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Reprint of: Effects of the antidepressant venlafaxine on fish brain serotonin and predation behavior☆

Joseph H. Bisesi Jr[a,*], William Bridges[b], Stephen J. Klaine[a]

[a] Institute of Environmental Toxicology, Department of Biological Sciences, Clemson University, 509 Westinghouse Road, P.O. Box 709, Pendleton, SC 29670, USA

[b] Department of Mathematical Sciences, 220 Parkway Drive, Clemson University, Clemson, SC 29634, USA

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ABSTRACT

Antidepressants that enter receiving waters through final treated wastewater effluent have exhibited relatively low acute toxicity in traditional fish tests at currently measured concentrations. However, the psychotropic mode of action of these compounds warrants examination of the behavioral effects these chemicals may have on aquatic organisms. Previous research has demonstrated that exposure to the antidepressant fluoxetine causes decreased brain serotonin levels in fish and results in a decreased ability to capture prey. Another antidepressant, venlafaxine, has been found at low μg/L concentrations in final treated wastewater effluent. The objective of this study was to quantify the effects of venlafaxine on fish predation behavior and determine if this effect was correlated with changes in brain neurotransmitter concentrations. The predator prey bioassay used hybrid striped bass (*Morone saxatilis x Morone chrysops*) as the predator and fathead minnows (*Pimephales promelas*) as prey. Bass were exposed to venlafaxine (0–500 μg/L) for a period of 6 days and then allowed to recover for 6 days. During both exposure and recovery, bass were fed four minnows every third day. The time to capture the minnows was quantified and compared among treatments to determine if there was an effect on predation behavior. Brain tissue was analyzed for serotonin, norepinephrine, and dopamine, to determine the relationship between exposure concentration, brain monoamine levels, and predation behavior. Results indicated that venlafaxine exposures increased time to capture prey 1 and 2 by day 6 for the 250 and 500 μg/L treatments. Time to capture prey 3 was increased for all venlafaxine treatments by day 6. Venlafaxine caused a statistically significant decrease in brain serotonin concentrations that initially decreased in a dose dependent manner before reaching a steady state by the end of exposures for all treatments. No significant, dose-dependent changes in dopamine or norepinephrine were seen. Brain serotonin alone did not adequately explain behavioral results. Serotonin response in other tissues as well as peripheral effects may have accounted for additional behavioral responses after brain serotonin reached a depressed steady state.

1. Introduction

Venlafaxine is commonly prescribed antidepressant pharmaceutical for the treatment of many depressive disorders (Bymaster et al., 2001). Recent analysis of antidepressants in environmental matrices has revealed that venlafaxine is commonly found in treated waste water effluent and receiving water due to inadequate removal during treatment. Concentrations measured in environmental samples have ranged from the low ng/L level to as high as 2 μg/L in wastewater effluent (Schultz et al., 2010; Gonzalez Alonso et al., 2010; Gracia-Lor et al., 2010; Metcalfe et al., 2010; Schultz and Furlong, 2008).

While maximum concentrations of venlafaxine found in aquatic matrices are higher than other antidepressants, such as fluoxetine (Vasskog et al., 2008; Kolpin et al., 2002; Metcalfe et al., 2003b), the toxicity of venlafaxine to aquatic organisms has not been extensively examined. Acute and chronic toxicity using US EPA standard methods has not currently been reported for venlafaxine. But sub chronic (6 weeks) exposure of zebrafish at 10 μg/L venlafaxine has been shown to decrease egg production and have effects on kidney tubule morphology (Galus et al., 2013). The potential of

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* Corresponding author at: Center for Environmental and Human Toxicology, Department of Environmental and Global Health, University of Florida, 2187 Mowry Road, Box 110885, Gainesville, FL 32611, USA. Tel.: +4103203967; fax: +8646462277.
E-mail address: jbisesi@phhp.ufl.edu (J.H. Bisesi Jr).

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venlafaxine to act as a behavior modulator has also been considered by a few researchers. Schultz et al. (Schultz et al., 2011) reported 40% and 25% mortality at 305 and 1,105 ng/L venlafaxine, respectively, though there was no effect on male fathead minnow reproductive behavior during these exposures. Exposures of embryonic fathead minnows to 500 ng/L venlafaxine caused increased time before initiation of escape response (latency), and decreased total escape response to stimuli after hatching. Fathead minnows exposed to 5,000 ng/L of venlafaxine for 12 days after hatching exhibited increased latency and decreased escape responses to stimuli (Painter et al., 2009).

In addition to studies examining the reproductive and behavioral effects of venlafaxine, efforts have been made to understand the mechanisms of venlafaxine both *in vitro* and *in vivo*. Venlafaxine has been shown to block epinephrine induced glucose production in rainbow trout (*Oncorhynchus mykiss*) hepatocytes which may have implications for stress response in exposed fish (Ings et al., 2012). Though uptake of venlafaxine into fish neural tissue has been shown to be limited during environmentally relevant exposures (Schultz et al., 2010; Schultz et al., 2011) gene expression patterns in the brains of fathead minnows exposed to venlafaxine reveal upregulation of genes related to neural development and regulation of action potentials (Thomas et al., 2012). But researchers have yet to examine the targeted outcome of venlafaxine, modulation of serotonin.

