2002). However, other chemoreceptors are also involved in the cardiorespiratory responses to hypoxia. In dogfish, *Scyliorhinus canicula*, receptors mediating bradycardia were distributed on all gill arches, but were also located throughout the

oro-branchial cavity, innervated by cranial nerves V (trigeminal) and VII (facial) (Butler et al., 1977). In *P. mesopotamicus*, the O_2 chemoreceptors mediating reflex bradycardia were located on all gill arches while the receptors mediating f_R responses are possibly located in the pseudobranch and/or in some extrabranchial sites (Leite et al., 2007). *Colossoma macropomum* also possesses extrabranchial O_2 chemoreceptors mediating ventilatory responses (Milsom, 2002). *Hoplias lacerdae* possesses receptors triggering alterations in f_R located exclusively in the 1st gill arch, while those mediating f_H are distributed over all gill arches (Micheli-Campbell et al., 2009). On the other hand, in the co-generic species *H. malabaricus* reflex bradycardia is mediated by receptors restricted to the 1st gill arch, whereas increases in f_R are induced by chemoreceptors distributed over the other arches (Sundin et al., 1999).

In the present study, excision of the first gill arch did not abolish ventilatory responses to graded hypoxia. However, the $\dot{V}_G$ and V_T of Hyp-opr at 17 mmHg were attenuated when compared to the Hyp-ctr at 16 mmHg. A decrease in the f_R of the Hyp-opr group was also observed at this P_iO_2, accompanied by a ~33% reduction in $\dot{V}O_2$.

When comparing both groups, the f_R of Hyp-opr was significantly higher at all P_iO_2, except at the deepest level of hypoxia. This strategy was probably needed to compensate the reduction in the respiratory surface area and ensure the O_2 uptake required to maintain metabolic rate.

The cardiorespiratory responses to NaCN are well documented in fishes (Burleson and Smatresk, 1990; Burleson and Milssom, 1995; Mckenzie et al., 1995; Sundin et al., 1999; Milsom, 2002; Leite et al., 2007; Micheli-Campbell et al., 2009). The NaCN acts as a blocker of oxidative phosphorylation in the mitochondria, thus stimulating the chemoreceptors (Burleson et al., 1992). In the present study gill excision attenuated, but did not abolish, reflex ventilatory responses in the NaCN-opr group, except for V_{amp} following the external injection (Fig. 6D). A similar attenuation was found in the V_{amp} of *H. lacerdae* after denervation of the first gill arch (Micheli-Campbell et al., 2009) and in the f_R of *P. mesopotamicus* in response to internal NaCN injection (Leite et al., 2007).

In both NaCN-ctr and NaCN-opr groups, a larger increase in V_{amp} was observed compared to f_R in response to external NaCN injections, as observed in hypoxia. However, the internal NaCN injections caused increases of same magnitude in f_R and V_{amp} in both experimental groups.

Fish species inhabiting hypoxic environments often need more O_2 sensitivity to rapidly detect and trigger compensatory mechanisms. As an example, *H. malabaricus*, which is found in hypoxic habitats, presents O_2 chemoreceptors mediating the f_R and V_{amp} on all gill arches, and also at extrabranchial sites (Sundin et al., 1999). By contrast, *H. lacerdae*, a co-generic species that typically inhabits well oxygenated waters, only possesses internally oriented chemoreceptors mediating f_R in the first gill arch (Micheli-Campbell et al., 2009). As a hypoxic tolerant species, *O. niloticus* possess O_2 chemoreceptors mediating the ventilatory variables (f_R and V_{amp}) with similar distribution and orientation to those found in the gills of *H. malabaricus*.

The high EO_2 of *O. niloticus* (70 to 80%) has been observed previously in this species exposed to hypoxia (Fernandes and Rantin, 1986a; Kalinin et al., 1999; Thomaz et al., 2009; Martins et al., 2011). Taking into consideration the fundamental equation of respiratory physiology proposed by Dejours (1981):

$$\left(\dot{V}_G/\dot{V}O_2\right).EO_2.P_iO_2 = 1,$$

the maintenance of a constant oxygen uptake when the environment O_2 concentration declines is possible by means of an increase in $\dot{V}_G$ and/or in EO_2. Due to a limited capacity to increase EO_2 increases in fishes, enhancements in $\dot{V}_G$ are required to maintain a constant $\dot{V}O_2$ (Kalinin et al., 1999; Martins et al., 2011). Thus, in hypoxia the Hyp-ctr group kept EO_2 constant until a P_iO_2 of 44 mmHg and the Hyp-opr fish were also able to maintain a constant EO_2 at all experimental P_iO_2.

