Journal of Hazardous Materials 187 (2011) 596–599

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Short communication

Does waterborne citalopram affect the aggressive and sexual behaviour of rainbow trout and guppy?

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ARTICLE INFO

Article history:
Received 15 November 2010
Received in revised form 11 January 2011
Accepted 11 January 2011
Available online 18 January 2011

Keywords:
Citalopram
SSRI
Guppy
Rainbow trout
Behaviour

ABSTRACT

Citalopram is one of several selective serotonin reuptake inhibitors (SSRIs) commonly found in treated sewage effluents. Accordingly, there are concerns about possible adverse effects of SSRIs on aquatic organisms, particularly behavioural effects similar to those associated with SSRI use in humans. Rainbow trout fry and adult male guppies were therefore exposed to waterborne citalopram, ranging from environmentally relevant to high concentrations (1, 10, 100 μg/L) for 3–7 days. Under these experimental conditions citalopram does not appear to cause significant effects on aggression in rainbow trout fry or on sexual behaviour in male guppies. This may be explained by a relatively low uptake of citalopram from water to fish.

1. Introduction

Both the function and organisation of the serotonin system is highly conserved among vertebrate classes [1–3]. Accordingly, fish serotonin transporters have high affinity to selective serotonin reuptake inhibitors (SSRIs) used in human therapy [4]. Several SSRIs have been reported in treated sewage effluent in concentrations up to 612 ng/L [5–8], and as an exceptional case, up to 840 μg/L citalopram has been detected in treated effluent from pharmaceutical manufacturers in India [9,10]. Furthermore, fluoxetine and sertraline, two other SSRIs, are found to bioconcentrate in fish [11–14]. Effects of SSRIs on aggression and reproduction have been reported in non-mammalian species, including fish [15–20]. In juvenile rainbow trout (*Oncorhynchus mykiss*), one-week feeding with citalopram (100 μg/kg) reduced aggressive behaviour [18]. It is, however, difficult to translate oral doses to water concentrations, and thus a risk assessment is difficult to perform without data from waterborne exposure experiments. The aim of this study was therefore to determine if waterborne citalopram, at concentrations regularly found in surface waters, is expected to alter the aggressive or sexual behaviour of fish using different behavioural models.

Established aggression tests, including mirror image stimulation (MIS) and dyadic contests, were used to examine the effects of citalopram on territorial aggression in rainbow trout fry [21]. To study the potential effects on loss of libido in fish, a common side effect of SSRIs in men, courting male guppies (*Poecilia reticulata*) were used as their sexual behaviour is distinct and well described [22]. A short-term bioconcentration test was also performed in trout to evaluate if citalopram is easily taken up from the water.

2. Materials and methods

2.1. Animals and chemical

Farmed rainbow trout fry (one to three months old at experimental date) and adult guppies were purchased from local dealers (Antens fiskodling AB, Alingsås, Sweden and Zoomässan, Kungsbacka, Sweden, respectively). The fish were kept in aerated indoor freshwater tanks with a pH of ~7.5 under a 12:12 h light dark cycle, and fed daily. The water temperatures were 12 °C for trout and 24–27 °C for the guppies. The exposures and analyses took place in batches over the course of several weeks as the behaviours of more than 500 fish (not including pilot studies) were evaluated

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doi:10.1016/j.jhazmat.2011.01.055

individually. During the exposure period 75% of the water was exchanged daily. Throughout the experiments the aquaria were semi-covered in black plastic to minimize disturbance. Furthermore, behaviours were scored blindly without access to the treatment key to avoid bias. There were no differences in mean wet mass (mg) or fork length (L_F mm) after conducted experiments among the four groups in either experiment (Kolmogorov–Smirnov test, One-way ANOVA, $P > 0.05$). All experiments were approved by the local animal ethics committee (36-2007 to D.G.J. Larsson).

2.2. Aggressive behaviour

At the beginning of both the MIS and dyad experiment rainbow trout fry were netted randomly to the four exposure aquaria (control, 1 μg/L, 10 μg/L or 100 μg/L citalopram, in the form of water-soluble citalopram hydrobromide C7861-50 mg, Sigma–Aldrich, Stockholm, Sweden) and exposed for six to seven days. On the experimental day, fry were transferred individually to the behavioural test aquaria without citalopram.

