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Serotonin antagonists induce anxiolytic and anxiogenic-like behavior in zebrafish in a receptor-subtype dependent manner

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ABSTRACT

Motor function and anxiety-like responses are easily quantifiable in zebrafish, a novel model organism for behavioral pharmacology. Activation of serotonin receptors through the use of selective agonists has been shown to alter anxiety-like behaviors in zebrafish. However, few studies have examined the effect of blockade of specific serotonin receptors. In the current study, we examine the effect of 4 serotonin receptor antagonists selective for 5-HT1$_A$, 5-HT1$_{B/D}$, 5-HT$_2$, and 5-HT$_3$ receptors on zebrafish motor and anxiety-like responses. Exposure to the receptor antagonists did not change baseline motor responses. However, when placed in a novel environment, zebrafish previously exposed to GR 55562 (5-HT1$_{B/D}$ antagonist) exhibited reduced anxiety-like behavior, whereas zebrafish previously exposed to p-MPPF (5-HT1$_A$ antagonist), Ketanserin (5-HT$_2$ antagonist), or Ondasetron (5-HT$_3$ antagonist) exhibited increased anxiety-like behaviors. These results show that drugs developed for mammalian serotonin receptors are efficacious in the zebrafish too, a finding that demonstrates evolutionary conservation of the serotoninergic system. The results also imply that zebrafish may be an appropriate animal model for examining the serotonergic neurotransmitter system in vertebrates.

Serotonergic neurotransmission mediates a large number of behaviors and processes including pain perception (Horjales-Araujo et al., 2013), learning (Izquierdo et al., 2012; Palminteri et al., 2012), aggression (Kulikov et al., 2012), and affect (Williams et al., 2006), including fear and anxiety (Maximino et al., 2013). At least 14 serotonin (5-hydroxytryptamine; 5-HT) receptor subtypes exist in vertebrates and are classified into seven families (5-HT$_1$–5-HT$_7$) based on their downstream effects (Barnes and Neumaier, 2011). All receptors are coupled to G-proteins with the exception of the 5-HT$_3$ receptor. 5-HT$_1$ and 5-HT$_5$ receptors are coupled to inhibitory G proteins which decrease intracellular levels of cAMP by inhibiting adenylyl cyclase. Activation of 5-HT$_4$, 5-HT$_6$ and 5-HT$_7$ receptors increases neuronal transmission by increasing intracellular levels of cAMP (Klee et al., 2012). 5-HT$_2$ receptors are excitatory receptor subtypes which couple to the G_q/G_{11} signal transduction pathway. Unlike the rest of the serotonin receptor families which are metabotropic, 5-HT$_3$ receptors are ionotropic ligand-gated ion channels. They are composed of five subunits which form a water-filled pore permeable to Na^+, Ca^{2+} and K^+. 5-HT$_3$ receptor activation leads to cation influx through the channel, causing depolarization in the post-synaptic terminal. (Walstab et al., 2010). The serotonergic system is believed to be highly conserved among vertebrates and may be a good candidate for pharmacological manipulation in a range of species including fish (Lillesaar, 2011; Marston et al., 2011).

Zebrafish have extensive homology with mammals at the genetic and neural level, and are rapidly becoming an important model organism for behavioral neuroscience. The serotonergic system in zebrafish has been shown to be physiologically and pharmacologically similar to the mammalian counterpart (Panula et al., 2010; Connors et al., 2014; Maximino et al., 2014). Furthermore, genes encoding serotonin receptors in zebrafish show a high nucleotide sequence homology to corresponding human genes (see Klee et al., 2012).

Serotonin is important in modulating a number of CNS processes including the function of neural networks that play roles in locomotion and motor function (Brustein et al., 2003; Maximino et al., 2011). For example, serotonin depletion in zebrafish larvae significantly reduces locomotor activity and induces a paralysis-like state (Airhart et al., 2012). Serotoninergic neurotransmission in zebrafish is also important in a number of other behavioral responses including anxiety-like responses (Maximino et al., 2013; Wong et al., 2013). Furthermore, pharmacological activation of serotonin receptors has been shown to reduce behavioral measures of anxiety (Bencan et al., 2009; Connors et al., 2014; Sackerman et al., 2010; Gebauer et al., 2011; Maaswinkel et al., 2012). However, few studies have examined the effects of different serotonin receptor antagonists on motor function or anxiety-like behavioral measures in adult zebrafish.