According to Duthie and Hughes (1987), the secondary lamellae of the first pair of gill arches are responsible for about 30% of the total respiratory surface area in *O. mykiss*. Assuming this is also true of *O. niloticus*, despite a reduction of this magnitude in the functional surface area, the Hyp-opr fish were still able to maintain an EO_2 of around 65%. This presumably required increased blood perfusion and lamellar recruitment in the other three gill arches. Fernandes and Rantin (1986b) reported that the second to fourth gill arches of *O. niloticus* have a high density of secondary lamellae, which will have received the entire cardiac output in the absence of the first arch, thus potentially eliciting greater than normal lamellar recruitment in the Hyp-opr fish.

4.3. Cardiac responses

The Hyp-opr fish showed higher resting f_H than the Hyp-ctr. The same tendency was found in *P. mesopotamicus* (Leite et al., 2007) and *H. lacerdae* (Micheli-Campbell et al., 2009) when these species had their first gill arch denervated. The increase in f_H could be related to some alteration in the resting vascular tone of the gill vessels or to an alteration of the afferent information from the gills, which modulated cardiac activity (Leite et al., 2007). After atropine injection, normoxic *H. malabaricus* with the first gill arch denervated increased f_H (Sundin et al., 1999). These authors suggested that cardiac cholinergic modulation was altered by denervation, although some degree of cardiac control remained even after complete gill denervation. Thus, it is most likely that the increase in f_H observed in the Hyp-opr group was related to both a decrease of branchial vascular resistance and to the removal of sensory information from the gills, as suggested by Leite et al. (2007).

Fish usually respond to hypoxia with a reduction in f_H, a hypoxic bradycardia (Randall, 1982; Taylor, 1992). This cardiac response has been documented for many teleosts species, but its physiological significance is not completely understood. Among suggested benefits, the reflex response (Fritsche and Nilsson, 1989; Burleson and Smatresk, 1990) would increase the blood residence time inside the secondary lamellae, leading to an improvement in O_2 diffusion from water to the blood, thus increasing the diffusing capacity and O_2 extraction by the gills (Farrell et al., 1980; Gilmour and Perry, 2007). Alternatively, Glass et al. (1991) suggested a causal relationship between cardiac dysfunction and hypoxic bradycardia in carp, *Cyprinus carpio*. To date, it is not entirely clear to what extent hypoxic bradycardia in fish is a regulatory response or a consequence of cardiac dysfunction, or both. For example, pharmacological or surgical abolition of hypoxic bradycardia did not significantly affect branchial gas exchange in *O. mykiss* (Perry and Desforges, 2006), or regulation of metabolism during hypoxia in Atlantic cod, *Gadus morhua* (Mckenzie et al., 2009) and European eel, *Anguilla anguilla* (Iversen et al., 2010). The hypoxic bradycardia may improve O_2 diffusion to cardiac myocytes by prolonging the diastolic time and, consequently, the residence time of the blood inside the heart lumen. Thus, hypoxic bradycardia could be a protective response to the highly aerobic cardiac muscle (Taylor et al., 1999). In the present study the Hyp-ctr group did not display consistent bradycardia and the Hyp-opr developed significant bradycardia only at a P_iO_2 of 25 mmHg and below (Fig. 4).

A different response was found in *O. mykiss*, in which bradycardia was abolished after excision of the first pair of gill arches (Perry and Reid, 2002) and in *H. malabaricus* after denervation of the first gill arches (Sundin et al., 1999). Denervation of the first gill arch did not, however, abolish hypoxic bradycardia in *P. mesopotamicus* (Leite et al., 2007), *H. lacerdae* (Micheli-Campbell et al., 2009) and *C. macropomum*, (Sundin et al., 2000), indicating the presence of chemoreceptors involved in the control of f_H in the other gill arches. In the present study,

the hypoxic bradycardia persisted after bilateral ablation of the first pair of gill arches.

