Ionoregulatory and oxidative stress issues associated with the evolution of air-breathing

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ARTICLE INFO

ABSTRACT

Aquatic areas frequently face hypoxic conditions. In order to get sufficient oxygen to support aerobic metabolism, a number of freshwater fish resort to aerial respiration to supplement gill respiration especially in situations with reduced oxygen availability in the water. In many species a concomitant reduction in gill surface area or in gill perfusion reduces possible loss of aerially acquired oxygen to the water at the gills, but it also compromises the ion regulatory capacity of gill tissue. In consequence, the reduced gill contact area with water requires appropriate compensation to maintain ion and acid-base homeostasis, often with important ramifications for other organs. Associated modifications in the structure and function of the gills themselves, the skin, the gut, the kidney, and the physiology of water exchange and ion-linked acid-base regulation are discussed. In air-breathing fish, the gut may gain particular importance for the uptake of ions. In addition, tissues frequently exposed to environmental air encounter much higher oxygen partial pressures than typically observed in fish tissues. Physostomous fish using the swimbladder for aerial respiration, for example, will encounter aerial oxygen partial pressure at the swimbladder epithelium when frequently gulping air in hypoxic water. Hyperoxic conditions or rapid changes in oxygen partial pressures result in an increase in the production of reactive oxygen species (ROS). Accordingly, in air-breathing fish, strategies of ionoregulation may be greatly modified, and the ROS defense capacity of air-exposed tissues is improved.

1. Introduction

In the history of our earth the photosynthesis and the development of photosystem II caused an increase in atmospheric oxygen concentration. This allowed a switch from the previously anaerobic metabolism to aerobic metabolism with a greatly increased the rate of ATP production from carbohydrates. It also opened the door for using fatty acids as a fuel, which cannot be metabolized in the absence of oxygen in eukaryotic organisms. Accordingly, metabolism of heterotrophic organisms became largely dependent on the availability of oxygen. Under conditions of reduced oxygen availability, some organisms or certain tissues may transiently refer to anaerobic metabolism. Hypoxia typically causes a significant reduction in ATP production and results in metabolic depression (St-Pierre et al., 2000; Guppy, 2004; van Ginneken and van den Thillart, 2009; Ali et al., 2012). Coastal areas and certain freshwater systems are prone to transient hypoxic situations (Diaz and Rosenberg, 2008; Diaz and Breitburg, 2009; Jenny et al.,

2016), and in our study we will focus mainly on the freshwater situation.

In aquatic systems, in particular in the Amazon Basin, the oxygen concentration is known to vary greatly, ranging from very low oxygen partial pressures in stagnant water during the night, to clearly hyperoxic conditions at day time when photosynthetic active organisms produce and release oxygen (Val and Almeida-Val, 1995; Muusze et al., 1998; Diaz and Breitburg, 2009; Welker et al., 2013). To supplement oxygen supply during aquatic hypoxia, many fish of the Amazon Basin rely on aquatic surface respiration or even use specific tissues or organs for aerial gas exchange (Val and Almeida-Val, 1995; Graham, 1997). A vascularized stomach or intestine is used for aerial gas exchange by some loricariid and the callichthyid fish. The tambaqui picks up oxygen from the water surface with a largely extended inferior lip under hypoxic conditions, and some species of the erythrinid family of fish use the swimbladder to extract oxygen from the air. Pharyngeal, branchial and mouth diverticula are found among the Electrophoridae and the

Synbranchidae, while the South-American lungfish *Lepidosiren paradoxa* takes most of the oxygen required for aerobic metabolism using its well-developed lung (Bemis et al., 1987; Val and Almeida-Val, 1995; Glass and Rantin, 2009).

In fish, venous blood is returned to a single atrium and, from the ventricle, the blood is pumped to the gills for reoxygenation. Oxygenated blood passes on to the systemic circulation, and then from the various organs returns to the heart. Accordingly, if under hypoxic conditions the gut or the swimbladder, for example, are used as an air-breathing organ, the oxygenated blood is returned to the heart and during subsequent passage through the gills oxygen may be lost again to the hypoxic water. In hypoxic water a loss of oxygen through the gills has indeed been recorded, for example, in spotted gar (*Lepisosteus osseus*) (Smatresk and Cameron, 1982; Smatresk, 1986), and in bowfin (*Amia calva*) (Johansen et al., 1970b; Randall et al., 1981b). A recent study also documented oxygen loss in some individuals (4 out of 11 animals) of the armoured catfish *Hypostomus aff. pyreneusi* at low PO_2 in the water (Scott et al., 2017).

In consequence, in many air-breathing fish a reduction in the surface area of the gills has been detected compared to purely water breathing species, thus reducing the possibility to lose oxygen obtained by aerial respiration during passage of the blood through the gills (Cameron and Wood, 1978; Graham, 1997; Brauner and Val, 2006; Gonzalez et al., 2010; Scott et al., 2017).

Fish gills, however, are multifunctional organs and serve for respiration, ion uptake and exchange, water homeostasis, acid-base regulation, and nitrogenous waste excretion (Laurent and Perry, 1991; Evans et al., 2005). Therefore, a reduction in gill surface area will compromise their capacity for ion exchange and acid-base regulation.

