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Air-breathing and excretory nitrogen metabolism in fishes

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ABSTRACT

During water-land transition, ancient fishes acquired the ability to breathe air, but air-breathing engendered problems in nitrogenous waste excretion. Nitrogen is a fundamental component of amino acids, proteins, and nucleic acids, and the degradation of these nitrogen-containing compounds releases ammonia. Ammonia is toxic and must be removed. Fishes in water excrete ammonia as the major nitrogenous waste through gills, but gills of air-breathing fishes are modified for air-breathing or largely replaced by air-breathing organs. Notably, fishes emerged from water can no longer excrete ammonia effectively because of a lack of water to flush the gills. Hence, ancient fishes that participated in water-land transition must have developed means to deal with ammonia toxicity. Extant air-breathing fishes, particularly amphibious ones, can serve as models to examine adaptations which might have facilitated the emergence of ancient fishes from water. Some of these fishes can actively emerge from water and display complex behaviors on land, while a few can burrow into mud and survive for years during drought. Many of them are equipped with mechanisms to ameliorate ammonia toxicity during emersion. In this review, the mechanisms adopted by air-breathing fishes to deal with ammonia toxicity during emersion were organized into seven disparate strategies. In addition, eight extant air-breathing fishes with distinctive terrestrial behaviors and peculiar natural habitats were selected to describe in detail how these seven strategies could be adopted in disparate combinations to ameliorate ammonia toxicity during emersion.

1. Introduction

The invasion of land by vertebrateswas a salient event of vertebrate evolution. Water-land transition necessitated many important physiological and biochemical adaptations to the terrestrial environment. Particularly, there were profound changes in the respiratory organs, from gills to lungs, to facilitate air-breathing. These adaptations facilitated the migration of fishes to land, leading to the evolution of tetrapods. During water-land transition, fishes had to deal with problems concerning metabolism and excretion of nitrogenous compounds. Nitrogen is a crucial element in biological systems; it is a fundamental component of amino acids, proteins, and nucleic acids. The catabolism of these nitrogen-containing compounds releases ammonia which is toxic and must be removed. Most aquatic animals excrete ammonia as the major nitrogenous waste in water, but fishes emerged from water cannot effectively excrete ammonia due to a lack of water to flush the body surfaces. Hence, ancient fishes involved in water-land transition must have developed ways to defend against ammonia toxicity. Among modern fishes, there are plenty of air-breathing examples, and some of them are amphibious in nature. They have developed mechanisms and strategies to deal with ammonia toxicity during emersion, and therefore can serve as models to examine adaptations which might have facilitated the invasion of the terrestrial habitat by ancient fishes.

2. Ammonia production and excretion in aquatic fishes

Animals digest dietary protein to amino acids. The quantity of amino acids exceeding what is needed for growth and development is degraded because animals cannot store excess amino acids. The majority of amino acids are catabolized in the liver (Campbell, 1991), while some can be broken down in the intestine (Karlsson et al., 2006; Tng et al., 2008). In fishes, a major portion (40–60%) of the dietary nitrogen intake is excreted as nitrogenous wastes within 24 h (Lim et al., 2004; Ip et al., 2004c). During fasting, muscle proteins can be hydrolyzed to release amino acid for ATP or carbohydrate production (Houlihan et al., 1995).

In fishes, amino acid catabolism occurs mainly in the liver, and the breakdown of amino acids releases ammonia (See Ballantyne, 2001 for a review). In aqueous solution, ammonia is present in two forms, the molecular NH_3 and the cationic NH_4^+, and the pK of the related equilibrium reaction ($NH_3 + H_3O^+ \Leftrightarrow NH_4^+ + H_2O$) is ~9.5. In liver cells, several amino acids can be catabolized by specific deaminases

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(histidase, asparaginase, serine dehydratase and threonine dehydratase) with the release of NH_3 in the cytosol (Youngson et al., 1982). Nonetheless, ammonia is generally released as NH_4^+ from the α-amino group of various amino acids through transdeamination, which requires the combined actions of cytosolic aminotransferases and mitochondrial glutamate dehydrogenase, in hepatocytes (Walton and Cowey, 1977; French et al., 1981; Ballantyne, 2001). The deamination of glutamate by glutamate dehydrogenase produces NH_4^+ inside the mitochondrial matrix (Campbell et al., 1983). The mitochondrial matrix of some fishes also have glutaminase, which releases NH_3 from the amide-N of glutamine (Ballantyne, 2001). Ammonia produced in the mitochondrial matrix of hepatocytes enters the blood after permeating the mitochondrial and plasma membranes.

