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Gill remodeling in three freshwater teleosts in response to high environmental ammonia

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ABSTRACT

The present study aimed to determine whether gill macro- and microstructure show compensatory responses in three freshwater fish differing in their sensitivity to high environmental ammonia (HEA). The highly ammonia-sensitive salmonid *Oncorhynchus mykiss* (rainbow trout), the less ammonia-sensitive cyprinid *Cyprinus carpio* (common carp) and the highly ammonia-resistant cyprinid *Carassius auratus* (goldfish) were used as test species and were exposed for 0 h (control), 3 h, 12 h, 24 h, 48 h, 84 h and 180 h to 1 mM ammonia (as NH_4HCO_3; pH 7.9). In cyprinids, dramatic alterations were initiated quickly evident by thickening of filaments and lamellae, retraction of lamellae, enlargement of interlamellar cell mass (ILCM), and increase in the water–blood diffusion distance; while in trout, these modifications were absent or developed very slowly. These reorganizations may attempt to reduce the surface area presumably protecting against the water borne ammonia; and were more pronounced in goldfish marked by momentous enlargement of ILCM volume and the presence of rudimental and almost fused lamellae. Extensive mucus production in the gills of goldfish and carp and to a limited extent in trout may have been part of general stress response and/or may have played a protective role. While goldfish and carp showed shrinkage of apical crypts of mitochondrion rich cells (MRCs), probably aiding to regulate ion status, trout showed enlarged apical crypts of MRCs. All species displayed changes in the pattern of the microridges on the surface of pavement cells (PVCs). Overall, the present results connote that the goldfish with its minimal respiratory surface area and a large population of the MRCs with small apical crypts located on the edge of ILCM is better prepared for survival in ammonia polluted water compared to carp which maintain larger lamellae and especially the trout that did not show gill remodeling.

1. Introduction

Fish gills are the primary site for gas exchange, ion regulation, acid–base balance and nitrogenous waste excretion (Evans et al., 2005). In teleosts, each branchial arch supports multiple filaments, which bear numerous lamellae that are the main sites of gas exchange. The epithelium covering the gill filaments and lamellae provides a distinct boundary between a fish's external environment and extracellular fluids and allows the branchial tissue to be exposed to variations of the external milieu. The multilayered filament epithelium is thick and stratified. Its outermost layers are composed of pavement cells (PVCs), mitochondrion rich cells (MRCs), accessory cells (ACs; only in marine fish), mucous cells (MCs), and underlined by a network of immature and undifferentiated cells. Lamellar epithelium is bi-layered and composed of PVCs and undifferentiated cells. PVCs are the most abundant cell type covering the epithelium (>90% of the surface area) also referred to as respiratory cells. They are multifunctional and considered as key units for gas exchange, and cells are involved in acid–base transport and Cl^- uptake in freshwater fish and as possible contributors to the gill mucus production (Evans et al., 2005; Marshall, 2002; Wilson and Laurent, 2002). PVCs have few mitochondria, well-developed rough endoplasmic reticulum and a Golgi apparatus producing vesicles filled with glycoproteins that may fuse with the apical cell membrane and release their content on the cell surface mixing it with mucous layer (Laurent and Perry, 1991; Wilson and Laurent, 2002). Highly specialized MCs, the major provider of mucus in the fish gills, produce mucous granules containing glycoproteins, mucopolysaccharides and carbohydrates. When these granules are discharged and rupture, their content forms a mucous layer onto the epithelial surface. Mucous substance may play an important role in ion and water balance, and excessive mucus production has been proposed as a part of the

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stress response in fish (Shephard, 1994; Wendelaar Bonga, 1997). Although MRCs represent approximately 8% of gill epithelium cells, they are considered as the primary sites of active physiological processes in the gills and play a central role in ion uptake in fresh water fish (Evans et al., 2005; Kaneko et al., 2008; Laurent and Perry, 1990; Marshall, 2002; Perry, 1997; Perry et al., 1992). These cells are found interspersed with the PVCs, especially in the interlamellar epithelium and mostly on the trailing edge of the filament (Heijden et al., 1997; Laurent, 1984). MRCs are characterized by a relatively high metabolic activity compared to PVCs (Perry and Walsh, 1989). They have numerous mitochondria and an extensive tubular system emanating from the basolateral membrane and associated with the localization of Na^+/K^+-ATPase, major transporter enzyme in osmoregulation (Karnaky et al., 1976a,b). MRCs open on the epithelial surface with apical crypts of different size and shape (Heijden et al., 1997; Laurent, 1984; Wilson and Laurent, 2002). These crypts contain some mucus that forms a microenvironment rich in cations that may facilitate ion exchange and limit water influx (Handy et al., 1980; Shephard, 1994). In response to a variety of environmental stressors, freshwater fish can alter the number and distribution of MRCs in gill epithelium (Goss et al., 1995; Laurent and Perry, 1991).

