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Dietary sodium protects fish against copper-induced olfactory impairment

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ABSTRACT

Exposure to low concentrations of copper impairs olfaction in fish. To determine the transcriptional changes in the olfactory epithelium induced by copper exposure, wild yellow perch (*Perca flavescens*) were exposed to 20 μg/L of copper for 3 and 24 h. A novel yellow perch microarray with 1000 candidate genes was used to measure differential gene transcription in the olfactory epithelium. While three hours of exposure to copper changed the transcription of only one gene, the transcriptions of 70 genes were changed after 24 h of exposure to copper. Real-time PCR was utilized to determine the effect of exposure duration on two specific genes of interest, two sub-units of Na/K-ATPase. At 24 and 48 h, Na/K-ATPase transcription was down-regulated by copper at olfactory rosettes. As copper-induced impairment of Na/K-ATPase activity in gills can be ameliorated by increased dietary sodium, rainbow trout (*Oncorhynchus mykiss*) were used to determine if elevated dietary sodium was also protective against copper-induced olfactory impairment. Measurement of the olfactory response of rainbow trout using electro-olfactography demonstrated that sodium was protective of copper-induced olfactory dysfunction. This work demonstrates that the transcriptions of both subunits of Na/K-ATPase in the olfactory epithelium of fish are affected by Cu exposure, and that dietary Na protects against Cu-induced olfactory dysfunction.

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1. Introduction

An intact sense of smell is crucial for many aquatic animals, including fish, as many vital activities such as finding food, avoiding predators, homing, and finding an appropriate mate are mediated by olfaction (Laberge and Hara, 2001). To efficiently detect odor molecules the olfactory system of fish, specifically olfactory sensory neurons (OSNs), is in direct contact with the surrounding water. As a consequence, sensitive neural tissue can easily come into contact with environmental contaminants and impair olfaction even at very low concentrations (Tierney et al., 2010).

Impairment of olfaction in fish due to contaminant exposure has received a considerable amount of attention. During the past five decades several studies have investigated the effects of various contaminants on fish olfaction, with metals being the most studied class of contaminant. While the effect of copper on olfaction has received most of the attention (Hara et al., 1976; Julliard et al., 1996; Beyers and Farmer, 2001; Baldwin et al., 2003; Carreau and Pyle, 2005; Bettini et al., 2006; Sandahl et al., 2006; Kolmakov et al., 2009; Dew et al., 2012, 2014), other metals such as nickel (Brown et al., 1982; Tallkvist et al., 1998; Dew et al., 2014), zinc (Brown et al., 1982; Cancalon, 1982; Kuz'mina, 2011), and cadmium (Baker and Montgomery, 2001; Scott et al., 2003; Blechinger et al., 2007) have also been studied. Regardless of the extensive efforts to explore the effects of copper on olfaction, the underlying mechanism of olfactory toxicity of copper remains unknown. Furthermore, studies discovering potential ways to protect olfaction against copper-induced impairment are rare.

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Measuring the effects of contaminants on the transcriptome of exposed animals has become an established method in toxicology studies (Walker, 2001; Schirmer et al., 2010). Highly sensitive molecular techniques provide the opportunity to reveal mechanisms of toxicity by investigating rapid and subtle changes at the molecular level (Denslow et al., 2007; Schirmer et al., 2010). Specifically, microarrays allow for the study of large numbers of genes simultaneously. Genes demonstrated to respond to the experimental treatment (e.g., Cu-exposure) on the microarray can be further explored individually using real-time PCR (Snape et al., 2004; Valasek and Repa, 2005; Lettieri, 2006; Schirmer et al., 2010; Pina and Barata, 2011).

Two microarray studies have investigated the effect of exposure to copper on olfactory tissues. Tilton et al. (2008, 2011),) exposed zebrafish (*Danio rerio*) to low concentrations of copper and/or chlorpyrifos. Tilton et al. (2008) exposed zebrafish to increasing concentrations of copper (6.3, 16, and 40 μg/L) for 24 h and found that the transcription of many genes was altered in response to copper exposure, with the largest number of genes having altered transcription at the highest tested concentrations. Tilton et al. (2011) exposed zebrafish to copper and/or chlorpyrifos (an organophosphate pesticide) for 24 h and both contaminants showed unique transcriptional signatures. However, when they used a mixture of copper and chlorpyrifos, the transcriptional signature was closer to that of copper, even at high concentrations of chlorpyrifos.

Like any toxicological endpoint, differential gene expression is influenced by exposure duration (Denslow et al., 2007; Heckmann et al., 2008; Ankley et al., 2009). However, to date no time series studies have been done at the transcriptional level in regards to olfactory toxicity. At other levels of biological organization, it has been demonstrated that low concentrations of copper will cause different effects on the olfactory system at different exposure durations. For example, fathead minnows (*Pimephales promelas*) exposed to low concentrations of copper for short durations (1, 3, 24, and 96 h) will initially have a decreased neurophysiological response to odors, which recovers over time (Dew et al., 2012). In terms of behavior, Colorado pikeminnows (*Ptychocheilus lucius*) have greater copper-induced impairment of behavioral response following 24-h exposures to copper compared to 96-hour exposures (Beyers and Farmer, 2001).