Another problem encountered with the development of a special air-breathing organ is related to oxygen. Because arterial oxygen partial pressure of water breathing fish typically is much lower than aerial oxygen partial pressure (Gilmour and Perry, 1994; Kristensen et al., 2010), oxygen partial pressure and concentration in the air-breathing structures of fish breathing air certainly are higher than in other tissues. Higher concentrations of oxygen may, however, result in the production of reactive oxygen species (ROS). The accumulation of ROS causes oxidative stress (Lushchak, 2014; Sies, 2015) and results in lipid per-oxidation, protein carbonylation, and/or DNA modifications, *i.e.* formation of oxidized bases, in particular 8-oxoguanine (Lushchak, 2011). In consequence, the inordinate accumulation of ROS causes serious tissue damage and is harmful for the whole organism.

The extensive changes in the anatomy and accompanying physiology of the organs involved in respiration, circulation, and excretion, connected to the evolutionary transition from breathing water to breathing air have been extensively studied in air-breathing fishes (Carter and Beadle, 1931; Johansen et al., 1970a, b; Johansen, 1972; Kramer et al., 1978; Dejours, 1981; Randall et al., 1981a; Ultsch, 1996; Graham, 1997; Martin and Bridges, 1999; Evans et al., 1999; Sayer, 2005; Glass and Rantin, 2009; Ishimatsu, 2012; Milsom, 2012; Lefevre et al., 2014; Wright and Turko, 2016). Far less attention has been given to two other issues: (i) the reduced availability of an aqueous environment as a major source of electrolytes for ionoregulation, counter-ions for acid-base regulation, and water for diffusive and osmotic exchanges through the gills, with important ramifications for other organs; (ii) the consequences for oxidative stress resulting from the intimate contact of tissues with a medium (air) which has many-fold higher O_2 concentration, and often a higher PO_2 than either the external water or the internal tissues. The evolution of air-breathing may have occurred independently 38–67 times in fish (Graham, 1997; Graham and Lee, 2004). Our goal here is to highlight overall trends that may illuminate common problems and convergent strategies for overcoming them that were employed in the evolution of air-breathing.

2. Ion and water homeostasis

2.1. Reduced contact with the aqueous environment: consequences for gills and other organs

Very simply, as the gills became smaller, less ventilated with water, in some fish more exposed to air, and less perfused with blood due to bypass shunting, critical branchial functions for ionoregulation, coupled acid-base exchange, and osmoregulation had to be modified and/or shifted to other organs. That organ was clearly not the swimbladder or incipient lung (Zheng et al., 2011) as it is not in contact with external water, ions, or acid-base equivalents.

2.2. Changes in gill ionoregulatory function

General trends seen in the gills of air-breathing fish include reductions in overall gill mass and gill surface area, the latter additionally due to stubbier filaments and lamellae, loss or clumping of lamellae, and infilling between lamellae with the interlamellar cell mass (ILCM), all of which contribute to increased blood-to water diffusion distance (Perna and Fernandes, 1996; Graham, 1997; Brauner et al., 2004a). The actual flow of blood through the lamellae is also likely reduced by bypass shunting, increasing physiological dead space. The net effect of these changes is to reduce O_2 uptake across the gills under normal circumstances, and perhaps more importantly, to minimize O_2 losses to external hypoxic water from venous blood entering the gills when the fish is ventilating the air-breathing organ (ABO) (Johansen et al., 1970a; Randall et al., 1981a, b; Smatresk and Cameron, 1982; Smatresk, 1986; Scott et al., 2017). CO_2 and ammonia excretion are less affected, presumably because of their greater lipid solubility, their potential transport by facilitated diffusion carriers such as Rh proteins (Wright and Wood, 2009; Perry et al., 2010), and the greater capacitance of the water for these respiratory gases. Indeed Janis and Farmer (Janis and Farmer, 1999) noted that no living fish has completely lost its gills, and speculated that this is because the gills remain essential for N-waste and CO_2 excretion. These morphofunctional changes accompanying air-breathing that reduce effective gill contact with the water would be expected to (i) compromise active ion uptake and ion *versus* acid-base exchanges at the gills, and (ii) reduce rates of diffusive ion loss and water exchange.

A useful approach to test these predictions is to compare closely related air-breathing *versus* water-breathing species such as the erythrinids jeju (*Hopleythrinus unitaeniatus*, a facultative air-breather) *versus* traira (*Hoplias malabaricus*, an obligate water breather) (*e.g.* (Cameron and Wood, 1978; Hulbert et al., 1978b; Fernandes et al., 1994; Wood et al., 2016) or the osteoglossids pirarucu (*Arapaima gigas*, an obligate air-breather) *versus* arowana (*Osteoglossum bicirrhosum*, an obligate water-breather) (Hulbert et al., 1978c; Brauner et al., 2004a; Gonzalez et al., 2010). The pirarucu may be particularly instructive as it represents an individual species where increased reliance on air-breathing occurs as development proceeds (Fernandes et al., 2012), such that it transitions from a water-breathing early life stage to an obligate air-breathing adult stage where the lamellae have been completely obliterated externally by formation of an ILCM, and greatly reduced internally by atrophy of the pillar cell channels (Ramos et al., 2013).

With respect to baseline ion fluxes under normoxic conditions, these predictions are only partially supported by the limited physiological data available. For example, branchial unidirectional Na^+ uptake and efflux rates in normoxic water were actually higher in the jeju (a facultative air-breather) than in the water-breathing traira, despite a 50% lower gill area in the former (Cameron and Wood, 1978). However, after a period of fasting, the traira was able to maintain positive net Na^+ and Cl^- balance with water (Fig. 1B), whereas the jeju could not (Fig. 1A), and plasma ions in the jeju were lower (Wood et al., 2016). The explanation for the higher unidirectional fluxes in the jeju may be