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Environmentally relevant concentrations of citalopram partially inhibit feeding in the three-spine stickleback (*Gasterosteus aculeatus*)

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ABSTRACT

Selective Serotonin Re-uptake Inhibitors (SSRI) are mood-altering, psychotropic drugs commonly used in the treatment of depression and other psychological illnesses. Many of them are poorly degraded in sewage treatment plants and enter the environment unaltered. In laboratory studies, they have been demonstrated to affect a wide range of behaviours in aquatic organisms. In this study we investigated the effect of a three-week exposure to 0.15 and 1.5 μg/l of the SSRI citalopram dissolved in the ambient water on the feeding behaviour in three-spine stickleback (*Gasterosteus aculeatus*). Feeding, measured as the number of attacks performed on a piece of frozen bloodworms during a 10-min period, was reduced by 30–40% in fish exposed to both 0.15 and 1.5 μg/l citalopram. The effects of the environmentally relevant concentration 0.15 μg/l on feeding, an important fitness characteristic, suggests that the ecological significance of environmental SSRI exposure may be pronounced.

1. Introduction

SSRIs (Selective Serotonin Re-uptake Inhibitors) are a class of mood altering, psychotropic drugs which are used in the treatment of several psychiatric diseases such as depression and obsessive-compulsive disorder. Many SSRIs are persistent in the environment (Kwon and Armbrust, 2005) and pass through sewage treatment plants (STPs) with very little or no degradation taking place (Vasskog et al., 2006). Citalopram and other SSRIs are frequently found in STP effluents and have been reported in concentrations between 0.02 and 840 μg/l (Larsson et al., 2007; Vasskog et al., 2008). They are commonly found in natural waters around the world (Fick et al., 2009; Vasskog et al., 2008; Woldegiorgis, 2011) and have been recorded in concentrations ranging from 0.5 ng/l to extremes around 76 μg/l (Fick et al., 2009).

SSRIs act on the serotonergic system which is highly evolutionarily conserved and is present in both vertebrates and invertebrates. The primary mode of action of SSRI is blockade of serotonin (5-HT) re-uptake into the pre-synaptic nerve ending, causing an elevated concentration of serotonin in the synaptic cleft. Serotonin is involved in a vast array of systems in the body and is known to affect satiety (Grignaschi et al., 1998; Ward et al., 1999), mood parameters such as aggression (Ricci and Melloni, 2012) and locomotor activity (Clissold et al., 2013; Olsén et al., 2014). In humans, sexual dysfunction is a common result of elevated serotonin levels (Keltner et al., 2002) and suppressed serotonin levels cause clinical depression (Van Praag, 1977). The effects of serotonin are exerted by serotonin binding on a number of 5-HT receptors with different functions, most of which transduce the serotonergic signal through G proteins (Hoyer et al., 2002). Human serotonin receptors are divided into seven different families and several families contain more than one receptor.

Many of the 5-HT receptors present in humans are also present in fish and accordingly, many of the effects of SSRIs and elevated serotonin levels found in mammals have also been detected in fish. SSRIs decrease territorial aggression (Lepage et al., 2005; Perreault et al., 2003) and the ability or willingness to catch prey is suppressed (Gaworecki and Klaine, 2008). Latency to the initiation of escape response is prolonged, escape velocity is lower (Painter et al., 2009) and SSRI treated piauçu fish (*Leporinus macrocephalus*) fail to react to a conspecific alarm substance (Barbosa et al., 2012).

The serotonergic system is also vital to the regulation of satiety and feeding. This regulation takes place at least partly in the hypothalamus (Steffens et al., 2010). In rats, intraperitoneal injection of citalopram has an anorectic effect (Grignaschi et al.,

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1998). Blockade of the 5-HT_{1A} receptor potentiates this effect (Grignaschi et al., 1998), probably through negative feedback on serotonin release from pre-synaptic 5-HT_{1A} receptors. Blockade of the 5-$HT_{2B/C}$ receptors antagonised the inhibitory effect on feeding caused by the combination of citalopram and a 5-HT_{1A} antagonist, suggesting that the hypophagic effect of citalopram is partially mediated by those receptors (Grignaschi et al., 1998). A more detailed picture is revealed by a recent study on rainbow trout (*Oncorhynchus mykiss*) in which stimulation of 5-HT_{1A} and 5-HT_{2C} receptors had a negative effect on food intake while stimulation of the 5-HT_{2B} receptor increased food intake (Pérez Maceira et al., 2014). Thus, the regulation of feeding and satiety is complex and the serotonergic system is heavily involved in this regulation. In rodents (Boisvert et al., 2011) and fish (De Pedro et al., 1998; Ortega et al., 2013), the effect of serotonin on feeding and satiety is at least in part mediated by the Corticotropin Releasing Hormone (CR) system. CRH is also known as the Corticotropin Releasing Factor (CRF). In tilapia (*Oreochromis mossambicus*), in vitro stimulation of telencephalic tissue by serotonin causes CRH release (Pepels, 2004). In goldfish (*Carassius auratus*) and rainbow trout (*Oncorhynchus mykiss*) (De Pedro et al., 1998; Ortega et al., 2013) the Corticotropin Releasing Factor (CRF) receptor antagonist α-helical $CRF_{(9–41)}$ partially blocks the inhibitory effect of serotonin on feeding. A possible mechanistic explanation for the connection between the CRH system and serotonin is supplied by a study which found that in rats, approximately half of the CRH-containing neurons in the paraventricular nucleus of the hypothalamus co-expressed 5-HT_{2C} mRNA. Genetic inactivation of 5-HT_{2C} downregulated CRH mRNA expression and blunted CRH and corticosterone release after 5-HT administration (Heisler et al., 2007).

