

**CARDIOVASCULAR AND RESPIRATORY REFLEXES IN THE  
AIR-BREATHING FISH, JEJU (*Hoplerythrinus unitaeniatus*):  
O<sub>2</sub> CHEMORESPONSES.**

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**EXTENDED ABSTRACT – DO NOT CITE**

The aim of the present study was to determine the location and distribution of chemoreceptors involved in the cardio-respiratory responses to hypoxia and in the uptake of oxygen from the air in a facultative air-breathing fish, the jeju (*Hoplerythrinus unitaeniatus*).

*Animals*

Adult specimens of jeju, weighing  $250 \pm 50$ g, were collected in the Paraná River Basin near Bataguáçu, Mato Grosso do Sul State. In the laboratory fish were maintained in 250 L outdoor tanks supplied with aerated water at  $25 \pm 1^\circ\text{C}$ . Fish were fed weekly with live food (smaller fish of various species).

### **Cardiorespiratory variables**

Heart rate ( $f_H$ ), arterial blood pressure (Pa), ventilation frequency ( $f_R$ ) and amplitude of opercular movements (or ventilation amplitude -  $V_{AMP}$ ) were measured during exposure to hypoxia or after application of NaCN to either water bathing the gills (external) or the ventral aortic blood (internal). This was done before and after selective denervation of branchial branches of the cranial nerves IX and X.

#### *Surgery*

After anesthesia, a PE 50 cannula was inserted into the caudal artery for measurements of  $f_H$  and Pa. To allow internal injections of NaCN (stimulation if internal  $O_2$  receptors), the caudal vein was also cannulated (PE 50). To add NaCN to the respiratory water stream for stimulation of externally oriented receptors on the gills, a hole was drilled between the nostrils and a flared buccal cannula (PE 60) was passed through the hole in the roof of the mouth and secured with a cuff.

#### *Denervation*

The cranial nerve IX and the pretrematic branch of cranial nerve X serving the first gill arch were accessed. In 10 fish, cranial nerve IX alone was sectioned (group IX) and in 10 fish, both cranial nerve IX and the pretrematic branch of cranial nerve X to the first gill arch were sectioned, completely denervating the first gill arch (group G1). For complete branchial denervation the cranial nerve IX and all branches of cranial nerve X going to the gills were sectioned, completely denervating the four gill arches (group G4; n = 10). Cardiac and visceral branches of cranial nerve X were left intact. The same procedure was then performed on the other side so that all denervations were bilateral. After the experiments all nerve sections were again confirmed *post mortem* by dissection.

#### *Recordings*

Heart rate ( $f_H$  - bpm) and arterial blood pressure (mmHg) were recorded by connecting the arterial cannula to a pressure transducer of an amplifier connected to a data acquisition system (DI 154 Dataq Instruments) of an IBM PC Pentium 166. Ventilation rate ( $f_R$  - breaths·min<sup>-1</sup>) and amplitude ( $V_{AMP}$  - % of control values) were recorded by connecting the buccal catheter to another pressure transducer and amplifier connected to a second channel of the same data acquisition system.

### *Experimental protocol*

After surgery the fish were left for at least 12 h (overnight) to allow ventilation, heart rate and blood pressure to come to steady levels. Following the recovery period, each fish was subjected to a series of internal (via the venous cannula) and external (via the snout cannula) injections of NaCN to stimulate O<sub>2</sub> chemoreceptors. Injections of saline (0.9% NaCl; internal) and saline or water (external) served as controls. The injections were administered as follows: 1. internal saline; 2. internal NaCN (0.2 ml 150 mg/ml); 3. external saline/water; 4. external NaCN (0.5 ml of 500 mg/ml). In each case, the cannula was flushed with a further 0.2 ml of saline (internal) or 0.5 ml of saline/water (external) to ensure complete NaCN delivery. Following each injection, cardiorespiratory variables were monitored for 3 min or until prior levels of ventilation, heart rate and blood pressure were restored. Hypoxic conditions were produced by bubbling nitrogen through the tank. The water PO<sub>2</sub> was monitored continuously and was allowed to fall to 17 mmHg before the tank was aerated once again.

### *Results and Discussion*

Control (intact) fish responded to both external and internal injections of NaCN and to progressive hypoxia with a bradycardia and an increase in  $f_R$  and  $V_{AMP}$ . Hypoxic bradycardia started when PO<sub>2</sub> fell below 35 mmHg accompanied by a progressive rise in  $f_R$  and  $V_{AMP}$ . The rise in amplitude (48.0 %) was far more prominent than the rise in frequency (24.2 %). Bilateral section of cranial nerve IX to the first gill arch significantly increased resting  $f_H$ . Denervation of the pretrematic branch of nerve X to the same arch (G1) followed by complete denervation of all gill arches (G4) did not completely abolish the hypoxic bradycardia. The increase in  $f_R$  in response to hypoxia was abolished by complete denervation (G4). No significant changes occurred in  $V_{AMP}$  after complete denervation of the first gill arch (G1). However, the increase in VAMP in response to hypoxia was abolished by total gill denervation (G4). Internal NaCN elicited bradycardia that remained even after total gill denervation. External NaCN evoked a similar cardiac response, which also required complete branchial denervation to be removed. There was a significant effect of internal NaCN on  $f_R$  detected in the control group and after denervation of nerve IX, but not in the groups G1 and G4. NaCN added to the inspired water evoked a significant increase in  $f_R$  that was abolished only after complete gill denervation. Internal and external NaCN evoked increases in  $V_{AMP}$  in intact fish and denervated groups IX and G1. However, the increases in  $V_{AMP}$  caused by internal and external injections of NaCN were completely abolished by total denervation of the gills (group G4).

