

**ACID-BASE RELATED REGULATORY RESPONSES IN THE SOUTH  
AMERICAN LUNGFISH, *Lepidosiren paradoxa* (FITZ.)**

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**Abstract**

The respiratory acid-base regulation of land vertebrates (Tetrapoda) is achieved by means of peripheral and central chemoreceptors. The last evolutionary ramification before terrestrial transition may have been the lungfishes (Dipnoi) that possess real lungs. In contrast to their relatives they never transgressed to terrestrial life. In this context we have evaluated acid-base related responses in *Lepidosiren*. Like Tetrapoda, the *Lepidosiren* modulates pulmonary ventilation within the brainstem region. Moreover, *Lepidosiren* increases ventilation in response to hypercapnia, whereas bicarbonate-related adjustments are of minor importance.

**Introduction**

*Lepidosiren* in South America, *Protopterus* in Africa and *Neoceratodus* (Australia) form the Dipnoi, a sister group of Tetrapoda that include amphibians, reptiles, birds and mammals (Meyer and Dolven, 1992). As a sub-group of Dipnoi, Lepidosirenidae (*Lepidosiren* and *Protopterus*) are obligate air breathers (Johansen et al., 1967). All are equipped with lungs and gills that are much reduced, in particular within Lepidosirenidae (Johansen and Lenfant, 1967). Gill ventilation adjustments cope with variable O<sub>2</sub> demands. The transition to terrestrial life re-directed the respiratory control to play a major role in acid-base regulation (Dejours, 1981; Ultsch, 1996). Obviously, the transition of vertebrates from the aquatic to the terrestrial environment had a large impact on

respiratory conditions (Dejours, 1981; Ultsch, 1996). Evidence exists for the presence of central acid-base chemoreceptors in all classes of land vertebrates (Tetrapoda) *i.e.* Amphibians; Reptiles; Birds; Mammals (Branco et al, 1991). These receptors are stimulated by alterations of H<sup>+</sup> and CO<sub>2</sub> levels of the cerebro-spinal fluid (CSF). The ventilatory control of acid-base status is a typical example of a negative feedback mechanism: Increased PCO<sub>2</sub> and decreased pH stimulate central and peripheral acid-base receptors, which increases pulmonary ventilation. In turn this reduces CO<sub>2</sub> levels, and acid-base status returns to its normal set-point values.

Although central chemoreceptor control seems essential to respiratory acid-base regulation in all the classes of Tetrapoda, data a non-existing for their sister group the Dipnoi. Therefore, we used classical mock CSF perfusion techniques (cf. Schläpke, 1981) in *Lepidosiren*. This involves that the ventricular cerebral system is infused into CSF to stepwise change pH of the perfusate, while ventilation was measured simultaneously.

### **Material and methods**

*Lepidosiren* (1000g ± 0,04) were collected close to Cuiabá, Mato Grosso State, Brazil, and transported to Ribeirão Preto, São Paulo State, where they were kept in 1000 l tanks containing shallow dechlorinated, aerated water at 25 °C and maintained on a diet of chicken liver.

#### *Measurement of pulmonary ventilation.*

Ventilation at 25 °C was measured directly as described by Lomholt and Johansen (1974). The animal was placed into a 10-l chamber, closed at the bottom. This chamber was filled with water to slightly above the cylindrical neck. Expansion of the lungs during inspiration increased the water level within the cylindrical neck of the chamber. Conversely, expiration decreased the water level. These movements were measured as hydrostatic changes. A PE90 catheter with its tip placed at the base of the cylindrical neck was attached to a highly sensitive pressure transducer LPU-0.1, coupled to the polygraph recorder. Injection and withdrawal of known volumes of water provided the relationship between recorded pressure and the corresponding volume change (Lomholt and Johansen, 1974).

#### *Surgical procedures*

The fourth cerebral ventricle was cannulated as described below; the animal was emerged for into a 1g/L solution of benzocain for 10 min (cf. Johansen et al,

1967). Anesthesia was maintained by passing benzocain-containing water through the gills (0.25 g/L) while access to the fourth ventricle was achieved. This involved longitudinal incision at the dorsal half of the skull. Muscles were separated by blunt dissection and the skull was exposed. Using a dental drill a 2-mm hole was drilled to access the fourth ventricle, identified according to Holmgren and Horst (1925). Two soldered and blunted needles were stereotaxically placed with their tips protruding into the fourth ventricle. This assembly served as furnished as an in- and outlet for perfusate. With the needle assembly in place, the hole was sealed with surgical wax and secured to the skull, using dental acrylic.