In plasma, ammonia is present mainly (> 95%) as NH_4^+ because the plasma pH (~7.4) is approximately 1–2 units below the p*K* of ammonia. Ammonia is circulated through plasma to other parts of the body. As ammonia has multiple deleterious effects on many cellular processes (see below), it must be excreted to prevent accumulation (Ip et al., 2001b; Chew et al., 2006b; Ip and Chew, 2010b). Fully aquatic fishes are predominantly ammonotelic in water as they excrete > 50% of the nitrogenous waste as ammonia. The primary organ of ammonia excretion in fishes is the gill (Evans and Cameron, 1986; Wilkie, 1997, 2002; Weihrauch et al., 2009), which has large surface area, extensive perfusion by blood, large ventilation rates, small diffusion distances, and close contact with a voluminous external medium (Evans et al., 2005). Ammonia is largely excreted as NH_3 across the branchial epithelium down a favorable blood-to-water PNH_3 gradient (Wilkie, 1997, 2002; Evans et al., 2005), with or without the participation of transporters/channels (Weihrauch et al., 2009; Wright and Wood, 2009). Rhesus glycoproteins (Rhag, Rhbg and Rhcg) are crucial NH_3 (and NH_4^+) channels in ammonotelic fishes (Nawata et al., 2007, 2010; Nakada et al., 2007a,b; Hung et al., 2007, 2008; Braun et al., 2009). At the gill, erythrocytic Rhag can facilitate NH_3 efflux from erythrocytes into plasma. NH_3 can then permeate the basolateral membrane of the branchial epithelial cells through Rhbg, and exit the apical membrane through a Na^+/NH_4^+ exchange complex. This exchange complex consists of Rhcg, Na^+/H^+ exchangers, vesicular-type H^+-ATPase, and Na^+ channel, which act concertedly to provide an acid trapping mechanism to enhance apical NH_3 excretion (Wright and Wood, 2009). In fact, the outwardly diffusive movement of NH_3 is dependent on acid trapping in the boundary-layer of water by H^+ released through the non-catalyzed or catalyzed (by carbonic anhydrase) hydration of metabolic CO_2 (Wright and Wood, 2009). With acid trapping, NH_3 excreted across the branchial epithelium is converted to NH_4^+ and stays in the external medium. There are indications that Aquaporin 1aa can also act as an ammonia channel in the gill of climbing perch (Ip et al., 2013b).

3. Constraints imposed by air-breathing on nitrogenous waste excretion in fishes

Some fishes adopt air-breathing as an adaptive response to live in aquatic habitats where dissolved PO_2 is low (Graham, 1997). Many of them have degenerate gills compensated with accessory breathing organs (Graham, 1997). The gill morphology and morphometry of these fishes are modified (Low et al., 1988, 1990; Graham, 1997) in pursuance of reducing the loss of O_2 taken up by the air-breathing organs to the hypoxic water. However, these modifications may also interfere with ammonia excretion in water, as degenerate gills impede branchial ammonia excretion. With accessory breathing organs and degenerate gills, many air-breathing fishes practice bimodal breathing during immersion. When they hold air in the buccal cavity without ventilation under water, the rate of branchial ammonia excretion would be drastically reduced. With air-breathing capability, some air-breathing fishes can survive passively in air during short periods of emersion, while others acquire the ability to actively emerge from water and make excursion onto land. A few of them can even burrow into mud to avoid desiccation as the external media dry up. When out of water, air-breathing fishes are confronted with problems of ammonia intoxication. The lack of water to flush the branchial and cutaneous surfaces leads to a build-up of excreted ammonia in the external unstirred-layer of water and disrupts the normally outwardly directed PNH_3 gradient. This would hinder ammonia excretion, leading to the accumulation of endogenous ammonia in the body.

