Molecular Genetics and Metabolism Reports 1 (2014) 373–377

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: http://www.journals.elsevier.com/molecular-genetics-and-metabolism-reports/

Short Communication

Development of catecholamine and cortisol stress responses in zebrafish ☆,☆☆

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ARTICLE INFO

Article history:
Received 13 August 2014
Accepted 15 August 2014
Available online 7 September 2014

Keywords:
Zebrafish
Stress response
Cortisol
Catecholamines
HPA axis

ABSTRACT

Both adrenal catecholamines and steroids are known to be involved in the stress response, immune function, blood pressure and energy homeostasis. The response to stress is characterized by the activation of the hypothalamus–pituitary–adrenal (HPA) axis and the sympathetic-adrenomedullary system, though the correlation with activation and development is not well understood. We evaluated the stress response of both cortisol and catecholamines during development in zebrafish. Zebrafish at two different stages of development were stressed in one of two different ways and cortisol and catecholamine were measured. Cortisol was measured by enzyme immune assay and catecholamine was measured by ELISA. Our results show that stress responses are delayed until after the synthesis of both cortisol and catecholamines. These observations suggest that the development of HPA axis may be required for the acquisition of the stress response for cortisol and catecholamines.

☆ Grant or fellowship support: None.
☆☆ Disclosure summary: The authors have nothing to disclose.
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http://dx.doi.org/10.1016/j.ymgmr.2014.08.003

1. Introduction

The stress response of cortisol or catecholamines in adult model organisms has been investigated with several stressors: shaking stress, immobilization, and cold or trauma [1–8]. In vivo studies of corticotrophin releasing hormone (CRH) knockout mice, which have reduced corticoid levels, showed interrupted stress-induced increases in both mRNA and protein levels of phenylethanolamine-N-methyltransferase (PNMT), the expression of which is regulated by glucocorticoids and neural input to the adrenal gland [3,4]. Tyrosine hydroxylase (TH) null-mice that lack production of adrenal catecholamines showed reduced levels of plasma corticosterone, and, in the electron micrograph of adrenocortical cells of TH-null mice, internal mitochondrial membranes, where the steroidogenesis takes place, were reduced [9].

The recent studies have described the development of cortisol or catecholamines and the correlation with the various stressors [10–15]. In zebrafish, the ability to synthesize cortisol by the interrenal organ, the adrenal equivalent, starts after hatch, though the response with cortisol elevation induced by stress does not exist at that time [10–12]. This discrepancy may be caused by the immaturity of the HPA-axis and its ability to respond to stress, as up-regulation of the expression of melanocortin type 2 receptor (MC2R) transcripts, which precedes cortisol synthesis, is already seen at the time around hatch [10–12]. The catecholamines are detectable from 1 h post fertilization (hpf) and significantly increased from 2 days post fertilization (dpf) toward 5 dpf in zebrafish development [14].

In this study, to better understand the stress response of the catecholamines and the correlation between cortisol and catecholamines during development, we analyzed both catecholamine and cortisol levels at several developmental stages under control conditions and after acute stressors using the zebrafish, *Danio rerio*, a powerful model for understanding organ development due to its ease of genetic and molecular manipulation, transparent embryos, and large number of progeny that provide statistical power [16,17].

2. Materials and methods

2.1. Zebrafish breeding and embryo maintenance

Wild-type zebrafish (AB line or Hybrid line) were maintained at the UCLA Zebrafish Core Facility. Embryos were obtained by natural spawning and cultured in culture aqua medium (fishwater) at 28 °C using standard zebrafish husbandry techniques [18]. Zebrafish were maintained in accordance with the Guide for the Care and Use of Laboratory Animals [19], and the studies were approved by the UCLA institutional committee on animal care.

2.2. Cortisol extraction and measurement

Wild type zebrafish embryos were cultured in fishwater with standard procedures [17]. A hundred embryos or larvae were gently collected into a 15 ml tube at 48 or 96 hpf, respectively. Stressed samples were prepared in one of the two ways: 30 s of hand swirling in 5 ml fishwater or 30 revolutions of roller swirling using a tube shaker/rotator (LABQUAKE, model 4002110: Barnstead International, IO, USA) at 8 rpm in 10 ml fishwater. After being stressed, samples were placed at 28 °C for 5 min and then on ice immediately, and embryos were transferred to a 1.5 ml tube and stored at −80 °C until use. For extraction, we followed the procedure as previously reported [13]. Cortisol enzyme immunoassay (EIA) (Cortisol EIA kit, Cat No. 500360: Cayman Chemical, MI, USA) was performed to determine the cortisol levels for each sample in duplicate.

2.3. Catecholamine extraction, acylation and measurement

Wild type zebrafish embryos were cultured in fishwater with standard procedures [17]. A hundred and fifty embryos at 48 hpf or a hundred larvae at 98 and 120 hpf were gently collected into a 15 ml tube and placed in the 28 °C incubator for 15 min. Stressed samples were prepared as follows: 30 s of hand swirling in 5 ml fishwater or 15 revolutions of roller swirling using the shaker/rotator in 10 ml fishwater. Tricaine was added soon after the stress to make a 0.02% final concentration in fishwater