Available online at www.sciencedirect.com

SCIENCE DIRECT®

Comparative Biochemistry and Physiology, Part A 141 (2005) 391 – 400

CBP

www.elsevier.com/locate/cbpa

Ontogeny of cardiovascular control in zebrafish (*Danio rerio*): Effects of developmental environment

Brian Bagatto *

Department of Biology, University of Akron, Akron, Ohio, 44325-3908, USA

Received 10 January 2005; received in revised form 26 June 2005; accepted 6 July 2005
Available online 8 August 2005

Abstract

The goal of this symposium paper was to identify and quantify developmental plasticity in the onset of cardiovascular responses in the zebrafish. Developmental plasticity was induced by altering the developmental environment in one of three ways: (1) by developing zebrafish in a constant current of 5 body lengths per second, (2) by developing zebrafish at a colder temperature (20 °C), and (3) by developing zebrafish in severe hypoxia (DO = 0.8 mg/L). Early morphological development was significantly affected by each of the treatment environments with hypoxia slowing development the most and producing the highest variation in measurements. Development in constant water current did not significantly affect the timing onset of cardiovascular responses to the pharmacological agents applied. Development at 20 °C significantly delayed the onset of all cardiovascular responses measured by 2–3 days. Development in hypoxia, however, not only delayed onset of all cardiovascular responses, but also shifted the onset relative to the developmental program. Hypoxia clearly has a profound affect on the onset of cardiovascular regulation and it will take many more studies to elucidate the mechanisms by which hypoxia is having its effect. Furthermore, long term studies are also needed to assess whether the plasticity measured in this study is adaptive in the evolutionary sense.

Keywords: Cardiovascular; Development; Heterokairy; Hypoxia; Plasticity; Temperature; Training; Zebrafish

1. Introduction

In fish, development under constant abiotic conditions is the exception rather than the rule. Perturbations in temperature, oxygen availability, water current etc. all have the potential of altering the developmental timing of specific events. The most common perturbation is an overall delay or acceleration in the developmental program. In some cases, the timing of one developmental parameter (such as the onset of cardiovascular regulation; Spicer and Burggren, 2003) may be accelerated or decelerated relative to others during development. The data presented in this study show general and specific developmental perturbations in the onset of cardiovascular regulation that depend on the environmental factor altered.

One of the first organ systems to function in the developing fish is the cardiovascular system. In all vertebrate species studied thus far, blood pressure, stroke volume, and cardiac output increase while total peripheral resistance decreases during development (Burggren and Warburton, 1994). Heart rate changes during development follow distinct species-specific patterns, while the mechanisms underlying this variation measured between species have not been established (Burggren and Warburton, 1994). Possible mechanisms could involve changed membrane permeability of the myocytes, resulting in altered frequency of the pacemaker action potentials, and the development of nervous and hormonal cardiovascular control systems (Burggren and Warburton, 1994; Fritsche and Burggren, 1996; Fritsche, 1997). Additionally, there are many who agree that the remodeling of the primary vascular system of the embryo into arteries and veins depends primarily on the influence of hemodynamic forces (Moyon et al., 2001;

* Tel.: +1 330 972 2571; fax: +1 330 972 8445.
E-mail address: bagatto@uakron.edu.

1095-6433/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2005.07.002

Jones et al., 2004). However, in some systems certain tissues can retain plasticity throughout development and thus will respond more readily to internal and external environmental changes. For example, avian endothelial cells remain plastic in terms of arterio-venous differentiation until late embryonic development (Moyon et al., 2001). In the frog, *Rana temporaria*, cardiovascular effects of acetylcholine and epinephrine can be seen before any innervation of the heart is demonstrated (Protas and Leontieva, 1992). This pattern is assumed to be present in fish, but studies that verify this assumption are limited. Data gathered on a single species, the golden mullet, *Liza auratus*, do suggest that the fish heart comes under sympathetic control well before parasympathetic control (Balashov and Soltitskij, 1991; Balashov et al., 1991). Additionally, Holeton (1971) has suggested that the drop in heart rate of rainbow trout larvae between 9 and 16 days (90–160 ATU — Accumulated Thermal Units) post hatch might be indicative of the heart coming under vagal control. This study begins to resolve not only the issues of timing in cardiovascular control, but also the relative flexibility of this timing.