4. Deleterious effects of ammonia

Ammonia is toxic to fishes because of multiply reasons. At the organismal level, it causes hyperventilation, hyper-excitability, coma, convulsions and, eventually, death of the fish (Hillaby and Randall, 1979; McKenzie et al., 1993). At the branchial level, $NH4^+$ can affect certain transporters and hinder ionoregulation by replacing K^+, and interfere with the operation of Na^+/K^+ -ATPase and $Na^+{:}K^+{:}2Cl^-$ co-transporter (Wilkie, 1997; Person Le Ruyet et al., 1997). It can also replace H^+ in Na^+/H^+ exchanger and affect acid-base balance (Randall et al., 1999). At the cellular level, ammonia can interfere with energy metabolism, as it can impair the tricarboxylic acid cycle (Arillo et al., 1981), by inhibiting pyruvate dehydrogenase, isocitrate dehydrogenase, or α-ketoglutarate dehydrogenase (Cooper and Plum, 1987). Ammonia can also activate phosphofructokinase I and stimulate glycolysis in fishes (Kloppick et al., 1967). In the central nervous system, ammonia asserts its acute effects through the activation of certain channels or transporters (Binstock and Lecar, 1969) and disruption of electrochemical gradients (Cooper and Plum, 1987). For instance, NH_4^+ can substitute for K^+ to activate the background K^+ channel and affect the resting membrane potential (Binstock and Lecar, 1969).

In mammalian brain, acute ammonia toxicity is attributable to glutamatergic dysfunction, glutamine accumulation leading to astrocyte swelling, and activation of *N*-methyl-D-aspartate (NMDA) receptors (Brusilow, 2002; Felipo and Butterworth, 2002; Rose, 2002; Albrecht and Norenberg, 2006; Albrecht et al., 2010). The excessive activation of NMDA receptors in response to ammonia (Hermenegildo et al., 1996; Kosenko et al., 1999) can lead to oxidative stress, neuronal degeneration, and death of neurons (Miñana et al., 1996). Nitrosative/oxidative stress and mitochondrial permeability transition also contribute to ammonia neurotoxicity (Reddy et al., 2009; Bemeur et al., 2010; Görg et al., 2010; Häussinger and Görg, 2010; Görg et al., 2013). As mitochondrial permeability transition involves the opening of a pore in the inner mitochondrial membrane, it would lead to the collapse of ionic gradients resulting in mitochondrial dysfunction. It can also enhance the entry of cytosolic glutamine into the mitochondrion, and cause an increase in the production of ammonia through the glutaminase reaction in the mitochondrial matrix of astrocytes (Albrecht and Norenberg, 2006). Although ammonia may also cause oxidative stress in fish brains (mudskipper; Ching et al., 2009), many of these mechanisms proposed for ammonia toxicity in mammalian brains have not been confirmed in fishes, and air-breathing fishes apparently have higher brain ammonia tolerance than mammals (Wee et al., 2007; Tng et al., 2009; Ip and Chew, 2010b; Ip et al., 2005a, 2013a).

5. Strategies adopted by air-breathing fishes to defend against ammonia toxicity during emersion

Many extant air-breathing fishes have high tolerance for both emersion (in air) and environmental ammonia in water, but the strategies utilized to defend against ammonia toxicity under these two different conditions are not exactly the same (see Ip et al., 2001b, 2004a,b; Chew et al., 2006b; Ip and Chew, 2010b; Chew and Ip, 2014 for reviews). For instance, some of the strategies adopted to deal with environmental ammonia (e.g. lowering environmental pH and reducing NH_3 permeability of the skin) may not be applicable to fishes emerged from water. Specifically for emersion, extant air-breathing fishes