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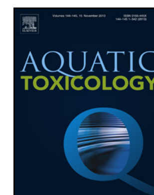

Environmental levels of the antidepressant venlafaxine impact the metabolic capacity of rainbow trout

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ABSTRACT

The antidepressant venlafaxine is detected at parts per billion levels in tertiary-treated municipal wastewater effluent. However, the impact of this serotonin–norepinephrine reuptake inhibitor (SNRI) on non-target aquatic animals is poorly understood. We tested the hypothesis that environmentally relevant levels of venlafaxine disrupt the highly conserved cortisol and glucose response to stress in rainbow trout (*Oncorhynchus mykiss*). Juvenile trout were exposed to venlafaxine (0, 0.2 and 1.0 μg/L) in a static system with daily renewal for seven days. The fish were then subjected to an acute handling disturbance and sampled either prior to (0 h) or 1, 4 and 24 h after stressor exposure. Venlafaxine exposure did not affect the handling disturbance-mediated transient elevation in plasma cortisol levels or target tissue glucocorticoid receptor expression. The drug exposure disrupted the interrenal steroidogenic capacity, including altered handling stressor-mediated changes in mRNA abundances of steroidogenic acute regulatory protein and cytochrome P450 side chain cleavage. The handling stressor-induced transient elevations in plasma glucose levels were significantly reduced in the venlafaxine-exposed fish. This was not accompanied by changes in liver glycogen content, glucose transporter 2 mRNA abundance or the glycolytic capacity, whereas the capacity for gluconeogenesis and amino acid catabolism were enhanced. Venlafaxine also brought about changes in the gill of trout, including enhanced lactate dehydrogenase activity and Na^+–K^+ ATPase protein expression, while the Na^+–K^+ ATPase enzyme activity was reduced. Collectively, our results demonstrate that venlafaxine at levels detected in the aquatic environment impacts tissue metabolic capacities and may compromise the adaptive responses to an acute stressor in rainbow trout.

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1. Introduction

Human pharmaceuticals are constantly being introduced into the aquatic environment through treated sewage effluent, leaching, and direct disposal (Daughton and Ternes, 1999). Even after tertiary treatment, municipal wastewater treatment plants are not able to remove all pharmaceuticals from the influent sewage, resulting in their frequent detection in municipal wastewater effluents (MWWEs) (see reviews by Corcoran et al., 2010; Metcalfe et al., 2010). Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), is the most commonly studied antidepressant in the aquatic environment and it has been shown to affect stress and reproductive performances in fishes, including rainbow trout (*Oncorhynchus mykiss*) (Mennigen et al., 2011). Recently, the use of venlafaxine, a serotonin–norepinephrine reuptake inhibitor (SNRI) antidepressant with improved therapeutic action and fewer side effects than fluoxetine (Horst and Preskorn, 1998), has been on the rise. Its major metabolite *O*-desmethylvenlafaxine has a similar potency for inhibiting neurotransmitter reuptake (Roseboom and Kalin, 2000). At present, venlafaxine and its major active metabolite are among the highest detected antidepressants in tertiary-treated MWWEs and aquatic systems. Environmental values of venlafaxine were reported as high as 2.19 μg/L in MWWE (St. Paul, MN, USA) and 1.31 μg/L in an effluent-dominated creek (Pecan Creek, Denton, TX) (Schultz et al., 2010). Locally, in the Speed River (Guelph, ON, Canada), venlafaxine and *O*-desmethylvenlafaxine were measured

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at 0.253 μg/L and 0.486 μg/L, respectively, at a site downstream of the tertiary sewage treatment plant (Ings, 2011). Despite this, very little is known about the impact of this pharmaceutical drug on non-target aquatic organisms, including fish.

Recently we showed that exposure to tertiary-treated MWWE impairs the cortisol and metabolic responses to an acute stressor in rainbow trout (Ings et al., 2011). The cortisol stress response in teleosts is part of an evolutionarily conserved adaptive response that allows animals to restore homeostasis when challenged by stressors. To that end, cortisol and catecholamines induce several metabolic alterations to help to overcome the threat. Briefly, after exposure to a stressor, the primary fight or flight response involves the stimulation of the sympathetic division of the autonomic nervous system and results in increased production of catecholamines, especially epinephrine and norepinephrine, within seconds of stressor perception (Fabbri et al., 1998; Reid et al., 1998; Vijayan et al., 2010). Catecholamines activate alpha- and beta-adrenergic signaling leading to rapid cardiovascular adjustments and glycogen breakdown to enhance oxygen and glucose delivery to tissues, respectively (Reid et al., 1998; Vijayan et al., 2010). The activation of the hypothalamus–pituitary–interrenal (HPI) axis in fish lags behind the sympathetic response and begins with the release of corticotropin releasing factor (CRF) from the hypothalamus triggering adrenocorticotropic hormone (ACTH) release from the pituitary, which stimulates cortisol biosynthesis and release from the interrenal cells of the head kidney (Vijayan et al., 2010). The key rate determining steps in steroidogenesis involve the steroidogenic acute regulatory protein (StAR), which transports cholesterol from the outer to the inner mitochondrial membrane, followed by conversion of cholesterol to pregnenolone by cytochrome P450 side chain cleavage (P450scc) (Aluru et al., 2005). The elevated cortisol levels in response to stress mediate target effects, including energy substrate mobilization, by activating glucocorticoid receptor (GR) and/or mineralocorticoid receptor (MR) signaling (Prunet et al., 2006; Bury and Sturm, 2007).