For MIS test, individual fry ($n = 20$ per treatment) were allowed to settle in the test aquarium for 1 h with a grey PVC sheet covering the mirror. Each aquarium was divided into five 2 cm wide zones. One minute before the PVC sheet was lifted the position of the fish was recorded every third second. The PVC sheet was carefully removed and agonistic behaviours (swim against mirror (SAM), lateral display (LD), frontal display (FD)) and position (D_a) were recorded every third second for 5 min. The number of times a fish swam into a new zone, i.e. the swimming activity (Z) before and after the mirror was exposed (Z_b and Z_a) was calculated from the distance measurements.

In the dyadic contests ($n = 38$ dyads per treatment) the two contestants were identified by visual cues and then allowed to interact for 1 h. The experimental fish were challenged with a fresh, non-exposed fish. After 1 h the fish were fed 10 pellets, one every 30 s, and the fish that took most pellets were considered to be the dominant fish. A pilot study demonstrated that in 14 out of 16 dyadic pairs the dominance/subordinates relationship was established within 1 h and was still stable after the contestants had been separated for 24 h and then again allowed to interact for 1 h before fed.

2.3. Sexual behaviour

Prior to any citalopram exposure, the baseline behaviour of each male guppy was observed by placing it, visually and physically separated, together with four females in an aquarium. After 30 min the divider was carefully removed and the number of gonopodial thrusts and gonopodial swings were studied for 20 min. Then each male was transferred randomly to one of the four exposure aquaria (control, 1 μg/L, 10 μg/L, 100 μg/L, $n = 40$–42 males per exposure). The male guppies were exposed for three days before put into a test aquarium and observed again using the same protocol and the same females as the first observation time. Only male guppies performing thrusts and swings prior to exposure were included.

2.4. Nominal and actual water concentration of citalopram in behavioural tests

Prior to the both guppy and trout experiment water samples were taken from the aquarium with the highest nominal concentration of citalopram (100 μg/L). This was repeated before and after the first water change and after the last behaviour test. Water samples were extracted and analyzed using a solid phase extraction (SPE) pre-treatment coupled to liquid chromatography–tandem mass-spectrometry (LC–MS/MS) analysis. Detailed description of the chemical analysis and the SPE extraction, including chemicals used, chromatographic details, selected reaction monitoring (SRM) transitions, etc., are available in the supporting information.

2.5. Bioconcentration of citalopram into blood plasma

Larger juvenile rainbow trout (average weight 70 g) were exposed to citalopram (control, 0.1 μg/L, 1 μg/L, 10 μg/L; five fish per concentration) for 24 h at 12 °C. Water samples were taken at the beginning and the end of the exposure (static). Levels of citalopram in both water and blood plasma were analyzed by LC–MS/MS (see supporting information).

3. Results

Analyses of water levels of citalopram in the behavioural test were reasonably similar to nominal levels (67% ± 6% SD, $n = 8$) and showed no clear tendency to change over time. Before the mirror was revealed, the fry were usually positioned in the middle of the aquarium (zone 3) but afterwards the fish swam significantly closer to the mirror (average ± SEM: D_b: D_a: control 3.16 ± 0.21: 2.01 ± 0.14; 1 μg/L 2.84 ± 0.19: 2.05 ± 0.18; 10 μg/L 2.87 ± 0.21: 2.24 ± 0.20; 100 μg/L 3.02 ± 0.22: 2.09 ± 0.22; Levene's test of equality Error variances, General linear model; $P < 0.05$, Fig. 1a). There was however no significant difference in the change in distance between the four exposure groups (average ± SEM: ΔD: control 1.15 ± 0.21; 1 μg/L 0.78 ± 0.22; 10 μg/L 0.63 ± 0.26; 100 μg/L 0.93 ± 0.25; Kolmogorov–Smirnov test, paired t-tests; $P > 0.05$). There was no significant difference in percentage time the fish displayed agonistic behaviours towards the mirror between control and citalopram-exposed fish (average ± SEM: control 30.84 ± 4.81; 1 μg/L 40.90 ± 7.01; 10 μg/L 23.05 ± 5.83; 100 μg/L 29.11 ± 5.51; Kolmogorov–Smirnov test, One-way ANOVA, Dunnett's post hoc test; $P > 0.05$, Fig. 1b). Swimming activity did not vary significantly before or after the fish were exposed to the mirror in either exposure (average ± SEM: Z_b: Z_a control 0.16 ± 0.04: 0.17 ± 0.03; 1 μg/L 0.13 ± 0.03: 0.19 ± 0.04; 10 μg/L 0.16 ± 0.05: 0.20 ± 0.04; 100 μg/L 0.25 ± 0.05: 0.16 ± 0.04; Levene's test of equality Error variances, general linear model; $P > 0.05$). Neither were there any differences in swimming activity between the exposure groups (average ± SEM: ΔZ control 0.00 ± 0.04; 1 μg/L 0.06 ± 0.05; 10 μg/L 0.04 ± 0.06; 100 μg/L 0.08 ± 0.05; Kolmogorov–Smirnov test, paired t-tests; $P > 0.05$). Similarly, in the dyadic contest, citalopram exposure did not affect the outcome (logistic regression test, percentage contest were control fish won: control 51%, 1 μg/L 42%, 10 μg/L 45%, 100 μg/L 51%, $P > 0.05$). Similar results were obtained even if fish had to take 75% or more of the pellets to be considered as the winner or if relative fork length was included as a covariate.