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Although the serotonergic system in zebrafish may be a potential pharmacological target, only two of the seven 5-HT receptor families (5-HT_1 and 5-HT_2) have been identified in this species (Norton et al., 2008; Schneider et al., 2012). 5-HT_1-like receptors have been examined in zebrafish and 2 genes, *ht1aa* and *ht1ab*, have been identified as zebrafish homologs of the mammalian gene that encodes the 5-HT_{1A} receptor. A third zebrafish gene, *ht1bd*, has been found to encode a receptor similar to the 5-HT_{1B} receptor in humans and 5-HT_{1D} receptor in puffer fish. All three genes show widespread expression in both the larval and adult zebrafish brain, with detected presynaptic autoreceptor and postsynaptic receptor activity (Norton et al., 2008). The second family of receptors recently characterized in zebrafish belongs to the 5-HT_2-like family. 5-HT_{2C} receptor cDNA was recently sequenced from the zebrafish, and was determined to be highly homologous to human and mouse sequences (Schneider et al., 2012). The potential role these receptor subtypes may play in anxiety-like behaviors in zebrafish is unclear.

The purpose of the current study was to characterize the behavioral function of the above receptor subtypes by using antagonists that are known to specifically target 5-HT_{1A}, 5-$HT_{1B/D}$, and 5-HT_2 receptors. In addition, we also employed an antagonist to target 5-HT_3 receptors, the only receptor not coupled to a G-protein. Although the gene encoding 5-HT_3 receptor has not been identified in zebrafish, behavioral alterations as a result of drug administration may provide indirect evidence for its existence in this species. To examine the behavioral functions of each serotonin receptor subtype, we first characterize the dose dependent effects of four different serotonin receptor antagonists on zebrafish motor responses: p-MPPF dihydrochloride (5-HT_{1A} antagonist), GR 55562 (5-$HT_{1B/D}$ antagonist), Ketanserin tartrate (5-HT_2 antagonist), and Ondansetron hydrochloride (5-HT_3 antagonist). We then focus our analyses on behavioral responses exhibited by zebrafish in a novel tank paradigm, a mildly aversive environment, in an attempt to quantify potential effects of these drugs on anxiety (Levin et al., 2007; Bencan and Levin, 2008; Egan et al., 2009). The distinction between fear and anxiety has been blurred in the past; nevertheless, these two phenomena have been distinguished in both the animal and human literature (for a recent review see Gerlai, 2010). For zebrafish, fear versus anxiety has been defined similarly as for mammals (Gerlai, 2010, 2013). Briefly, fear is operationally defined as a set of responses directly induced by the appearance/delivery of specific aversive stimuli, whereas anxiety is defined as responses that do not require the actual appearance or presence of such stimuli.

Although serotonergic neurotransmission mediates a large number of behaviors, we restrict our analysis to motor and anxiety related responses which have been well defined and found quantifiable in zebrafish (Tran and Gerlai, 2013; Tran et al., in press; Levin et al., 2007).