Many fish species experience a broad range of water current either over time or from location to location. This common environmental challenge has the potential of inducing plasticity in fish both during development (Bagatto et al., 2001; Pelster et al., 2003), and during adult stages (for review, see Davison, 1997; Kieffer, 2000). For example, swim training enhances swimming efficiency, increases mitochondrial density in red and intermediate muscle, increases capillarization, and increases tolerance to hypoxia in zebrafish larvae (Bagatto et al., 2001; Pelster et al., 2003). In adult salmonids, such training leads to increases in growth rate and food-conversion efficiency (Davison, 1997). Thus, it is clear that water current can cause a vast array of phenotypically plastic responses however; it is unclear what effect, if any, this plasticity during development has on the onset of cardiovascular regulation.

Temperature is arguably the most important abiotic factor affecting development. Eggs and larvae of developing poikilotherms can experience wide temperature fluctuations in their surrounding micro-environments (Pelster, 1997), profoundly affecting the rate and overall success of development. In fish, lower temperatures may delay hatching or even prevent embryogenesis (Schirone and Gross, 1968), and although higher temperatures will accelerate development, an upper limit is signified by increased morphological abnormalities and increased mortality. In the zebrafish, regular successive cleavages and a distinct continuity of morphological features proceed normally between 23 and 34 °C. Embryos kept at temperatures outside these limits immediately following fertilization may not complete embryogenesis (Schirone and Gross, 1968). Superimposed on thermal tolerance is a significant increase in the viable temperature range within the first few days of embryo development in zebrafish (Rombough, 1996). Thus, thermal tolerance for normal embryonic development is more restricted than in adult fish (Rombough, 1996; Hochachka and Somero, 2002).

The thermal environment during development can have an enormous impact on the adult phenotype, and this, in turn, may affect the adaptation of successive generations. Egg incubation temperature has been shown to affect the number of muscle fibers produced in a number of fish species (for review, see Johnston et al., 2001). The effects of egg incubation temperature on fiber number and myogenic precursor density was shown to differ in reproductively isolated populations of Atlantic salmon in the same river system, providing evidence for local adaptation of trait plasticity (Johnston et al., 2000). In zebrafish, maternal thermal environment has been shown to significantly affect developmental rate of offspring. Bagatto (2001) show that at 25 °C , zebrafish embryos from parents that are hatched and raised at 25 °C develop faster than embryos from parents that are hatched and raised at 28 °C. However, this does not necessarily mean that the physiological development has also altered its developmental trajectory.

Hypoxia is a common stress in many fish populations. Reflecting a history of evolution in a range of oxygen environments, hypoxia appears to play a key role in a suite of organismal, cellular, and genetic responses in fish. Hypoxia has a profound effect on fish behavior (Marks et al., in press), cardiovascular physiology (Jacob et al., 2002), most ionic and substrate membrane transport systems (for review, see Nikinmaa, 2002), intracellular signaling systems (Salama and Nikinmaa, 1990), and gene expression in fish (Gracey et al., 2001). Because of these complex interactions during development, hypoxic exposure during development is likely to show more than just an overall decrease in the developmental program.

The goals of this study were not only to determine when the zebrafish heart and vasculature would respond to pharmacological agents, but also if the timing of these responses could be altered by specific environmental changes. The ontogeny of cardiovascular regulation in the zebrafish is likely to be altered by the environmental perturbations. This study attempts to identify the magnitude and direction of these changes, and to comparatively discuss these changes in the context of fish development.

2. Materials and methods

2.1. Animals

Adult zebrafish, *Danio rerio*, were maintained and bred according to well documented procedures (Westerfield, 1994; Bagatto et al., 2001). True wild type zebrafish were used in this study in order to maximize the amount of genetic variation. Zebrafish were hand caught in various Ganges River tributaries in India and imported through Poseidon Aquatics where genus and species were confirmed