Given that venlafaxine is the predominant antidepressant detected in MWWE lately, and since very little is known about its role in non-target animals, we hypothesized that levels of this drug detected in the aquatic environment elicits a stress response and also were sufficient to disrupt the adaptive cortisol and glucose performances to subsequent stressors in fish. This was tested using rainbow trout as a model with waterborne exposure to two concentrations of venlafaxine [0.2 μg/L (0.64 nM) or 1.0 μg/L (3.18 nM)] based on levels detected in the aquatic environment (Metcalfe et al., 2010). We examined the impact of the drug on basal and an acute handling stressor-stimulated steroid biosynthetic capacity in the interrenal tissue as well as target tissue metabolic capacities. At the interrenal level, *StAR* and *P450scc* transcript abundance was measured along with plasma cortisol levels to assess cortisol biosynthetic capacity, as these are targets for xenobiotic impact (Aluru et al., 2005; Arukwe, 2008; Sandhu et al., 2014). Tissue metabolic capacity was assessed by measuring plasma glucose, glycogen content, glucose transporter type 2 (*GLUT2*) transcript abundances and activities of enzymes involved in key metabolic pathways, including glycolysis [hexokinase (HK), glucokinase (GK), pyruvate kinase (PK) and lactate dehydrogenase (LDH)], gluconeogenesis [phosphoenolpyruvate carboxykinase (PEPCK)] and amino acid catabolism [alanine aminotransferase (AlaAT) and aspartate aminotransferase (AspAT)] in the liver of trout (Vijayan et al., 2006). Also, metabolic capacity of the gill, a key target tissue for glucose utilization during stress, was assessed by measuring Na^+–K^+ ATPase activity and protein expression, as well as activities of key glycolytic enzymes, including HK, PK and LDH (Mommsen, 1984; Soengas et al., 1995). In addition liver GR protein expression was used as a marker of target tissue responsiveness to cortisol (Vijayan et al., 2005).

2. Material and methods

2.1. Animals

Juvenile rainbow trout (average body mass 8.5 ± 2.5 g) were obtained from Rainbow Springs Hatchery (Thamesford, ON) and maintained in flow-through well water (13 °C, 400 mg $CaCO_3$/L, pH 8.0, Alsop et al., 2009). Fish were acclimated for two weeks in 2000 L tanks and photoperiod was on a 12 h day/night cycle. Fish were fed to satiety daily during the acclimation period with a commercial trout chow (Martin Feed Mills, Elmira, ON).

2.2. Experimental design

The study involved exposing trout to different concentrations of venlafaxine over a period of seven days. There were duplicate tanks (30 L) for each treatment and 16 fish in each tank. The tanks were aerated and their water temperature maintained by leaving them in a larger tank (2000 L) with flow-through well water exactly as mentioned above. Fish were acclimated in the experimental setup tanks for one week prior to the exposure. During acclimation, the fish were fed to satiety (once daily) and the tanks were cleaned and 40% water replenished (12 L) daily. Fish were exposed to either 0 (control), 0.2 μg/L (0.64 nM) or 1.0 μg/L (3.18 nM) nominal venlafaxine concentrations (venlafaxine hydrochloride; Sigma-Aldrich, St. Louis, MO; dissolved in water) to mimic environmental levels (Metcalfe et al., 2010). Food was withheld for the duration of the experiment and the 40% daily replacement water was supplemented with venlafaxine at the appropriate concentrations before addition to the fish tanks. This static exposure system with daily water renewal was shown to accurately maintain nominal venlafaxine concentrations over the 7-d exposure period (Melnyk-Lamont, 2014).

Following the 7-d exposure, an initial group of fish were sampled (0 h) while the rest were subjected to a handling disturbance (consisting of 30 s netting and 5 m crowding at 2 fish/L) and sampled at 1, 4 and 24 h post-handling stressor exposure. The 7-d drug exposure period was sufficient to affect brain monoamine levels in trout (Melnyk-Lamont, 2014). Sampling consisted of quickly netting four fish from each replicate tank (eight per treatment) at the respective time-points. Fish were euthanized with an overdose of 2-phenoxyethanol (1:1000 dilution; Sigma-Aldrich), weighed and bled by caudal severance into heparinized capillary tubes. The whole procedure took less than 5 min for each tank. The plasma was collected after centrifugation of the capillary tubes at approximately 5000 × *g* for 4 min and was stored in microfuge tubes at −80 °C for later hormone and metabolite assay. Liver, gill, and head kidney were removed and snap frozen on dry ice and were stored at −80 °C for later analysis. The experimental protocol was approved by the University of Waterloo Animal Care Committee and was conducted in accordance with the Canadian Council for Animal Care guidelines for the use of fish in research.

2.3. Plasma glucose and cortisol levels

Plasma glucose was measured colorimetrically according to Hancock et al. (2004), while plasma cortisol levels were determined using a commercially available competitive ELISA kit (Neogen, Lexington, KY, USA) according to the manufacturer's instructions.

2.4. Tissue preparation

Liver and gill tissues were sonicated in ice cold 50 mM Tris buffer (pH 7.5) with protease inhibitors (Roche Diagnostics GmbH, Mannheim, Germany). Protein concentrations of the samples were determined using the bicinchoninic acid (BCA) reagent

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