Regarding O_2 chemoreceptors orientation, according to Burleson et al. (1992) the O_2 chemoreceptors mediating f_H reflexes are externally oriented and located on the first gill arch in most water-breathing teleosts, such as *G. morhua* (Fritsche and Nilsson, 1989) and sea raven, *Hemitripterus americanus* (Saunders and Sutterlin, 1971). However, more recent studies have revealed various different orientations for these receptors. *Hoplias malabaricus* possess chemoreceptors modulating the f_H responses that are internally oriented, located exclusively in the first gill arch (Sundin et al., 1999). Moreover, *C. macropomum* (Sundin et al., 2000), *M. cephalus* (Shingles et al., 2005), *P. mesopotamicus* (Leite et al., 2007), *H. lacerdae* (Micheli-Campbell et al., 2009) and *H. unitaeniatus* (Lopes et al., 2010), exhibit internally and externally oriented chemoreceptors distributed over all gill arches, that elicit reflex bradycardia.

The attenuated bradycardia in the NaCN-opr is similar to responses observed in the catfish, *Ictalurus punctatus* (Burleson and Smatresk, 1990) and after external NaCN injections in *P. mesopotamicus* (Leite et al., 2007), both with the first pair of gill arches denervated. These findings suggest that in *O. niloticus* the O_2 chemoreceptors modulating the f_H responses are internally and externally oriented, located in the first gill arch, but also present in other gill arches.

5. Conclusions

In summary, the cardiorespiratory variables involved on the responses to graded hypoxia were not completely abolished by the excision of the first pair of gill arches of *O. niloticus* and the $\dot{V}O_2$ and EO_2 were not drastically reduced. Moreover, responses to internal and external injections of NaCN were attenuated, but not abolished, in *O. niloticus* with the first pair of gill arches excised. The data suggest that *O. niloticus* possess internally and externally oriented O_2 chemoreceptors, distributed over all gill arches, mediating reflex homeostatic cardiorespiratory adjustments in response to hypoxia.

Acknowledgments

This research was supported by The São Paulo State Research Foundation – FAPESP (Proc. 2010/03599-2). The authors are thankful to Juliana Thomaz and Rafael Zaneli for their technical support and Dr. David J. McKenzie for the text revision.

This study was performed in accordance with the rules of the Brazilian Federal Legislation and was approved by the Ethical Committee on Animal Experimentation, Federal University of São Carlos (Authorization No. 015/2010).

References

Axelsson, M., Fritsche, R., 1994. Cannulation techniques. In: Hochachka, P.W., Mommsen, T.P. (Eds.), Analytical Techniques. Biochemistry and Molecular Biology of Fishes. Elsevier, Amsterdam, pp. 17–36.

Barret, D.J., Taylor, E.W., 1984. Changes in heart rate during progressive hypoxia in the dogfish, *Scyliorhinus canicula* L.: evidence for a venous oxygen receptor. Comp. Biochem. Physiol A 78, 697–703.

Burleson, M.L., Milsom, W.K., 1993. Sensory receptors in the 1st gill arch of rainbow-trout. Resp. Physiol. 93, 97–110.

Burleson, M.L., Milsom, W.K., 1995. Cardio-ventilatory control in rainbow trout: I. Pharmacology of branchial, oxygen-sensitive chemoreceptors. Resp. Physiol. 100, 231–238.

Burleson, M.L., Smatresk, N.J., 1990. Evidence for two oxygen-sensitive chemoreceptor loci in channel catfish, *Ictalurus punctatus*. Physiol. Zool. 63, 208–221.

Burleson, M.L., Smatresk, N.J., Milsom, W.K., 1992. Afferent inputs associated with cardioventilatory control in fish. Fish Physiol 12 B, 389–426.

Butler, P.J., Taylor, E.W., Short, S., 1977. The effect of sectioning cranial nerves V, VII, IX and X on the cardiac response of the dogfish *Scyliorhinus canicula* to environmental hypoxia. J. Exp. Biol. 69, 233–245.

