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Effects of chronic ammonia exposure on ammonia metabolism and excretion in marine medaka *Oryzias melastigma*

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ABSTRACT

Ammonia is highly toxic to aquatic organisms, but whether ammonia excretion or ammonia metabolism to less toxic compounds is the major strategy for detoxification in marine fish against chronic ammonia exposure is unclear to date. In this study, we investigated the metabolism and excretion of ammonia in marine medaka *Oryzias melastigma* during chronic ammonia exposure. The fish were exposed to 0, 0.1, 0.3, 0.6, and 1.1 mmol l^{-1} NH_4Cl spiked seawater for 8 weeks. Exposure of 0.3–1.1 mmol l^{-1} NH_4Cl had deleterious effects on the fish, including significant reductions in growth, feed intake, and total protein content. However, the fish could take strategies to detoxify ammonia. The tissue ammonia (T_{Amm}) in the 0.3–1.1 mmol l^{-1} NH_4Cl treatments was significantly higher than those in the 0 and 0.1 mmol l^{-1} NH_4Cl treatments after 2 weeks of exposure, but it recovered with prolonged exposure time, ultimately reaching the control level after 8 weeks. The amino acid catabolic rate decreased to reduce the gross ammonia production with the increasing ambient ammonia concentration. The concentrations of most metabolites remained constant in the 0–0.6 mmol l^{-1} NH_4Cl treatments, whereas 5 amino acids and 3 energy metabolism-related metabolites decreased in the 1.1 mmol l^{-1} NH_4Cl treatment. J_{Amm} steadily increased in ambient ammonia from 0 to 0.6 mmol l^{-1} and slightly decreased when the ambient ammonia concentration increased to 1.1 mmol l^{-1}. Overall, marine medaka cope with sublethal ammonia environment by regulating the tissue T_{Amm} via reducing the ammonia production and increasing ammonia excretion.

1. Introduction

There are two forms of ammonia in water, unionized (NH_3) and ionized (NH_4^+). Since the late 20th century, the ammonia concentration in natural waters has been widely elevated by an influx of ammonia from multiple anthropogenic activities, including the discharge of sewage effluent and industrial waste, and the overuse of chemical fertilizer in agriculture [6]. Additionally, the ammonia concentration can be elevated in intensive fish farming operations due to fish excretion, biological degradation of uneaten feed, and restricted water flow. For instance [5], reported that the concentration of ammonia reached 46 mg l^{-1} in an intensive breeding aquaculture system. Excessive ammonia in water is often a threat to fish health, although ammonia is the major end product of protein catabolism (>50%) in teleost fish [10]. High environmental ammonia (HEA) could lead to reduction of growth rate, physical stamina [9], disruption of the ionic balance [36], increased vulnerability to disease [1], histopathological changes in gill epithelia [4], and oxidative damage [35] in fish.

Acute ammonia exposure usually greatly influences ammonia metabolism and excretion in many fish species [23,48,55]. Acute ammonia exposure leads to an increase in the total tissue/plasma ammonia concentration upon initial exposure, which subsequently plateaus or decreases in later exposure. For example, the plasma T_{Amm} significantly increased from about 80 to 200 μmol l^{-1} after 4 h

Abbreviations: HEA, high environment ammonia; SGR, specific growth rate; IR, feed intake rate; T_{Amm}, total ammonia ($NH_4^+ + NH_3$); T_{Urea}, total urea; MO_2, oxygen consumption rate; J_{Amm}, ammonia excretion rate; J_{Urea}, urea excretion rate; GSase, glutamine synthetase.

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and then slightly increased to about 250 μmol l^{-1} at 12 h - 24 h of exposure for seawater acclimated rainbow trout (*Oncorhynchus mykiss*) exposed to 1000 μmol l^{-1} NH_4HCO_3 [47]. The plasma T_{Amm} of increased 100-fold to 5000 μmol l^{-1} at 24 h and recovered to control level after 24–48 h in Pacific hagfish (*Eptatretus stoutii*) exposed to 20 mmol l^{-1} ammonia [8]. Many fish have developed various defense strategies against acute HEA. Firstly, converting ammonia into less toxic nitrogenous compounds such as glutamine and urea is an important detoxification strategy against acute ammonia exposure [3,37,44] and chronic ammonia exposure [15]. For example, glutamine synthetase (GSase) that catalyzes ammonia and glutamate to glutamine works in many tissues including brain, liver, intestine, and muscle to detoxify ammonia in rainbow trout [3]. The high ammonia tolerance of gulf toadfish was dependent on its ability to convert ammonia to urea [44]. Secondly, increasing ammonia excretion is crucial for defending against both endogenous and exogenous ammonia [8,20]. Lastly, decreasing endogenous ammonia production is another important strategy to detoxify ammonia [17]. By this strategy, muderskippers could detoxify endogenous ammonia under aerial exposure [24]. To date, most of these studies have been conducted in freshwater fish at acute exposure. However, it is not clear which strategy plays the major role during chronic ammonia exposure (>1 month) in marine fish.

In realistic situations, fish are usually chronically subjected to HEA at sublethal ammonia levels in natural or agricultural waters. The findings obtained from acute ammonia exposure studies have yielded limited information on chronic ammonia exposure. It is therefore more environmentally relevant to examine the toxic effects and ammonia metabolism in chronic ammonia exposure. Moreover, marine fish could have different strategies from those of freshwater fish when subjected to HEA [47,54]. For example, rainbow trout acclimated to seawater had faster recovery of ammonia excretion than that acclimated to freshwater after being exposed to 1000 μmol l^{-1} ammonia for 24 h [47]. It is therefore important to study the toxicity and detoxification mechanisms of marine fish under chronic ammonia exposure.