Most studies of effects of serotonin and SSRI effects on feeding behaviour have used very high concentration of SSRI or injections of SSRI or serotonin into the test subject (De Pedro et al., 1998; Ortega et al., 2013). Mennigen et al. (2010) included an environmentally relevant concentration of fluoxetine (540 ng/l) but found no effect on food intake at this concentration. While injection of active substances, or very high concentrations of active substances in the ambient water provide valuable control over the involved variables, they are less relevant in an environmental context where uptake, particle binding, photolysis and other factors influence the bioavailability of drugs and drug metabolites. Furthermore, most studies done on the subject have used fluoxetine which is generally regarded as the least selective SSRI on the market. Apart from increasing extracellular serotonin levels, fluoxetine also increases extracellular levels of norepinephrine and dopamine (Bymaster et al., 2002). At least in the rat, blockade of dopamine receptors is known to decrease feeding (Sudakov and Bashkatova, 2013). In the fish sea bass (*Dicentrarchus labrax*), the dietary administration of L-dopa (a dopamine precursor) has an inhibitory effect on feeding behaviour (Leal et al., 2013).

In Sweden and many other countries, citalopram is much more commonly used in the treatment of depression than fluoxetine. Citalopram is a racemic mixture and the S-enantiomer (known as Escitalopram) is considered to be the most selective SSRI developed so far. The high citalopram usage and known potential to influence fish behaviour along with the poor degradation in STPs are good reasons for environmental concern. In this study we exposed three-spine sticklebacks to the environmentally relevant concentrations 0.15 μg/l and 1.5 μg/l citalopram dissolved in the ambient water for three weeks and measured the effects of this exposure on feeding behaviour. We found that citalopram dissolved in the surrounding water had a pronounced inhibitory effect on the feeding behaviour of the three-spine stickleback which implicates that it should be considered an environmentally unsafe pharmaceutical.

2. Materials and methods

2.1. Fish

Three-spine sticklebacks were caught in the small harbour of Skåre (55°22,30 N 13°3,18 E) in southern Sweden in November 2013. A landing net with small mesh size was used. Adults of 5–6 cm overall length were chosen for absence of disease. The following day, the fish were transported to Södertörn university where they were gradually acclimatised to fresh water and room temperature over a period of 48 h. The fish were then divided by sex and housed in 400 l holding tanks containing pieces of garden clay pots for shelters. The tanks were cleaned daily and fresh, aerated water was supplied continuously by a flow-through system. The fish were fed Chironomus larva (bloodworms) daily and kept on a 8:16 light:dark cycle to avoid sexual maturation. Fish from the same catch were used in both experiments. Only females were used in this study.

2.2. Experimental setup

Experiments took place between the beginning of December 2013 and mid-March 2014. On the first day of the experiment the fish were transferred to 9 l exposure aquaria. There were 27 exposure aquaria standing side by side on five shelves. Black plastic sheets were placed between the aquaria to avoid visual contact between the aquaria. The exposure aquaria contained one piece of garden clay pot each for shelter. Fish were fed bloodworms to satiation daily.

2.2.1. Experiment 1

Fish were weighed and then moved to their exposure aquaria ($n_{control} = 14$ and $n_{treated} = 13$) where they were kept solitarily. In order not to hurt the fish in the weighing process, they were weighed by lifting them from the water using a landing net, shaking them gently twice and then putting them in a pre-weighed water-filled 500 ml beaker. Citalopram bromide (kindly donated by Lundbeck A/S) dissolved in MilliQ water was added to every second aquarium to yield a final nominal concentration of 1.5 μg/l. The aquaria not receiving citalopram received a corresponding amount of MilliQ water. The water regime was semi-static and half of the water was exchanged with new room-temperated and aerated water every second day. At the same time, the aquaria were cleaned from faeces and food residues. New citalopram solution or milliQ water was added to compensate for the amount removed during cleaning. A baseline reading of feeding behaviour was done when the fish had been in the experimental aquaria for 24 h. After the baseline reading, citalopram or milliQ water was added to the aquaria. Feeding behaviour was then recorded for 10 min once a week for three weeks, starting one week after the beginning of the exposure. Recording always took place between 9 a.m. and 1 p.m. When the fish were filmed, they had been food-deprived for approximately 24 h. Recording of feeding behaviour was done with digital video cameras and all staff left the room while recording. The analysis was performed manually afterwards, by counting the number of attacks on food items. While recording feeding behaviour, the aquaria were backlit using a light rack in order to see the fish and their food better.

2.2.2. Experiment 2

Like experiment 1, experiment 2 tested effects on feeding behaviour but included a lower, more environmentally relevant concentration (0.15 μg/l) in addition to the 1.5 μg/l concentration used in experiment 1. In this experiment the fish were housed three fish in each aquarium and were treated with either 0 μg/l, 0.15 μg/l or 1.5 μg/l for three weeks. Feeding behaviour was recorded during the two final days of the experiment. As in experiment 1,