Progress in Neuro-Psychopharmacology & Biological Psychiatry 56 (2015) 221–226

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Acute and chronic ethanol exposure differentially alters alcohol dehydrogenase and aldehyde dehydrogenase activity in the zebrafish liver

Steven Tran [a,*], Magda Nowicki [b], Diptendu Chatterjee [b], Robert Gerlai [a,b]

[a] Department of Cell and Systems Biology, University of Toronto Mississauga, Canada
[b] Department of Psychology, University of Toronto Mississauga, Canada

ARTICLE INFO

Article history:
Received 13 August 2014
Received in revised form 16 September 2014
Accepted 29 September 2014
Available online 5 October 2014

Keywords:
Alcohol dehydrogenase
Aldehyde dehydrogenase
Chronic ethanol
Tolerance
Zebrafish

ABSTRACT

Chronic ethanol exposure paradigms have been successfully used in the past to induce behavioral and central nervous system related changes in zebrafish. However, it is currently unknown whether chronic ethanol exposure alters ethanol metabolism in adult zebrafish. In the current study we examine the effect of acute ethanol exposure on adult zebrafish behavioral responses, as well as alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activity in the liver. We then examine how two different chronic ethanol exposure paradigms (continuous and repeated ethanol exposure) alter behavioral responses and liver enzyme activity during a subsequent acute ethanol challenge. Acute ethanol exposure increased locomotor activity in a dose-dependent manner. ADH activity was shown to exhibit an inverted U-shaped curve and ALDH activity was decreased by ethanol exposure at all doses. During the acute ethanol challenge, animals that were continuously housed in ethanol exhibited a significantly reduced locomotor response and increased ADH activity, however, ALDH activity did not change. Zebrafish that were repeatedly exposed to ethanol demonstrated a small but significant attenuation of the locomotor response during the acute ethanol challenge but ADH and ALDH activity was similar to controls. Overall, we identified two different chronic ethanol exposure paradigms that differentially alter behavioral and physiological responses in zebrafish. We speculate that these two paradigms may allow dissociation of central nervous system-related and liver enzyme-dependent ethanol induced changes in zebrafish.

1. Introduction

Ethanol (alcohol or ethyl alcohol) is primarily metabolized in the liver in a two-step process by alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH). In humans, ethanol is converted into acetaldehyde by a class 1 alcohol dehydrogenase (ADH1), a class of enzymes responsible for the oxidation of alcohols to aldehydes (Hoog and Ostberg, 2011). Class III alcohol dehydrogenases have also been reported to contribute to ethanol metabolism but to a lesser extent (Haseba et al., 2003, 2006). The catalytic oxidation of ethanol to acetaldehyde by alcohol dehydrogenase requires nicotinamide adenine dinucleotide (NAD^+), a co-enzyme which is reduced to NADH. In the second step, acetaldehyde is converted into acetic acid by aldehyde dehydrogenase 2 (ALDH2), the primary enzyme in the liver responsible for the oxidation of aldehydes to its corresponding carboxylic acids also by reducing NAD^+ to NADH (Hoog and Ostberg, 2011).

Current evidence suggests that zebrafish metabolize ethanol in a similar manner. In zebrafish, 3 different genes encoding alcohol dehydrogenases have been identified. The first cDNA cloned was ADH3, a class III alcohol dehydrogenase with a predicted amino acid sequence with 81% similarity to the human sequence (Dasmahapatra et al., 2001). ADH3 mRNA was detected in the adult zebrafish liver and may encode a protein that metabolizes ethanol at a lower rate compared to class I alcohol dehydrogenases similar to humans (Dasmahapatra et al., 2001). Subsequently, two class I alcohol dehydrogenases were identified in zebrafish. The two cDNA sequences were cloned (ADH8A and ADH8B) and their predicted amino acid sequence was 72 and 68%, respectively, similar to the human ADH1 (Reimers et al., 2004). Notably, the ADH8B isoform was unable to metabolize ethanol, whereas the ADH8A metabolized ethanol at a similar rate to that of the human ADH1 (Reimers et al., 2004). In addition, the gene encoding the zebrafish aldehyde dehydrogenase was also recently identified. cDNA for the zebrafish ALDH2 gene was cloned and the

Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; ADH1, class 1 alcohol dehydrogenase; ADH3, class 3 alcohol dehydrogenase; NAD^+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide reduced; cDNA, complementary deoxyribonucleic acid; ALDH1, aldehyde hydrogenase 1; ALDH2, aldehyde dehydrogenase 2; k_m, Michaelis constant; ZFIN, Zebrafish Information Network; ANOVA, analysis of variance; HSD, Honestly Significant Difference; S.E.M., standard error of the mean.