Campbell, H.A., Egginton, S., 2007. The vagus mediates cardiorespiratory coupling that changes with metabolic demand in a temperate nototheniod fish. J. Exp. Biol. 210, 2472–2480.

Chervinsk, J., 1982. Environmental physiology of tilapias. In: Pullin, R.S.V., Lowe-Mcconnel, R.H. (Eds.), The Biology and Culture of Tilapias, ICLARM Conference Proceedings 7. International Center for Living Aquatic Resources Managements, Manila, Philippines, p. 432.

Coolidge, E.H., Ciuhandu, C.S., Milsom, W.K., 2008. A comparative analysis of putative oxygen-sensing cells in the fish gill. J. Exp. Biol. 211, 1231–1242.

Daxboeck, C., Holeton, G.F., 1978. Oxygen receptors in the rainbow trout, *Salmo gairdneri*. Can. J. Zool. 56, 1254–1256.

Dejours, P., 1981. Principles of Comparative Respiratory Physiology, 2nd ed. Elsevier, New York.

Duthie, G.G., Hughes, G.M., 1987. The effects of reduced gill area and hyperoxia on the oxygen consumption and swimming speed of rainbow trout. J. Exp. Biol. 127, 349–354.

Farrell, A.P., Sobin, S.S., Randall, D.J., Crosby, S., 1980. Intralamellar blood flow patterns in fish gills. Am. J. Physiol. 239, 428–436.

Fernandes, M.N., Rantin, F.T., 1986. Lethal temperatures of *Oreochromis niloticus* (Pisces, Cichlidae). Rev. Bras. Biol. 46, 589–595.

Fernandes, M.N., Rantin, F.T., 1986. Gill morphometry of cichlid fish, *Oreochromis (Sarotherodon) niloticus* (Pisces, Teleostei). Ciência. e Cult. 38, 192–198.

Fernandes, M.N., Rantin, F.T., 1987. Respiratory responses of *Oreochromis niloticus* (Pisces, Cichlidae) to environmental reductions of dissolved oxygen. Bol. Fisiol. Anim. 11, 51–60.

Fernandes, M.N., Rantin, F.T., 1989. Respiratory responses of *Oreochromis niloticus* (Pisces, Cichlidae) to environmental hypoxia under different thermal conditions. J. Fish. Biol. 35, 509–519.

Fernandes, M.N., Rantin, F.T., 1994. Relationships between oxygen availability and metabolic cost of breathing in Nile Tilapia (*Oreochromis niloticus*): aquacultural consequences. Aquacult. 127, 339–346.

Florindo, L.H., Leite, C.A.C., Kalinin, A.L., Reid, S.G., Milsom, W.K., Rantin, F.T., 2006. The role of branchial and orobranchial O_2 chemoreceptors in the control of aquatic surface respiration in the neotropical fish tambaqui (*Colossoma macropomum*): progressive responses to prolonged hypoxia. J. Exp. Biol. 209, 1709–1715.

Fritsche, R., Nilsson, S., 1989. Cardiovascular responses to hypoxia in the Atlantic cod, *Gadus morhua*. Exp. Biol. 48, 153–160.

Gilmour, K.M., Perry, S.F., 2007. Branchial chemoreceptor regulation of cardiorespiratory function. Fish Physiol 25, 97–120.

Glass, M.L., Rantin, F.T., Verzola, R.M.M., Fernandes, M.N., Kalinin, A.L., 1991. Cardiorespiratory synchronization and myocardial function in hypoxic carp, *Cyprinus carpio*. J. Fish. Biol. 39, 142–149.

Hughes, G.M., 1973. Respiratory responses to hypoxia in fish. Am. Zool. 13, 475–489.

Hughes, G.M., Saunders, R.L., 1970. Responses of respiratory pumps to hypoxia in the rainbow trout, *Salmo gairdneri*. J. Exp. Biol. 53, 529–545.

Iversen, N.K., Mckenzie, D.J., Malte, H., Wang, T., 2010. Reflex bradycardia does not influence oxygen consumption during hypoxia in the European eel (*Anguilla anguilla*). J. Comp. Physiol. B 180, 495–502.

