Comparative Biochemistry and Physiology, Part C 154 (2011) 296–304

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Changes of glycogen metabolism in the gills and hepatic tissue of tilapia (*Oreochromis mossambicus*) during short-term Cd exposure

Yu-Siang Lin [a], Shu-Chuan Tsai [b], Hui-Chen Lin [c], Chung-Der Hsiao [d], Su Mei Wu [a,*]

[a] Department of Aquatic Biosciences, National Chiayi University, Chiayi 600, Taiwan
[b] Institute of Life Sciences, Central Taiwan University of Science and Technology, Taichung 40601, Taiwan
[c] Department of Life Science, Tunghai University, Taichung 40704, Taiwan
[d] Department of Bioscience Technology, Chung Yuan Christian University, Jhongli 32023, Taiwan

ARTICLE INFO

Article history:
Received 16 February 2011
Received in revised form 11 May 2011
Accepted 24 June 2011
Available online 2 July 2011

Keywords:
Cadmium
Glucagon
Teleost
Cortisol
Glucose
Ion regulation

ABSTRACT

The aim of the study was to test the hypothesis that the mechanism of glycogen metabolism has taken place in gills rather than in liver during Cd exposure. Male tilapia were exposed to 44.45 μM ambient Cd for 12 h, and we found blood glucose significantly increased, however, lactate levels showed no significant changes. The glycogen phosphorylase (GP) activity increased immediately after 0.75 to 3 h of Cd exposure in the gills, and after 1 to 6 h in the liver, respectively. In addition, the glycogen level depleted faster in the gills than in the liver. Plasma cortisol level increased from 0.25 to 1 h and recovered after 3 h, while the glucagon did not significantly change during Cd exposure. Glucocorticoid receptor (GR) mRNA expression decreased after 0.75 h in the gills, while it significantly increased after 6 h in the liver. Ca^{2+}, Na^{+}, Cl^{-}, and K^{+} significantly decreased upon Cd exposure within 6 h following Cd-induced toxic stress. We suggested that the cortisol is the spontaneous stimulation of glycogen metabolism in the gills, and it triggers a subsequent energy supply later in the liver. Taken together, the profile of glycogen metabolism between gills and liver during Cd-exposure stress provide good support to our hypothesis.

1. Introduction

Animals increase blood glucose levels and regulate energy metabolism in response to stress. Glycogen metabolism is the principal energy source in both vertebrates and invertebrates, especially during responses to environmental fluctuations and stress (Bacca et al., 2005). Glucose (or glucose 6-phosphate) is released through the degradation of glycogen by glycogen phosphorylase (GP) (Roach et al., 1998), and energy is mainly supplied by the oxidation of glucose and lactate as a result of carbohydrate metabolism (Morgan et al., 1997).

Heavy metals, including cadmium (Cd), exert a wide range of pathological effects on fish (Iger et al., 1994). Teleosts are highly sensitive to Cd, as decreased growth rates were commonly observed due to the impairment of growth hormone activation by Cd (Jones et al., 2001). In addition, stress responses upon Cd exposure including increases in metallothionein expression, lysozyme content, and cortisol levels all need energy costs (Wu et al., 2007). Generally speaking, heavy metals cause changes in metabolic rate (oxygen consumption), but the direction of the effect varies with the metal: some stimulate, while others inhibit (Heath, 1995). However, the profile of energy support system in gills and hepatic tissues during hours of Cd exposure remains unclear.

In addition to functioning in ion/osmotic regulation, acid–base balance, and gas exchange (Evans et al., 2005), gills are also the first line of defense against ambient heavy metal accumulation (McDonald and Wood, 1993). However, following heavy metal stress, there was a rise in the number of mucus-secreting cells (Wu et al., 2007), and a down-regulation in Ca^{2+}-ATPase activity (Wong and Wong, 2000). The functional morphology of chloride cells (CC) changed after Cd exposure during development of tilapia (*O. mossambicus*) (Lee et al., 1996; Wu, 2000). Various ion transporters and enzymes in CC of gills were also affected by heavy metals (Hirose et al., 2003). Tseng et al. (2007) reported that a group of cells rich in glycogen deposits, called glycogen-rich cells, were neighbored with CC. These cells expressed a specific form of GP in tilapia gills. In their study, both GP activity and protein expression in gills were up-regulated in tilapia within 1–3 h after acute transfer from fresh water to 2.5% seawater. On the contrary, glycogen levels in both gills and liver were significantly depleted after their transfer to seawater, but the depletion occurred significantly earlier in gills than in the liver. These data suggested that glycogen-rich cells are initially stimulated to rapidly provide energy for neighboring CC that triggers ion-secretion mechanisms (Chang et al., 2007; Tseng et al., 2008). Several species—including tilapia, goldfish, and rainbow trout—all experienced a modest decrease in plasma Na^{+}, K^{+}, Ca^{2+} and Cl^{-}