Parallel to understanding the different aspects of toxicity of any contaminant, many researchers are trying to understand the factors that affect toxicity to make better ecological risk assessments. For example, calcium has been shown to reduce the lethal effects of copper (Chen et al., 2012); however, calcium is not protective against olfactory impairment caused by exposure to low concentrations of copper (Green et al., 2010; Dew et al., 2012). Sodium may also protect against copper-induced olfactory dysfunction. Increasing dietary sodium has been demonstrated to decrease the uptake of waterborne copper in gill tissues of rainbow trout (*Oncorhynchus mykiss*; Pyle et al., 2003). Interestingly, in the same study it was demonstrated that copper-induced impairment of Na/K-ATPase in gill tissue was reversed by increased dietary sodium. This protective effect of dietary sodium has yet to be studied in olfactory tissue (Pyle et al., 2003).

In the current study, the effects of copper on olfactory tissue at the transcriptional level were investigated using a novel 1000 candidate gene yellow perch microarray (Bougas et al., 2013). Real-time PCR was used to confirm the results of microarray analyses and to measure the transcription of both subunits of Na/K-ATPase in response to different exposure durations of copper. Due to the reason that wild yellow perch do not feed on in captivity, we chose another species for testing the effect of increased dietary sodium on copper-induced olfactory dysfunction. Rainbow trout were fed with control and sodium-rich diets and then were exposed to waterborne copper to determine if an increase in dietary sodium in fish could be protective against exposure to copper.

2. Materials and methods

2.1. Experimental animals

Yellow perch is indigenous to North American freshwater lakes and enjoys a nearly ubiquitous distribution east of the Rocky Mountains in Canada and the northern United States (Scott and Crossman, 1973). Because of its known tolerance to metals, it is often one of the first species to recolonize metal-contaminated habitats or the last to be extirpated (Couture and Pyle, 2008). Consequently, yellow perch was studied here for its relevance to Canadian freshwater ecosystems. Regarding the feeding experiment, it has been our experience that yellow perch are not amenable to long-term holding in an aquatic facility. Therefore, we were required to use another species for the feeding experiments as we were required to hold fish for a minimum of four weeks (three weeks for acclimation, one week for the feeding experiments). Considering the difficulties of feeding wild fish in captivity, we selected a model species, rainbow trout, which feed more actively in captivity and consequently we were able to feed them with different diets.

2.2. Gene transcription experiment

2.2.1. Collection and acclimation of fish

Yellow perch were collected from Geneva Lake, near Sudbury, Ontario (46°45′59″N, 81°32′41″W) using seine nets and angling during June 2012 for microarray experiments, and during June 2013 for real-time PCR experiments. Fish were transported in aerated native lake water to the Cooperative Freshwater Ecology Unit, Sudbury, and acclimated to laboratory conditions in water from Geneva Lake for 24 h in 12 L plastic tanks. In each container, four fish were held in 8 L of water. All tanks were aerated during acclimation and the temperature was kept close to Geneva Lake water temperature (22–23 °C).

2.2.2. Exposure protocol

To test the effects of copper on olfactory tissue at the transcriptional level (microarray experiments), after 24 h of acclimation fish were randomly assigned to two treatment groups: control (Geneva Lake water), and copper (Geneva Lake water with 20 μg/L (0.32 μM) of elevated copper). For each group, four aerated tanks containing three fish were set up for 3 or 24 h.

To confirm the results of microarray analyses and to measure the transcription of genes of interest (real-time PCR experiments), fish were exposed to either control water (i.e., Geneva Lake water) or control water plus 20 μg/L (0.32 μM) copper, and held for either 1, 3, 12, 24, or 48 h. Three fish were randomly assigned to each of 30 experimental replicates (tanks) containing 6 L of aerated exposure water.

2.2.3. Water quality analysis

To measure temperature and pH in the exposure tanks, a YSI 6600 V2 multiparameter sonde (YSI Inc., Yellow Springs, Ohio) was used. Water samples were collected from exposure tanks at the end of the exposure period. From each exposure or control tank, three water samples were analyzed for dissolved metal concentrations, dissolved organic carbon (DOC), and alkalinity. Total dissolved metal concentrations were determined by first passing unfiltered water samples through a 0.45 μm nylon syringe filter. Filtered water samples were acidified by adding 200 μL of trace metals grade high purity nitric acid (Fisher Scientific, Nepean, ON) to 50 mL of water sample. Samples were stored at 4 °C until

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