Jia, X.X., Burggren, W.W., 1997. Developmental changes in chemoreceptive control of gill ventilation in larval bullfrogs (*Rana Catesbeiana*). J. Exp. Biol. 200, 2237–2248.

Johansen, K., Lenfant, C., Grigg, G.C., 1967. Respiratory control in lungfish. Comp. Biochem. Physiol. 20, 835–854.

Jonz, M.G., Nurse, C.A., 2003. Neuroepithelial cells and associated innervation of the zebrafish gill: a confocal immunofluorescence study. J. Comp. Neurol. 461, 1–17.

Jonz, M.G., Fearon, I.M., Nurse, C.A., 2004. Neuroepithelial oxygen chemoreceptors of the zebrafish gill. J. Physiol. 560, 737–752.

Kalinin, A.L., Glass, M.L., Rantin, F.T., 1999. A comparison of directly measured and estimated gill ventilation in the Nile tilapia, Oreochromis niloticus. Comp. Biochem. Physiol. A 122, 207–211.

Lague, S.L., Speers-Roesch, B., Richards, J.G., Farrell, A.P., 2012. Exceptional cardiac anoxia tolerance in tilapia (*Oreochromis hybrid*). J. Exp. Biol. 215, 1354–1365.

Laurent, P., Rouzeau, J.D., 1972. Afferent neural activity from the pseudobranch of teleost. Effects of PO_2, pH, osmotic pressure and Na^+ ions. Resp. Physiol. 14, 307–331.

Leite, C.A.C., Florindo, L.H., Kalinin, A.L., Milsom, W.K., Rantin, F.T., 2007. Gill chemoreceptors and cardio-respiratory reflexes in the neotropical teleost pacu, Piaractus mesopotamicus. J. Comp. Physiol. A 193, 1001–1011.

Lopes, J.M., Boijink, C.H., Florindo, L.H., Leite, C.A.C., Kalinin, A.L., Milsom, W.K., Rantin, F.T., 2010. Hypoxic cardiorespiratory reflexes in the facultative air-breathing fish jeju (*Hoplerythrinus unitaeniatus*): role of branchial O_2 chemoreceptors. J. Comp. Physiol. B 180, 797–811.

Martins, N.D., Colvara, W.A., Rantin, F.T., Kalinin, A.K., 2011. Microcystin-LR: How it affects the cardio-respiratory responses to hypoxia in Nile tilapia, *Oreochromis niloticus*. Chemosphere 84, 154–159.

McKenzie, D.J., Burleson, M.L., Randall, D.J., 1991. The effects of branchial denervation and pseudobranch ablation on cardio-ventilatory control on an air-breathing fish. J. Exp. Biol. 161, 347–365.

McKenzie, D.J., Taylor, E.W., Bronzi, P., Bolis, C.L., 1995. Aspects of cardioventilatory control in the adriatic sturgeon (*Acipenser naccarii*). Resp. Physiol. 100, 45–53.

McKenzie, D.J., Skov, P.T., Taylor, E.W., Wang, T., Steffensen, J.F., 2009. Abolition of reflex bradycardia by cardiac vagotomy has no effect on the regulation of oxygen uptake by Atlantic cod in progressive hypoxia. Comp. Biochem. Physiol. A 153, 332–338.

Micheli-Campbell, M.A., Campbell, H.A., Kalinin, A.L., Rantin, F.T., 2009. The relationship between O_2 chemoreceptors, cardio-respiratory reflex and hypoxia tolerance in the neotropical fish *Hoplias lacerdae*. Comp. Biochem. Physiol. A 154, 224–232.

Milsom, W.K., 2002. Phylogeny of CO_2 and pH chemoreception in vertebrates. Respir. Physiol. Neurobiol. 131, 29–41.

Milsom, W.K., 2012. New insights into gill chemoreception: receptor distribution and roles in water and air breathing fish. Respir. Physiol. Neurobiol. 184, 326–339.

Perry, S.F., Desforges, P.R., 2006. Does bradycardia or hypertension enhance gas transfer in rainbow trout (*Oncorhynchus mykiss*)? Comp. Biochem. Physiol. A 144, 163–172.