The data suggest that hypoxia elicits a bradycardia that arises from internally and externally oriented O<sub>2</sub> receptors distributed in all gill arches. They also indicate the participation of extra-branchial O<sub>2</sub>-chemoreceptors in the control of the cardiac adjustments. Hypoxia induced increases in  $f_R$  arose from receptors oriented both internally and externally and located exclusively to the first gill arch. Increases in  $V_{AMP}$  also involved receptors internally and externally oriented and distributed on the four gill arches.

### **Air-breathing frequency and duration**

#### *Recordings*

Air-breathing frequency and air-breathing duration of intact and denervated fish were recorded using an experimental setup as shown in figure 1. The upper part of the experimental chamber consisted of an “inverted funnel”, the neck of which housed an electric bulb positioned in front of a photoelectric cell. To breathe air fish were forced to pass throughout the neck, interrupting the light circuit between the bulb and the photocell. This interruption was detected by a decoder/amplifier in which a square wave was generated and recorded by the data acquisition system. Different hypoxic levels were achieved by bubbling the lower part of the experimental chamber with controlled amounts of N<sub>2</sub>. The water PO<sub>2</sub> was continuously monitored by the electrode of an oxygen analyzer connected to a micro-processed system controlling a solenoid valve.

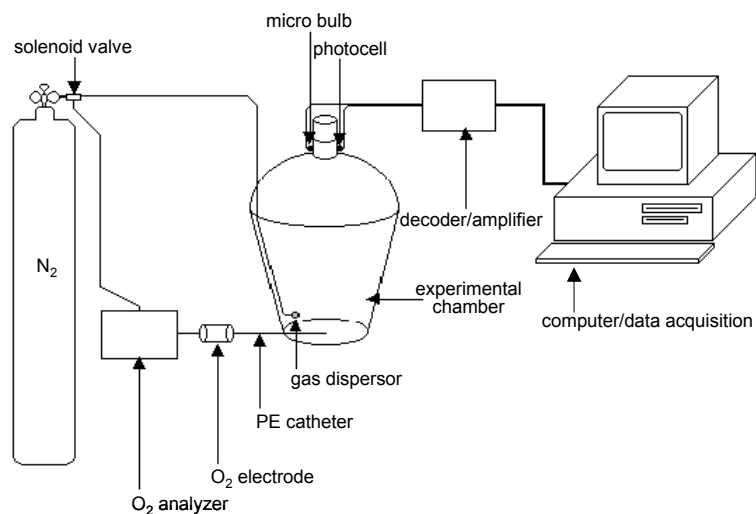


Figure 1. Diagram of the experimental system used to record the air-breathing frequency and air-breathing duration of jeju, *Hoplerythrinus unitaeniatus*, during normóxia and exposure to different levels of hypoxia.

#### *Experimental protocol*

Before experimentation fish were kept overnight period in the experimental chamber. After recording eventual air-breathing episodes during normóxia (140 mmHg), fish were subjected to the following hypoxic tensions: 94, 64, 45, 35, 26 and 17 mmHg for 30 min each tension.

#### *Results*

The air-breathing frequency and air-breathing duration of intact and denervated jeju (IX, G1 and G4), as a function of the O<sub>2</sub> tensions of inspired water (PiO<sub>2</sub>) are presented in figure 2.

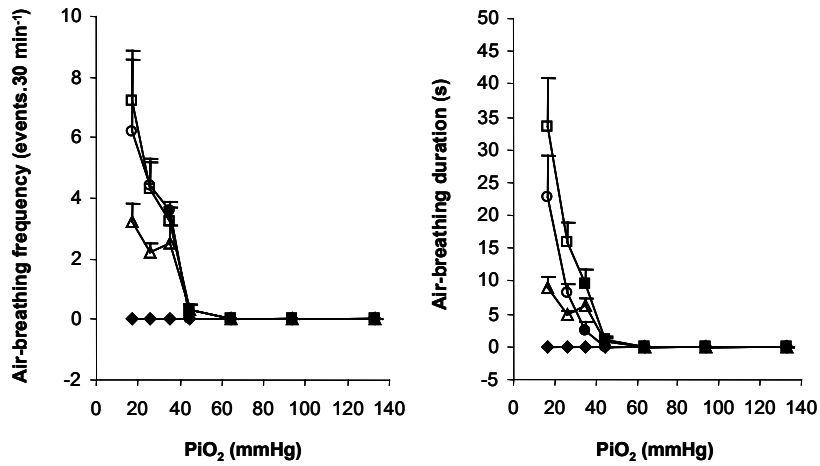


Figure 2. Air-breathing frequency and air-breathing duration of intact (●) and denervated (IX - ▲, G1 - ■, and G4 - ◆) jeju, *Hoplerythrinus unitaeniatus*, as a function of the  $PO_2$  of inspired water ( $PiO_2$ ). Open symbols represent statistical differences in relation to normoxia. Mean  $\pm$  SEM; n = 10.

Denervation of the cranial nerve IX and the pretrematic branch of cranial nerve X serving the first gill arch did not abolish air-breathing in jeju. This response, however, was completely eliminated after complete branchial denervation (cranial nerve IX and all branches of cranial nerve X going to the four gill arches - group G4). This indicates that the control of air-breathing in jeju involves  $O_2$  chemoreceptors distributed on all the gill arches.