Recently, a number of studies have shown that in response to stressful environments, some teleosts have the ability to modify their gill morphology through plasticity of the branchial components (Brauner et al., 2004; Ong et al., 2007; Sollid et al., 2003) and can be greatly altered within hours to days (Sollid and Nilsson, 2006; van der Meer et al., 2005). This ability of some fish species to undergo modifications in their gill morphology in an attempt to attain homeostasis is often termed as 'gill remodeling'. The morphological changes in the gills and the changes in specialized cells in the gill epithelia associated with the gill remodeling have been well documented in response to hypoxia in Crucian carp (*Carassius carassius*) (Nilsson, 2007; Sollid et al., 2003, 2005; Sollid and Nilsson, 2006), goldfish (*Carassius auratus*) (Mitrovic and Perry, 2009; Mitrovic et al., 2009; Sollid et al., 2005), Amazonian oscar (*Astronotus ocellatus*) (Matey et al., 2011), rainbow trout (*Oncorhynchus mykiss*) (Matey et al., 2011) and scaleless carp (*Gymnocypris przewalskii*) (Matey et al., 2008). It was seen that under normoxic conditions, in order to prevent diffusive ion loss to the water, the crucian carp maintains a cell mass enveloping the gill lamellae, deemed the interlamellar cell mass (ILCM) (Sollid and Nilsson, 2006; Sollid et al., 2003). However, during hypoxia, fish appears to 'grow lamellae' by gradually losing the ILCM through a combination of a lowered rate of mitosis and heightened rate of apoptosis (Sollid and Nilsson, 2006), thereby increasing surface area by many folds and effectively increases the gas exchange (Sollid et al., 2003). High temperature and salinity changes seem to induce similar effects (Brown, 1992; Cioni et al., 1991; Franklin, 1990; Kultz et al., 1995; Pisam et al., 1988; Rissanen et al., 2006; Sollid et al., 2005). Metal exposure is also reported to induce morphological changes in the gills, including reversible thickening of the epithelium. Thickening leads to an increase in the diffusion distances between ambient water and gill vascular system, thus repress the rate of toxicant influx (De Boeck et al., 2007; Lappivaara et al., 1995). Thickening of gill epithelium also tends to reduce the respiratory surface area which might be a protective response since smaller respiratory surface makes fish less accessible for toxic substances (ammonia, for example). Though a reduced respiratory surface area undoubtedly limit gas exchange, it benefits by reducing the costs of osmo-regulation by lowering the amount of ions that has to be replenished. Thus, gill morphology is likely to be a compromise between opposing demands and fish often experience an osmo-respiratory compromise, especially under stressful conditions (Nilsson, 2007; Randall et al., 1972).

Despite of extensive arrays of studies on gill morphological changes in response to a number of adverse environments, very limited information is available (e.g. Cardoso et al., 1996; Kirk and Lewis, 1993; Lang et al., 1987; Lease et al., 2003; Smart, 1976) on this aspect when fish are subjected to waterborne ammonia. The build-up of high environmental ammonia (HEA) is a serious threat for aquatic animals, including fish. Numerous studies on different fish species concerning acute and chronic ammonia toxicity already exist, but possible adaptive morphological modifications of fish gill that respond to HEA are not yet fully understood. We postulated that the ability to undergo gill remodeling in response to HEA may have a protective effect and will vary among fish species which have different tolerance limits to ammonia toxicity. In our previous experiments (Liew et al., 2013; Sinha et al., 2012a,b, 2013a,b, 2014) we revealed differential physiological, metabolic, biochemical and molecular compensatory mechanism in trout, common crap and goldfish determining their sensitivity to HEA; however, a morphological approach is still lacking. Therefore, a closer examination into the changes at gill morphology and the associated cellular components will offer a better insight in determining the reasons for ammonia sensitivity. The present investigation paralleling our previous studies (Liew et al., 2013; Sinha et al., 2013a,b, 2014), was performed to provide a comprehensive investigation of the macro- and ultra-structural responses of the gills of three commercially important freshwater fish differing in their sensitivities to ammonia: a sensitive salmonid, the rainbow trout *O. mykiss*, a less sensitive cyprinid, the common carp, *Cyprinus carpio*, and a very resistant cyprinid, the goldfish, *C. auratus*, when exposed acutely (3 h) and chronically (up to 180 h) to high environmental ammonia (1 mM; pH 7.9). The reported ammonia 96 h LC_{50} values (expressed as total ammonia) for goldfish, common carp and rainbow trout juveniles are ~4.2 mM (pH 8.0), 2.6 mM (pH 7.5–7.8) and 1.7 mM (pH 8.0) respectively (Dowden and Bennett, 1965; Hasan and MacIntosh, 1986; Thurston et al., 1981; Schenone et al., 1982; Wilkie et al., 2011).

In brief, the aim of the present study was to determine, using light microscopy (LM) and more extensive scanning electron microscope (SEM) (i) the morphological and morphometric alterations in gills including the occurrence of ILCM and (ii) and modification of the surface ultrastructure of pavement, mitochondrion-rich and mucous cells comprising the gill epithelium. Overall, we hypothesize a differential gill remodeling response among these three experimental fish species during ammonia threat.

2. Materials and methods

2.1. Experimental system and animals

Rainbow trout, *O. mykiss*, were obtained from a fish farm – Pisciculture Collette, Bonlez, Belgium; goldfish, *C. auratus*, were obtained from Aqua Hobby, Heist op den Berg, Belgium; common carp, *C. carpio*, were obtained from the fish hatchery at Wageningen University, The Netherlands. Fish were kept at the University of Antwerp in aquaria (200 L) for at least a month before the exposure started. Thereafter, a total of 96 goldfish, 96 common carp and 96 rainbow trout were distributed species wise into four 200 L tanks ($n = 24$ per tank). Average mass (mean ± standard deviation) of rainbow trout was 15 ± 2 g (4–4.5 months old), of common carp 18 ± 3 g (6–6.5 months old), and of goldfish 17 ± 3 g (6–7 months old). Each of the tanks was equipped with a recirculating water supply in a climate chamber where temperature was adjusted at 17 ± 1 °C and photoperiod was 12 h light and 12 h dark. Water quality was ensured through an additional bio-filter containing wadding, activated charcoal and lava stones. Water parameters were: pH 7.4 ± 0.2, dissolved oxygen 6.9–7.4 mg/L, total ammonia 0.006–0.009 mM, nitrite 0.0015–0.0021 mM, nitrate 0.015–0.042 mM, Ca^{2+} 0.8–1.0 mM, Mg^{2+} 0.19–0.21 mM, Na^+