Perry, S.F., Reid, S.G., 2002. Cardiorespiratory adjustments during hypercarbia in rainbow trout *Oncorhynchus mykiss* are initiated by external CO_2 receptors on the first gill arch. J. Exp. Biol. 205, 3357–3365.

Porteus, C.S., Brink, D.L., Milsom, W.K., 2012. Neurotransmitter profiles in fish gills: putative gill oxygen chemoreceptors. Respir. Physiol. Neurobiol. 184, 316–325.

Randall, D.J., 1982. The control of respiration and circulation in fish during exercise and hypoxia. J. Exp. Biol. 100, 275–288.

Randall, D.J., Jones, D.R., 1973. The effects of deafferenation of the pseudobranch on the respiratory response to hypoxia and hyperoxia in the trout (*Salmo gairdneri*). Resp. Physiol. 17, 291–302.

Randall, D.J., Burggren, W., French, K., 1997. Gas exchange and acid–base balance. In: Randall, D.J., Burggren, W., French, K. (Eds.), Animal Physiology – Mechanisms and Adaptations. WH Freeman and Co., New York, pp. 517–570.

Rantin, F.T., Kalinin, A.L., Glass, M.L., Fernandes, M.N., 1992. Respiratory responses to hypoxia in relation to mode of life of two erythrinid species (*Hoplias malabaricus* and *Hoplias lacerdae*). J. Fish. Biol. 41, 805–812.

Saunders, R.L., Sutterlin, A.M., 1971. Cardiac and respiratory responses to hypoxia in the sea raven, *Hemitripterus americanus*, and an investigation of possible control mechanisms. J. Fish. Res. Board. Can. 28, 491–503.

Shingles, A., McKenzie, D.J., Claireaux, G., Domenici, P., 2005. Reflex cardioventilatory responses to hypoxia in the flathead grey mullet (*Mugil cephalus*) and their behavioural modulation by perceived threat of predation and water turbidity. Physiol. Biochem. Zool. 78, 744–755.

Smatresk, N.J., Burleson, M.L., Azizi, S.Q., 1986. Chemoreflexive responses to hypoxia and NaCN in longnose gar: evidence for two chemoreceptive loci. Am. J. Physiol. 251, 116–125.

Smith, F.M., Davies, P.S., 1984. Effects of sectioning cranial nerves IX and X on the cardiac response to hypoxia in the coho salmon, *Oncorhynchus kisutch*. Can. J. Zool. 62, 766–768.

Speers-Roesch, B., Sandblom, E., Lau, G.Y., Farrell, A.P., Richards, J.G., 2010. Effects of environmental hypoxia on cardiac energy metabolism and performance in tilapia Am. J. Physiol. Regul. Integr. Comp. Physiol. 298, 104–119.

Steffensen, J.F., 1989. Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish Physiol. Biochem. 6, 49–59.

Sundin, L.I., Reid, S.G., Kalinin, A.L., Rantin, F.T., Milsom, W.K., 1999. Cardiovascular and respiratory reflexes: the tropical fish, traira (*Hoplias malabaricus*) O_2 chemoresponses. Resp. Physiol. 116, 181–199.

Sundin, L., Reid, S.G., Rantin, F.T., Milsom, W.K., 2000. Branchial receptors and cardiorespiratory reflexes in a neotropical fish, the tambaqui (*Colossoma macropomum*). J. Exp. Biol. 203, 1225–1239.

Taylor, E.W., 1992. Nervous control of the heart and cardiorespiratory interactions. Fish Physiol 12B, 343–387.

Taylor, E.W., Jordan, D., Coote, J.H., 1999. Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. Physiol. Revs. 79, 855–916.

Thomaz, J.M., Martins, N.D., Monteiro, D.A., Rantin, F.T., Kalinin, A.L., 2009. Cardiorespiratory function and oxidative stress biomarkers in Nile tilapia exposed to the organophosphate insecticide trichlorfon (NEGUVON). Ecotoxicol. Environ. Saf. 72, 1413–1424.

Zachar, P.C., Jonz, M.G., 2012. Neuroepithelial cells of the gill and their role in oxygen sensing. Respir. Physiol. Neurobiol. 184, 301–308.