* Corresponding author at: National Chiayi University, 300 University Road, Chiayi 600, Taiwan. Tel.: +886 5 2717854; fax: +886 5 2717847.
E-mail address: sumei@mail.ncyu.edu.tw (S.M. Wu).

1532-0456/$ – see front matter.
doi:10.1016/j.cbpc.2011.06.014

under Cd exposure (Heath, 1995). It is known that heavy metal exposure causes a rather rapid decrease in plasma electrolytes and/or osmolality in fish. We have thus hypothesized that there might be a similar profile of glycogen metabolism upon Cd exposure like the salinity stress, which also has the GP activation and glycogen depletion occurring significantly earlier in gills than in the liver.

Heavy metal-induced stress is a state characterized by a specific syndrome that is induced and causes changes within a biological system. The costs of heavy metal-induced stress are associated with three phases of stress: alarm, adaptation, and exhaustion; and the alarm phase involves immediate increases in catecholamine and cortisol levels (Newman, 1995). Both endocrine pathways trigger the release of additional energy required for stress acclimation. Catecholamine is known for its role in the activation of hepatic GP and the inhibition of pyruvate kinase (PK), which increases glycogenolysis and gluconeogenesis while reducing glycolysis (Reid et al., 1992). Similarly, cortisol might have a modulatory effect on the catecholamine-stimulated adenylyl cyclase system in trout hepatocytes (Reid et al., 1992). It is well known that cortisol is a classic indicator of stress in fish. Cortisol played multiple functions during stress, such as ion balance regulation (Mommsen et al., 1999); enhancement of Na^+-K^+-ATPase activity by stimulating CC differentiation (Veillette and Young, 2004); suppression on immune response and induce in metallothionein (Wu et al., 2006); and energy metabolism during stress (Potllant and Tort, 1997). As regards to the effect of Cd on cortisol levels, some previous studies found that cortisol was induced upon Cd exposure (Hontela et al., 1996; Wu et al., 2006; 2007). However, there were other studies that did not find cortisol to be induced by Cd treatment (Pelgrom et al., 1995; Dang et al., 2001). The cortisol levels were not the same during Cd treatment in fish, and the cause of that may be due to Cd developing a pathological and stress responses from fish. It is possible that dietary Cd accumulates more slowly and to a lesser extent in fish gills than does waterborne Cd. Certain Cd doses fed to fish did not induce a stress response at all (Dang et al., 2001). Besides, Cd might impact the HPI (hypothalamous–pituitaty–interrenal); it significantly suppressed ACTH-stimulated cortisol production (Sandhu and Vijayan, 2011). These findings result in more enigmatic roles of cortisol after Cd treatment in fish.

Glycemia is also one of the classic plasma indicators of stress in fish (Roche and Bogé, 1996). Plasma glucose was raised from fish exposed to Cd (Hontela et al., 1996; Wu et al., 2007). Cd exposure also induced increased glucose concentration in white muscle of fish (Almeida et al., 2001). The glucose concentration was proposed to be mediated by endocrine release such as cortisol (Pelgrom et al., 1995; Hontela et al., 1996). However, in cortisol and plasma glucose content, both were nonspecific stress responses as they did not exhibit a parallel change during the time course experiment. For example, the stressed juvenile common carp (*Cyprinus carpio*) did not change its cortisol and glucose levels compared to the pre-challenge values (Hosseini and Hoseini, 2010). When exposed to 3.67 μM Cd for 2 h, the rainbow trout (*Oncorhynchus mykiss*) had a significant increase in plasma cortisol levels and glucose (Hontela et al., 1996). Thus, the level of blood glucose was probably mediated by other endocrine release during Cd stress and not only by cortisol.