The guppy males performed, on average, 24 gonopodial thrusts and swings over 20 min pre-exposure. Analyses of the changes in individual male's behaviour after three days waterborne citalopram exposure revealed no significant effects at any of the tested concentrations: control −6.9 ± 3.66; 1 μg/L 1.93 ± 2.71; 10 μg/L −6.33 ± 3.17; 100 μg/L −4.48 ± 3.25 (homogeneity test, Univariate ANOVA; $P > 0.05$, Fig. 1c). An analysis of thrusts and swings separately gave similar results ($P > 0.05$).

In the bioconcentration test, measured water concentrations of citalopram were 0, 0.1, 1.2 and 9.7 μg/L, i.e. very close to nominal levels. Concentrations were similar at the beginning and the end of the exposure, suggesting that the static exposure protocol did not compromise the interpretation. Citalopram was undetectable in all blood plasma samples, except in 2 out of 5 samples from the 10 μg/L group (0.044 and 0.080 ng/mL), corresponding to a bioconcentration factor from water to fish blood plasma of 0.004–0.008 or less.

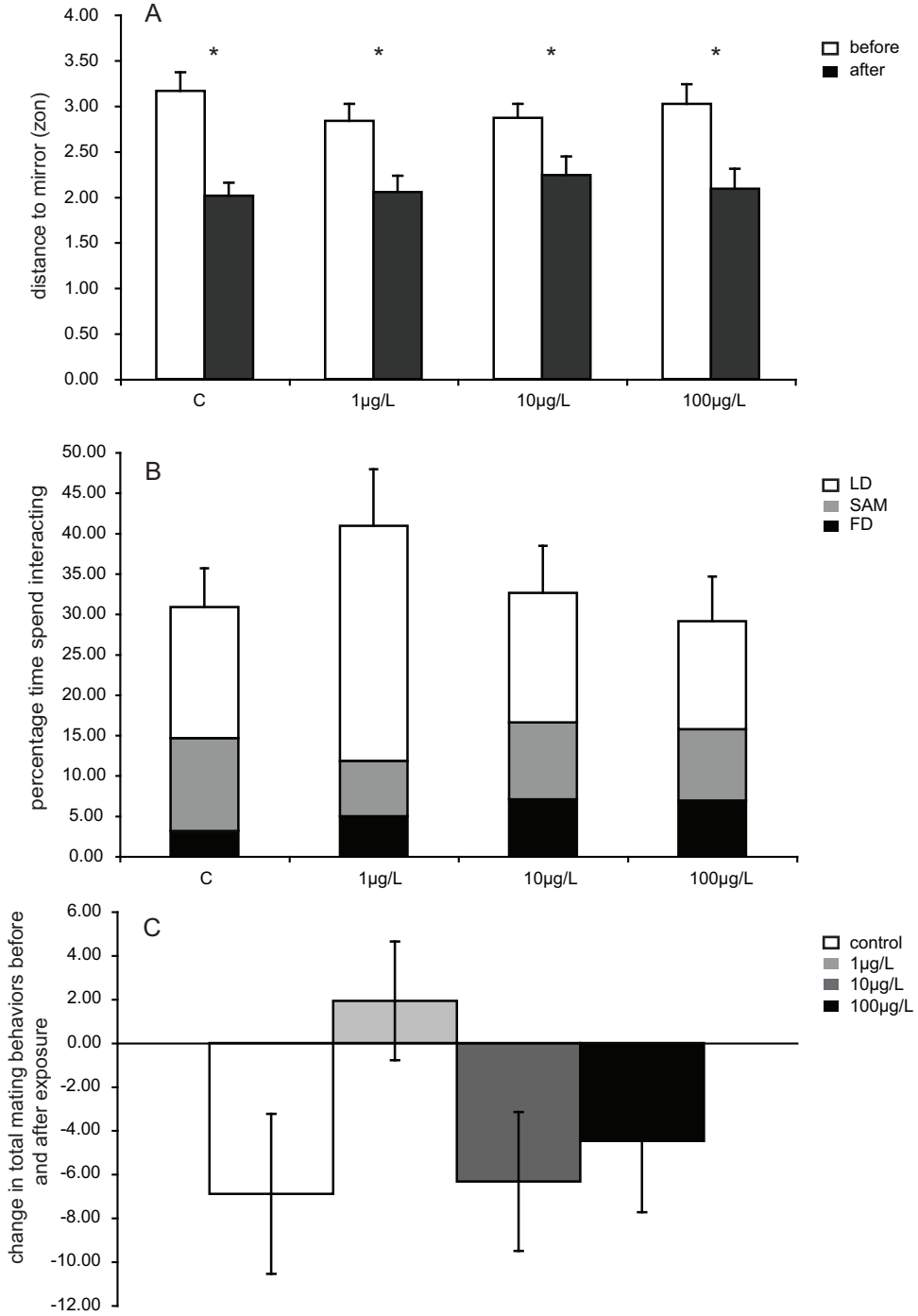

Fig. 1. Aggressive behaviour of rainbow trout fry (A, B) and sexual behaviour of adult guppies (C) were not affected when exposed 6–7 days to citalopram. (A) The aquarium was divided into five zones (2 cm wide, zone 1 is closest to the mirror) and the distance (D) to the mirror was noted before and after the mirror was revealed (average ± SEM). (B) The bars indicate the total time spent interacting towards the mirror (average ± SEM) also separated into the following behaviours: swim against mirror (SAM), lateral display (LD) and frontal display (FD). (C) Individual changes in total mating behaviours (gonopodial swings and thrusts) of adult male guppies before and after three days exposure to citalopram (average ± SEM).

4. Discussion and conclusion