Venlafaxine is designed to impact human behavior by modulating levels of the brain neurotransmitters serotonin and norepinephrine. This is achieved by blocking the serotonin and norepinephrine reuptake transporter on presynaptic neurons effectively defeating the negative feedback mechanism of these neurotransmitters, thus increasing levels in the chemical synapse (Schafer, 1999). Venlafaxine is referred to as a serotonin and norepinephrine reuptake inhibitor (SNRI) and like other selective serotonin reuptake inhibitors (SSRIs) has been shown to have a positive effect on depressive disorders (Schafer, 1999; Kreke and Dietrich, 2008). These monoaminergic biochemical pathways are highly conserved in fish (Kreke and Dietrich, 2008) and have been implicated in a number of behaviors including feeding (Gaworecki and Klaine, 2008), locomotion (Winberg and Nilsson, 1993), and aggression (Overli et al., 1998).

Previous work by Gaworecki and Klaine (Gaworecki and Klaine, 2008) showed that exposure to the antidepressant fluoxetine decreased brain serotonin levels of hybrid striped bass (*Morone saxatilis x Morone chrysops*). Decreased serotonin was correlated with an increased time to capture prey, though concentrations were two orders of magnitude higher than reported values in environmental samples. The fact that venlafaxine shares a common mode of action to fluoxetine, yet has an additional receptor target warrants investigation of its effect on brain serotonin and behavior in fish. The objective of this study was to quantify the effects of venlafaxine on fish predation behavior and determine if this effect was correlated with changes in brain neurotransmitter concentrations. We hypothesized that exposure to venlafaxine would cause a dose dependent decrease in brain serotonin and norepinephrine concentrations correlating with increases in time to capture prey. We also hypothesized that the biochemical mode of action was reversible after a depuration period and would result in recovery of prey capture ability.

2. Materials and methods

2.1. Test chemicals

Venlafaxine hydrochloride (LKT laboratories), sodium hydroxide, monochloroacetic acid, HPLC grade methanol, acetone, acetonitrile, glacial acetic acid, perchloric acid, tetrahydrofuran, and triethylamine were purchased from Fisher Scientific (Pittsburgh, PA, USA). Trace metal grade concentrated hydrochloric acid was purchased from Spectrum Chemicals (Gardena, CA, USA). MS-222 (Tricaine-S) was purchased from Western Chemical (Ferndale, WA, USA). Serotonin creatinine sulfate complex, dopamine HCl, norepinephrine HCl, 3,4-dihydroxybenzlamine (DHBA), 5-hydroxyindoleactic acid (5-HIAA), sodium octyl sulfate, and disodium EDTA dihydrate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Water used for analytical procedures was ultrapurified using a Milli-Q Super-Q Filtration system (Millipore™, Billerica, MA, USA) with a measured resistivity of 18.2 MΩ cm.

2.2. Test fish

All experiments performed on fish in this study were approved by the Clemson University Institutional Animal Care and Use Committee. Hybrid striped bass (*Morone saxatilis x Morone chrysops*) were generously donated from Southland Fisheries (Columbia, SC, USA) as fingerlings. Fish were held in 450 L circular holding tanks in the cherry farm aquatic research lab at Clemson University (Clemson, SC, USA). Holding tanks were maintained as flow through systems, constantly supplied with fresh water (pH 6.28 ± 0.17, hardness 24 mg/L as $CaCO_3$, alkalinity 10 mg/L as $CaCO_3$) from Lake Hartwell (Clemson, SC, USA). Before reaching the holding systems, water was filtered through a gravel bed, and sterilized with UV radiation. Water temperature was maintained between 22 and 25 °C using a mixture of ambient and heated (via inline heater) or chilled water (via inline chiller), depending on incoming water temperature. Water was constantly aerated with air stones and agitators (Boatcycle Inc., Henderson, TX, USA). During holding, bass were fed a commercial diet (Finfish Silver 4.5 mm slow sink) purchased from Zeigler Bros, Inc. (Gardners, PA, USA).

Fathead minnows were purchased from Anderson Minnow Farm (Lonoke, AR, USA). Minnows were held in 100 L holding tanks for using the same water as described above. During holding, minnows were fed a commercial diet (Tetramin® Tropical Flakes) purchased from Dr's Foster and Smith, Inc. (Rhinelander WI, USA).

2.3. Bass training

Because bass were grown on a pelleted feed, conditioning to live diet was required before the initiation of behavioral assays. Hybrid striped bass (mass: $249.68\,g \pm 80.09$, length: $232.81\,mm \pm 25.63$) were randomly removed from holding tanks and placed into a separate 300 L rectangular holding tank for group training. Water conditions in this tank were the same as the holding tanks. Bass were starved for 3 days prior to the start of group training. On day 0 of training, bass were fed four minnows/bass in the group training tank. Bass were fed an additional four minnows/bass on day 3 and day 6. Hybrid striped bass were removed from the group training tank and placed into individual aquaria (one bass/tank). Bass were allowed to acclimate to the aquaria for 9 days. During the acclimation period, bass were fed four fathead minnows (approximately four cm long) on days 3 and 6. The time to capture each minnow was recorded for selection of fish for exposures.

2.4. Venlafaxine exposures

Exposures took place in 113 L aquaria measuring $92.1 \times 32.4 \times 40$ cm purchased from Deep Sea Aquatics (Garland, TX, USA). Tanks were filled to 80 L and marked to ensure uniform exposure volume. Each tank had a 1.9 cm PVC vertical standpipe drilled into the front panel for control of water volume when maintained as a flow through system. Water for exposures was the same as described above with additional filtration supplied by a multi-resin filtration system (Water and Power