Glucagon is another powerful metabolic hormone, often opposing the actions of insulin, regulating glucose metabolism. It is involved in the regulation of hepatic glycogenolysis and gluconeogenesis in fishes (Moon, 1998). *In vitro,* glucagon exposure increased GP activity 3.1-fold in fish hepatocytes (Hallgren et al., 2003). So far, it was not clear about the role of glucagon on glycogen metabolism during metal treatment in fish. We therefore measured the changes of cortisol and glucagon in order to confirm their roles on glucose releasing and GP activation within hours of Cd exposure in the present study.

In fish, cortisol plays a dual role in carbohydrate and mineral metabolism in fish. Bury and Strum (2007) reported that corticosteroids are mediated *via* corticosteroid receptors, which include the glucocorticoid (GR) and mineralocorticoid receptors (MR). Both of them showed distinct expression patterns and transcriptional activities among tissues (Greenwood et al., 2003). But, there were many researchers who reported that the cortisol signaling in teleosts is thought to be mediated predominantly by the GR (Mommsen et al., 1999; Vijayan et al., 2003), and its expression appeared to be a tissue-specific GR transcript response in the gill and liver (Singer et al., 2007). Our previous study suggested that the multiple functions of cortisol leads to all target tissues including gills, liver, kidneys, and intestines for this hormone (Wu et al., 2005). In addition, it is known from previous studies that GR signals appeared more in chloride, pavement, respiratory and undifferentiated cells in Cd treated fish (Dang et al., 2001). It was suggested that GR expression in gills and liver was related with induction of metallothionein (one of metal binding protein) synthesis by cortisol since it occurs only after cortisol binding to GR (cited by Dang et al., 2001). Cortisol treatment *in vivo* and *in vitro* results in significant decreases in hepatic GR protein levels; but increase in hepatic GR mRNA levels suggested an autoregulation of hepatic GR mRNA by GR protein abundance (Vijayan et al., 2003). Hence, GR mRNA expression was related to the activation of cortisol.

As described above, the gills are the first target organ affected by metal exposure in fish (McDonald and Wood, 1993), and the liver is one of the major organs that stores glycogen. However, no study has clarified the partitioning of energy supplies between the energy requirements of the liver and gills during acclimation to ambient Cd. Hence, there is little understanding of the relationships among the endocrine system, the energy supply between tissues, and GP activity during the process of Cd exposure in fish.

In the present study, male adult tilapia were challenged with 44.45 μM Cd, which is approximately half of the 96 h LC_{50} (94.84 μM) for adult tilapia (*O. mossambicus*) (Gaikwad, 1989; cited from Yorulmazlar and Gül, 2003). Following this treatment, we compared many parameters, including changes of blood, endocrine, and biochemistry tissues of gills and hepatic, in order to examine the profile of energy applied system. In addition, ions such as Na^+, K^+, Ca^{2+}, and Cl^- blood levels investigated during Cd exposure.

2. Materials and methods

2.1. Fish

Male Mozambique tilapia (*O. mossambicus*) were used as the animal models in the present study because there is a sex-specific difference between male and female fish exposed to waterborne Cd (Sellin et al., 2007). These fish were obtained from the Mariculture Research Center of the Taiwan Fisheries Research Institute, Tainan, Taiwan. The fish were reared in 182-L glass aquaria using plastic chips for gravel. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee, approval number 95021. Each tank was supplied with dechlorinated, circulated, aerated local tap water (FW) at 26–28 °C under a photoperiod of 12–14 h. Fish were fed commercial fish food pellets. The water quality parameters included a total hardness of 146.6 ± 5.6 mg/L, Na^+, 35.6 ± 0.3 mg/L, K^+, 3.3 ± 0.1 mg/L, Ca^{2+}, 30 ± 2.3 mg/L, and a pH of 8.2 ± 0.3 Cd concentration was kept < 1 μg/L.

2.2. Experimental design and sampling

The Cd medium was prepared using completely dried $CdCl_2$ (Sigma-Aldrich, St, Louis, MO, USA) dissolved in 1 mL of concentrated HCl; double-deionized water was used to prepare the 0.09 mM Cd stock solution, which was then diluted with tap water before the experiments. Adult fish of 10–12 cm total length and 60–80 g of body mass were used in the present study. The time course experiment was repeated at least 4 times. In each experiment, two aquaria (at 30 × 15 × 24 cm^3) were used for each experimental time period after being cleaned with 10% HNO_3,