#### *Preparation of mock CFS*

Solutions for perfusion of the fourth cerebral ventricle were prepared according to the classical procedure by Loeschcke et al (1958) as modified by Jones and Taylor (1984). For details see also Branco et al. (1991) and Hitzig and Jackson (1978). Using a GF 3/MP Gas Mixing Flow Meter (Cameron Instr., Port Arkansas, Texas, USA), the perfusate was equilibrated to 1.5% CO<sub>2</sub> both during preparation and application. Bicarbonate concentrations were adjusted to obtain the pH of each mock CSF (Branco et al., 1991), *i. e.* 7.4, 7.6, 7.8 and 8.0. A gravitation-controlled perfusion rate was kept close to 0.1 ml/min (For details see Hitzig and Jackson, 1978).

#### *Experimental protocol*

The animals were left in an aerated aquarium to recover from surgery for at least 20 h. One day before experimentation; the animal was placed into the chamber containing aerated, dechlorinated water and was left to adjust to the environment. Subsequently, series of perfusions were applied leaving 1 hour for each pH value. Initially the sequences were randomized to assure that the responses were reversible. Then, the standard sequence was chosen to be: pH 7.8, 7.6, 7.4 and 8.0. *Calculations and Statistics*

The data were analyzed using repeated measures ANOVA, followed by the test of Tukey for comparison between individual means. Significance was accepted for  $P < 0.05$ .

## Results

The relationships between CSF pH and ventilatory variables, *i. e.* respiratory frequency, tidal volume and their product - pulmonary ventilation - are shown in figure 1. A reduction of CSF pH from 8.0 to 7.4 caused a more than three-fold increase of ventilation that, subsequently, returned to lower levels when the mock CSF pH was lowered to 8.0. (figure 1). The horizontal lines of the figure indicate control levels of ventilation before onset of mock CSF perfusion procedures.

## Discussion

*Lepidosiren* increased pulmonary ventilation in response to decreases of CSF pH, that were achieved by flow-through infusion of mock CSF via the central cerebral ventricular system. This suggests that central acid-base receptors are present.

The earliest known members of Dipnoi date back to the lower Devonian period about 400 million years ago, when many of the highly distinct morphological and anatomical features of this group were already present (Carroll, 1988). It is, therefore, notable that the ventilatory responses of *Lepidosiren* to central perfusion are similar to those of Tetrapoda.

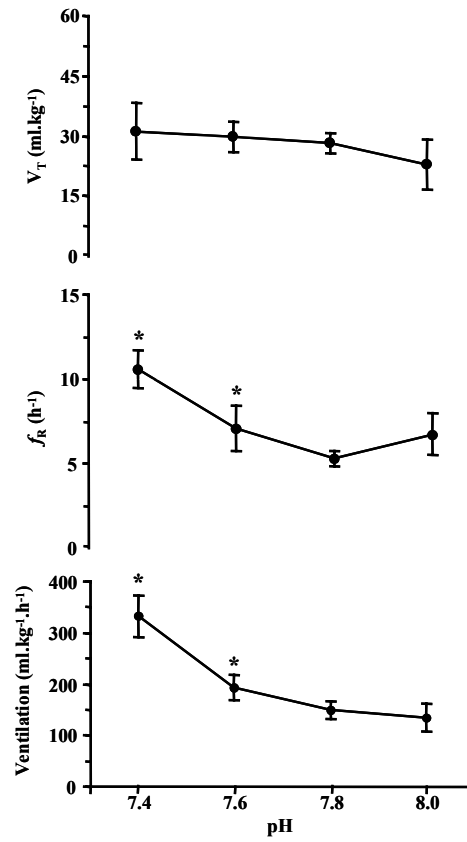


Figure 1. The effects of altered CSF pH on the ventilatory variables of the South American lungfish, *Lepidosiren paradoxa*.  $V_T$  = tidal volume;  $f_R$  = respiratory frequency; Mean  $\pm$  SEM; n = 9. Asterisks indicates  $P < 0.05$  for mean values being identical to baseline values before perfusion.

Recent experiment on chronic hypercapnia document (Sanchez et al. in prep) that *Lepidosiren* is unable of any significant acid-base compensation through bicarbonate exchange. This contrasts with the situation in typical teleost fish (carp), but is very much like the situation in amphibians. Evidently, the presence of lungs has profound consequences for the overall respiratory regulation in *Lepidosiren*.

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