* Corresponding author at: Department of Cell and Systems Biology, University of Toronto Mississauga, 3359 Mississauga Road North, Rm 1022D, Mississauga, Ontario L5L 1C6, Canada. Tel.: +905 569 4277 (office), +905 569 4257 (lab).
E-mail address: stevenhuy.tran@mail.utoronto.ca (S. Tran).

http://dx.doi.org/10.1016/j.pnpbp.2014.09.011
0278-5846/

predicted amino acid sequence was found to be 75% similar to that of the mammalian ALDH2 (Lassen et al., 2005). The gene was then expressed using the baculovirus expression system and the purified protein was found to break down acetaldehyde at a rate comparable to the human ALDH2 (Lassen et al., 2005). Since the primary enzymes responsible for the breakdown of ethanol and its metabolite are present in zebrafish and are functionally similar to the mammalian enzymes, ethanol metabolism is likely to occur in a similar manner in these species.

Zebrafish have become a popular animal model for examining the mechanisms underlying ethanol's actions in vivo (Dlugos and Rabin, 2003; Damodaran et al., 2006; Rico et al., 2007). It is a small vertebrate with similar physiology and neurochemistry compared to mammals including humans (Rico et al., 2011b). A number of practical advantages including small size and high fecundity make it an attractive animal model for high-throughput research (Gerlai et al., 2000). However, the greatest advantage (in our opinion) is the method of drug delivery. Water soluble drugs such as ethanol can be mixed directly with the tank water and is subsequently taken up by the immersed zebrafish (Dlugos and Rabin, 2003; Rosemberg et al., 2012; Tran et al., in press). Drug immersion is less invasive compared to injection or inhalation–vaporization based procedures used in rodents and have been successfully utilized in both larvae (Lockwood et al., 2004; Ramcharitar and Ibrahim, 2013) and adult zebrafish (Dlugos and Rabin, 2003; Kily et al., 2008; Mathur and Guo, 2011).

Studies have shown that acute ethanol exposure alters a number of behavioral responses in adult zebrafish including locomotor activity, preference for the bottom, and erratic movement (Pannia et al., 2014; Rosemberg et al., 2012; Tran and Gerlai, 2013). These behavioral responses are accompanied by changes in the levels of neurotransmitters (Chatterjee et al., 2014; Gerlai et al., 2009; Tran et al., in press), enzyme activity and gene expression in the zebrafish brain (Rico et al., 2007; Rosemberg et al., 2010). Chronic (long-term) exposure to ethanol has also been shown to alter behavioral and neurochemical responses in zebrafish (Chatterjee et al., 2014; Dlugos and Rabin, 2003; Dlugos et al., 2011; Gerlai et al., 2009; Rico et al., 2011a; Tran et al., in press). Although chronic alcohol exposure has not been operationally defined in zebrafish, current studies suggest that as few as 7 days of continuous or 8 days of repeated ethanol exposure may be enough to induce observable changes in zebrafish behavior (Egan et al., 2009; Mathur and Guo, 2011). For example, zebrafish continuously housed in ethanol for 3 weeks respond to a subsequent acute ethanol challenge with an attenuated locomotor and neurochemical response (Chatterjee et al., 2014; Tran et al., in press). Long-term continuous ethanol exposure alters neurochemical levels, enzymatic activity, gene and protein expression in the adult zebrafish brain (Chatterjee et al., 2014; Damodaran et al., 2006; Pan et al., 2011; Rico et al., 2011a; Tran et al., in press). Repeated intermittent exposure to ethanol has also been shown to alter behavioral responses and gene expression (Blaser et al., 2010; Kily et al., 2008; Mathur and Guo, 2011). Changes within these systems suggest the development of tolerance at the level of the central nervous system. However, following chronic ethanol exposure genes encoding liver enzymes responsible for the breakdown of ethanol such as alcohol dehydrogenase and aldehyde dehydrogenase have been shown to be upregulated in the zebrafish brain (Pan et al., 2011). Although these changes were detected in the brain, it may suggest adaptation in the periphery as well (i.e. the liver). It is currently unknown whether chronic ethanol exposure alters the activity of alcohol dehydrogenase and aldehyde dehydrogenase in the zebrafish liver.

