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Vascular parameters continue to decrease post-exposure with simultaneous, but not individual exposure to BPA and hypoxia in zebrafish larvae

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ABSTRACT

How fish respond to hypoxia, a common stressor, can be altered by simultaneous exposure to pollutants like bisphenol A (BPA), a plasticizer. BPA is cardiotoxic and interferes with the hypoxia inducible factor pathway (HIF-1α), therefore disrupting the hypoxic response. Co-exposure to hypoxia and BPA also causes severe bradycardia and reduced cardiac output in zebrafish larvae. The purpose of this work was to determine how the cardiovascular effects of co-exposure vary with BPA concentration and persist beyond exposure. Zebrafish embryos were exposed to 0, 0.01, 0.1, 1, and 100 μg/L of BPA during normoxia (> 6.0 mg/L O_2) and hypoxia (2.0 ± 0.5 mg/L O_2) between 1 h post fertilization (hpf) and late hatching (72–96 hpf). Heart rate, cardiac output, and red blood cell (RBC) velocity were determined through video microscopy and digital motion analysis at late hatching and 10 days post fertilization (dpf), several days post exposure. In comparison to the hypoxic control, RBC velocity was 25% lower with 0.01 μg/L BPA and hypoxia at late hatching. At 10 dpf, the difference in RBC velocity between these treatments doubled, despite several days of recovery. This coincided with a 24% thinner outer diameter for caudal vein but no effect on cardiac or developmental parameters. Statistical interactions between BPA and oxygen concentration were found for arterial RBC velocity at both ages. Because the co-occurrence of both stressors is extremely common, it would be beneficial to understand how BPA and hypoxia interact to affect cardiovascular function during and after exposure.

1. Introduction

The fish cardiovascular system is responsive to common environmental perturbations such as hypoxia. Maintaining oxygen homeostasis in response to environmental hypoxia can be achieved through molecular, metabolic, cardiovascular, and behavioral mechanisms (Randall, 1982; Mandic et al., 2009; Richards, 2009). This includes upregulation of transcription factors like the hypoxia inducible factor (HIF-1α; Semenza, 2000), re-balancing of oxygen supply and demand (Richards, 2009), reflexive bradycardia (Farrell, 2007), and reduced swimming activity (Widmer et al., 2006). How organisms respond to this common and historic stressor can be disrupted by co-exposure to novel pollutants that are present at high concentrations and in mixtures (Heugens et al., 2001; Holmstrup et al., 2010). Co-exposure can decrease hypoxia tolerance through alterations in gill morphology and oxygen demand (Holmstrup et al., 2010; Whitehead, 2013). Conversely, co-exposure can increase pollutant toxicity through upregulation of metabolism and therefore epithelium contact (Laskowski et al., 2010; Whitehead, 2013). These interactions can have negative consequences for health and survival during and after the exposure (Laskowski et al., 2010). Because exposure to multiple stressors is the norm in natural environments, it is imperative, particularly for wildlife management, to understand how stressor interactions affect individuals throughout their lifetime.

Bisphenol A (BPA) is a ubiquitous compound in aquatic environments and therefore commonly occurs with hypoxia (Rocha et al., 2012). This small hydrophobic molecule is used in the production of polycarbonates and epoxy resins and is known for its ability to mimic estrogen (Vandenberg et al., 2009). It also impairs cardiomyocyte contractility and results in the degradation of HIF-1α, the transcription factor that controls over 50 hypoxia response genes (Kubo et al., 2004; Pant et al., 2011; Gao and Wang, 2014). BPA exposure during hypoxia has the potential to disrupt the molecular, metabolic, and cardiovascular mechanisms that provide tolerance to hypoxia. Previously, we showed that exposure to BPA during hypoxia resulted in severe bradycardia and reduced cardiac output in larval zebrafish (Cypher et al., 2015). This could be due to HIF-1α degradation, cardiotoxicity, oxygen constraints, or some combination of interacting mechanisms. It remains to be seen, however, whether co-exposure has a dose-response effect on heart rate and cardiac output across lower concentrations. It is also

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unknown if cardiovascular function is temporarily affected or remains altered post-exposure. The purpose of this study was to determine how cardiovascular function and development are affected during and after an embryonic exposure to a range of environmentally relevant concentrations of BPA with normoxia and hypoxia.

2. Methods

2.1. Ethical procedures

Animal care protocols were approved by the Institutional Animal Use and Care Committee at The University of Akron (15-05-7BFC).

2.2. Chemicals

Bisphenol A (BPA) (96%) was purchased from Sigma Aldrich (133027) and was used to create several environmentally relevant concentrations using DMSO (99%, 472,301) as a solvent. A non-solvent control was not used because the parameters chosen for this study are unaffected by the concentrations of DMSO used in this exposure (Hallare et al., 2006). The BPA concentrations used here have been found to occur in aquatic environments and are often used in topical exposures with similar physiological end points (Staples et al., 1998; Crain et al., 2007; Vandenberg et al., 2013). Concentrations deemed as environmentally relevant range from ng/L up to 17.2 mg/L (Staples et al., 1998; Vandenberg et al., 2013). The concentrations used here are 1000 fold lower than the maximum environmentally relevant concentration and those found to exert a cardiotoxic effect (Pant et al., 2011).