1. Methods

1.1. Animal housing

Adult zebrafish of the AB strain were bred at the University of Toronto Mississauga Vivarium (Mississauga, Ontario, Canada). The progenitors of this population were originally obtained from the ZFIN Center (Eugene, Oregon, USA). The AB strain was selected for its homozygozity at over 80% of loci (Guryev et al., 2006), and its frequent use in behavioral neuroscience and mutagenesis studies (Gerlai et al., 2000). Furthermore, the AB strain has been reported to exhibit more robust and quantifiable anxiety-like responses in a novel environment compared to other strains (Sackerman et al., 2010). Zebrafish eggs were collected 2 hours post-fertilization and placed in 2.7 L tanks on a high-density rack system (Aquaneering Inc.). The rack system had multi-stage filtration including a mechanical filter, fluidized glass bed biological filter, active carbon filter, and fluorescent UV light sterilizing unit. Ten percent of the system water (reverse-osmosis deionized water supplemented with 60 mg/L Instant Ocean Sea Salt (Big Al's Aquarium)) was replaced daily. Water quality parameters were maintained at optimal conductivity levels (100–300 microsiemens), temperature (28 °C–30 °C), and pH (6.8–7.2). Upon hatching, larvae were fed Larval AP 100. At 21 days post-fertilization, juvenile fish were fed brine shrimp twice a day. Starting at 30 days post-fertilization, zebrafish were fed brine shrimp and a mixture of flake food (2:1 ratio of tetramin: spirulina). Zebrafish were tested at 8 months of age (approximately 50% males and 50% females).

1.2. Experimental design and procedure

The experiment was intended to characterize the behavioral responses to a range of doses of four serotonin receptor antagonists: p-MPPF, GR 55562, Ketanserin, and Ondansetron, in a novel tank exploration task. On the day of testing, 2.7 L housing tanks were moved to the testing room and animals were allowed to habituate for 30 minutes prior to testing. Individual zebrafish were first exposed to different concentrations of p-MPPF, GR 55562, Ketanserin, and Ondansetron (0.0, 0.1, 0.5, 1.0 mg/L) for 30 minutes in a 1 L tank containing the appropriate drug concentration ($n = 16$) to examine changes in baseline motor responses. Drug immersion was chosen as the method of delivery over other methods (e.g. injection) because of its less invasive nature and frequent use in assays using adult zebrafish (Levin et al., 2007; Sackerman et al., 2010; Connors et al., 2014). For example, drug uptake for citalopram (a serotonin reuptake inhibitor) from the water bath into zebrafish brain tissue confirmed by radioligand binding assays is approximately 1/1000 (Sackerman et al., 2010). The novel tank paradigm used in the current study is sensitive to measures of anxiety and the use of an invasive drug delivery method would have introduced potential confounds. Since the pharmacodynamic and pharmacokinetic properties of these drugs are unknown in zebrafish, the concentrations and duration of exposure chosen for this study are based on unpublished pilot data from our laboratory showing significant reductions in whole brain serotonin and its metabolite (5-hydroxyindolacetic acid) 5-HIAA following 30 minutes of drug administration at the highest dose (Tran et al., unpublished data). Following the 30 minute drug exposure, zebrafish were subsequently placed in a 37 L novel tank (drug-free system water) to quantify anxiety-like behaviors. The back and lateral surfaces, of both the drug exposure and novel tanks, were covered with white corrugated plastic sheets to obscure external cues and to provide a uniform testing environment. Video recordings were made from the front view during both the drug exposure period and novel tank exploration. Recordings commenced immediately after the fish was placed in either tank. Water quality parameters for both the drug exposure and novel tanks matched those of housing tanks.

1.3. Drug administration

All chemicals were obtained from Sigma-Aldrich. Stock solutions (5 mg/mL) of the water soluble drugs; p-MPPF, GR 55562, and Ondansetron, were made and were further diluted to obtain the appropriate concentration for the drug exposure tanks (0, 0.1, 0.5, 1.0 mg/L) using system water. Ketanserin is insoluble in water and was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution of 5 mg/ml, which was further diluted to the appropriate concentrations for the drug exposure tank (0, 0.1, 0.5, 1.0 mg/L Ketanserin) using system water. A final DMSO concentration in all tanks containing Ketanserin including the Ketanserin control was 0.2% (vol/vol). The technique of immersion based drug delivery with zebrafish using DMSO has been implemented by previous studies. The concentration of DMSO employed in these studies reached 0.4% (vol/vol) and has been shown not to significantly alter the motor responses of zebrafish larvae (Irons et al., 2013; Giacomini et al., 2006) or adults (Connors et al., 2014).