In the current study we first examine the effect of acute ethanol exposure on the locomotor responses and total ADH and ALDH activity in the zebrafish liver. Subsequently, we determine whether chronic ethanol exposure (repeated and continuous) alters locomotor responses and the activity of both enzymes in a subsequent acute ethanol challenge.

2. Methods

2.1. Animals and housing

9 and 24 month old zebrafish (50% males and females) of the AB strain (progenitors obtained from the ZFIN Center (Eugene, Oregon, USA)) were used for the current study (24 month old zebrafish were used for experiment 1 and 9 month old zebrafish were used for experiment 2). The AB strain was chosen based on previous studies demonstrating robust behavioral and neurochemical changes in response to acute and chronic ethanol exposure (Chatterjee et al., 2014; Tran et al., in press). Zebrafish were raised and housed in 37 L glass tanks containing system water supplemented with 60 mg/L of instant ocean sea salt (Big Al's Aquarium) with biological filtration prior to ethanol exposure. Water quality parameters were monitored on a weekly basis and maintained at optimal conditions (Conductivity: 100–300 μS, Temperature: 26–28 °C, pH: 6.8–7.2). Additional details on housing conditions and maintenance are described elsewhere (Tran and Gerlai, 2013).

2.2. Experimental design and procedure

2.2.1. Experiment 1

Ethanol naïve zebrafish were netted from their group housing tanks and were individually exposed to 0.0%, 0.25%, 0.50%, or 1.0% v/v ethanol in a 37 L tank containing 28 L water (n = 16 per group) for 30 min. The duration of exposure is based on prior studies demonstrating the biphasic effect of ethanol, with maximal locomotor stimulation at 30 min in 1.0% v/v ethanol (Tran and Gerlai, 2013). The behavioral tests were conducted in 37 L glass tanks with white corrugated plastic sheets on the back and sides of these experimental tanks, which obscured external cues and provided a uniform testing environment. Water quality parameters in the experimental tanks matched those of the housing tanks with the exception of the addition of ethanol. Zebrafish motor responses were recorded using a video camera from the front view during acute exposure. Following the 30 min acute ethanol exposure, zebrafish were immediately netted and decapitated with the body stored at −80 °C until processing.

2.2.2. Experiment 2

A group of zebrafish (n = 20) was continuously housed in ethanol to induce ethanol tolerance using a previously established chronic ethanol exposure paradigm that utilized a dose escalation procedure (Tran and Gerlai, 2013, in press). Briefly, zebrafish were initially housed in 37 L tanks containing 0.125% v/v ethanol for 4 days, followed by 0.25% v/v for 4 days and 0.375% for 4 days, for a total of 12 days. Starting on day 13, zebrafish were then housed in 0.50% v/v ethanol for 10 days, i.e. the experimental subjects were continuously exposed to ethanol for a total of 22 days. During continuous ethanol exposure, biological filtration was turned off to prevent the death of bacterial fauna in the filters. Water changes occurred every other day to remove biological waste and ensure appropriate ethanol concentrations.

On day 13, a second group of zebrafish (n = 22) received intermittent exposure to ethanol (fluctuating concentration) on a daily basis for 10 consecutive days, an exposure regimen that more closely resembled human binge drinking. Briefly, zebrafish were exposed to 1.0% ethanol for 1 h every day from 11:00 to 12:00 for 10 consecutive days. The concentration, exposure duration and number of exposures were based on a number of previous studies showing behavioral and gene expression related changes following repeated intermittent exposure to ethanol (Blaser et al., 2010; Kily et al., 2008; Mathur and Guo, 2011). Finally, a third group (n = 20) was handled in an identical manner as group 2 but without ethanol to serve as controls (i.e. repeated exposure to freshwater for 10 consecutive days). All tanks were aerated using aeration stones to ensure maximal oxygen levels, specifically during the 1 h acute exposures. On day 23, all groups were challenged with an acute dose of 1.0% v/v ethanol in a 37 L tank for 30 min. The