Recently, the marine medaka (*Oryzias melastigma*) has been strongly proposed as a new model fish for marine ecotoxicological research [19]. Yet few studies have investigated the ecotoxicology of ammonia in this species. According to the above studies, we hypothesize that the metabolism and excretion of ammonia are the strategies in the marine fish to counteract chronic HEA. The objectives of the present study were therefore to examine the chronic toxicity, metabolism and excretion of ammonia in marine medaka. Specifically, the sublethal ammonia toxicity was evaluated through the SGR, IR, total protein, and MO_2 of the fish. Additionally, ammonia metabolism (tissue T_{Amm}, amino acid catabolism, metabolites content, GSase activity) and nitrogen excretion (J_{Amm} and J_{Urea}) were determined in order to demonstrate the ammonia detoxification mechanisms.

2. Materials and methods

2.1. Laboratory-reared fish

Marine medaka had been raised in our laboratory since 2012 (more than five generations). They were maintained in aerated seawater (composed of recrystallized sea salt; Landebao Co., China) at a salinity of 30 ± 1 ppt (mean ± SD), pH of 8.0 ± 0.1, temperature of 25 °C ± 1, and photoperiod of 12 h light:12 h dark. Marine medaka in 0.04 ± 0.004 g wet weight, (1.67 ± 0.12 cm in length, 6-months old) were selected for experiments and fed every day (3%–5% ration relative to body mass) with a ground commercial diet (crude protein, >= 44%; crude ash, <=15%; crude fiber, <= 5%; moisture, <=12%; total phosphorus, 0.5%–3%; calcium, 0.5%–3%; NaCl, 0.3%–3%; lysine, <=2%, Foshan Shunde Fenghua Feedstuff Industry Co., Ltd., Foshan, China). All procedures were approved by the Animal Research Ethics Board of the Chinese Academy of Sciences and were in accordance with the Guidelines of the Chinese Council on Laboratory.

2.2. Ammonia exposure

Ten glass tanks (50 × 29 × 29 cm) with 15 L seawater were prepared as two replicates for five ammonia exposure treatment. Two hundred fish were transferred into each tank and exposed to different concentrations of NH_4Cl for 8 weeks. A 4 mol l^{-1} NH_4Cl (CNW; Shanghai, China) stock (adjusted to pH 8.0 with NaOH) was used to prepare solutions with five concentrations of ambient ammonia, nominal 0, 0.1, 0.3, 0.6 and 1.1 mmol l^{-1} (actual concentrations: 0.02 ± 0.01, 0.10 ± 0.01, 0.31 ± 0.01, 0.61 ± 0.03 and 1.11 ± 0.02 mmol l^{-1} respectively, sampled before and after renewing water everyday and measured by the indophenol blue method [18]). The pH of the water was also checked every day throughout the experiment using a pH electrode (Ohaus, Starter 3C). The water pH could maintain at a constant value of 8.0 ± 0.1 for the buffer system of CO_2/HCO_3^- in the seawater. The 96-h LC50 of marine medaka was approximately 2.6 mmol l^{-1} NH_4Cl based on our preliminary 96-h lethal concentration test. A concentration of 1.1 mmol l^{-1} NH_4Cl, calculated as 40% of the 96-h LC50 [32], was chosen as the chronic LC50 of NH_4Cl. A concentration of 0.1 mmol l^{-1} NH_4Cl is an environmentally relevant concentration that occurs frequently in freshwater and marine environments [25]. The fish fed once daily and uneaten pellets were collected 1 h after feeding. Half of the seawater was renewed daily and an extra amount of NH_4Cl was added to maintain the ammonia concentration during the exposure.

2.3. Specific growth rate, feed intake and oxygen consumption rate measurements

To determine the specific growth rate (SGR), fifteen fish from each treatment were randomly selected at the start of the exposure and reared in parallel. Each fish was weighed at the start and end of the exposure, and the SGR was calculated as follows:

$$SGR = [\ln W_f - \ln W_i] / \text{days} \times 100 \quad (1)$$

where W_i and W_f are the initial and final mean body weight during the experimental period, respectively.

After 8 weeks of exposure, twenty-five fish were randomly selected from each treatment to measure the feed intake rate (IR). The fish in each treatment were held in a glass tank (30 × 19 × 16 cm) containing 2 L water with corresponding HEA concentration for 7 days. They were fed every day with weighed pellets, and the uneaten pellets were collected 1 h after the feeding, dried and re-weighed. The fish in each treatment were weighed, and the IR, (body weight day^{-1}) was measured daily during a 7-day experimental period.

To determine the MO_2, three replicates of fifteen fasted fish (24 h of fast) were selected randomly from each treatment after eight weeks of ammonia exposure. The fifteen fish were batch-weighed and placed into a plastic box (700 ml) that could maintain a good seal, with the probe of the oxygen meter inserted into the box. A magnetic stirring rotor was placed into the box, and a perforated metal sheet was used to separate the magnetic stirring rotor from the above water column in the box. During measurement of the oxygen consumption rate, the box was kept on the magnetic stirrers (RCT basic, IKA) with the magnetic stirring rotor