2.3. Animals

Wild-type zebrafish were used because of their transparency during early development which allows for non-invasive optical measurement of cardiovascular parameters. Eggs were collected by introducing breeding baskets to tanks with up to 50 adults housed in The University of Akron Research Vivarium at a light cycle of 14 L:10 and maintained at a temperature of 27 ± 0.5 °C. Once collected, eggs were maintained at 28 ± 0.5 °C and 14 L: 10 D throughout the experiment.

2.4. Exposure

Eggs were randomly distributed among flasks of 0, 0.01, 0.1, 1, and 100 μg/L of BPA with 0.02% DMSO after they were bubbled with nitrogen or compressed air until they reached the appropriate oxygen concentration (n ≥ 8 flasks). No > 15 eggs were allocated per flask and were treated as a pseudoreplicate that was nested within flasks. Treatment flasks were bubbled with nitrogen until they reached an oxygen concentration of 2.0 ± 0.5 mg/L O_2 and monitored with a YSI Model 55 handheld dissolved oxygen meter. Control flasks were bubbled with compressed air to reach a concentration that was > 6 mg/L O_2. Once treated, flasks were sealed with stoppers to prevent oxygen fluctuations. They were monitored at least twice a day with additional bubbling of nitrogen or compressed air occurring as needed. BPA solutions were created daily to maintain a consistent exposure. Once the exposure ended at late hatching, larvae were transferred to fresh flasks with dechlorinated tap water, incubated at 28 ± 0.5 °C, and fed twice a day until 10 dpf.

2.5. Measurements

Exposures ended at late hatching when > 50% of the larvae in a flask had reached the end of the hatching period, which was indicated by a protruding mouth (Kimmel et al., 1995). At this time, they were transferred to a 1 mL well containing untreated, dechlorinated, tap water incubated to 28 ± 0.5 °C. Video was recorded five minutes after transfer in order to allow cardiovascular parameters to return to a resting state. Recording of beating hearts and blood flowing through caudal vessels was conducted using an inverted microscope (Leica DMIRB) with a temperature controlled (28 °C) stage (Harvard Apparatus) and a high speed camera (Red Lake MASD) recording at 125 frames/s (Morgan Hill, CA). Individuals were recorded up to 10 s at 10× magnification. Videos were recorded at late hatching and 10 dpf. At 10 dpf, larvae were anesthetized with 0.01% MS 0.222 solution for no > 2 min in order to reduce movement. Unanesthetized animals were photographed daily so that eye size could be used as indicators of development until 10 dpf.

Videos were analyzed for a number of parameters including heart rate (ƒH), stroke volume (end diastolic volume and end systolic volume), cardiac output (Q), and red blood velocity in caudal vessels. Images were analyzed for eye area using Image Pro Software (Silver Spring, MD). Cardiac parameters were calculated as described by Bagatto and Burggren (2006). Briefly, ventricular volume was measured at systole and diastole by drawing lines across the major length and width axes for 3 beats per individual. The equation for volume of a parabolic spheroid ($V = 4/3\pi ab^2$) was used to calculate ventricular volume. Stroke volume (S_v) was calculated by subtracting end systolic volume (ESV) from end diastolic volume (EDV). ƒH was calculated by measuring the length of time for at least 8 heart beats. Cardiac output (Q) was calculated by multiplying ƒH and S_v.

Red blood cell velocity was measured by taking the differential of subsequent video frames of caudal arteries and veins for digital motion analysis (Schwerte and Pelster, 2000). In the differential, grey scale correlates with the amount of movement, with whiter areas representing the most movement. The distance an erythrocyte traveled from one frame to the next can be measured by drawing a line across that area. This was performed seven times per vessel for each individual. Eye area was measured by drawing a perimeter around the eye.

2.6. Statistics

Because individual larvae were nested within flasks with up to 15 embryos (n ≥ 8), a nested two-way analysis of variance (ANOVA) with a post hoc Tukey's multiple comparison test was used to explore between treatment differences. BPA, oxygen concentration, and their interaction were also included in the ANOVA as a means to determine if the treatments interacted to affect cardiovascular variables. An analysis of covariance (ANCOVA) was conducted for eye area with age as the continuous covariate and BPA, oxygen, and their interactions as discrete effects. Statistical analysis was completed with JMP Pro 12 (SAS Institute) with an alpha of $p < 0.05$.

3. Results

3.1. Late hatching

At late hatching (72–96 hpf), larvae exhibited a typical response to hypoxia in comparison to previous work at similar oxygen concentrations (Moore et al., 2006). With hypoxia alone, ESV and EDV decreased by 23.4 and 18.8%, respectively, while ƒH decreased 11.3% with no corresponding change in cardiac output ($p < 0.0001$, $p = 0.0007$, $p < 0.0001$, $p = 0.1$, Fig. 1). BPA and hypoxia did not interact to affect ESV, EDV, S_v, ƒH, or Q ($p = 0.13$, $p = 0.58$, $p = 0.77$, $p = 0.27$, $p = 0.82$).

While no patterns were observed with increasing concentration of BPA, some dose specific effects were observed. S_v, for instance, was 22% greater with 1 μg/L BPA during hypoxia in comparison to the hypoxia control ($p = 0.03$, Fig. 1A). ƒH was also 14 and 8% lower with hypoxia at the 0.1 and 1 μg/L concentration, respectively ($p = 0.01$, $p = 0.002$, Fig. 1B). Cardiac output increased by 23.5 and 22.2% with 0.01 and 1 μg/L, respectively, in comparison to the hypoxia control