The aim of the present study was to assess if environmental concentrations of citalopram are likely to affect the sexual and aggressive behaviour of fish, using adult male guppies and rainbow trout fry as experimental models. The present study cover more than one relevant endpoint, it includes two different fish species and the number of biological replicates is relatively high. Also, the concentrations used ranged from just above levels found in certain sewage effluents [5–7] to about a hundred-fold higher levels. Nevertheless, no effect of citalopram was recorded.

It cannot be excluded that the exposure time applied in the present study (3–7 days) is too short for citalopram to elicit an effect. However, in Japanese medaka exposed to waterborne fluoxetine (0.64 μg/L), fluoxetine was detected in tissues already after five hours and peaked after three days [12]. Importantly, earlier studies on different SSRIs and fish have shown effects after a few days or even as short as 50–70 min after administration

[17–19,23]. In an acute toxicity test with juvenile rainbow trout an LC_{50} value of 0.38 mg sertraline/L were obtained after 96 h of sertraline-contaminated water exposure [24], but this may reflect a non-specific mode of action, with little relevance for concentrations found in the environment.

To our knowledge, effects of waterborne SSRIs on sexual behaviour in fish have not been extensively studied. Fluoxetine (32 μg/L) reduced ovarian estrogen levels and reduced the number of spawned eggs in zebrafish [25], possibly as a consequence of a reduced gonadotropin release [26]. Foran and co-workers [16] exposed Japanese medaka to waterborne fluoxetine (0.1, 0.5, 1 and 5 μg/L) for 4 weeks without effects on reproductive success. In agreement, we could not find any evidence for a reduced libido in the male guppies exposed to citalopram, a common side effect of SSRI use in men.

L-Tryptophan or high doses of citalopram fed to juvenile trout affect their aggressive behaviour [18,20]. We therefore suspected that the lack of effects on aggressive or sexual behaviour in our experiment was due to limited bioconcentration of citalopram from water. Indeed, very low levels of citalopram were detected in fish blood plasma after 24 h of waterborn exposure. The human therapeutic plasma level is 10 ng/mL [27], i.e. more than 100 times higher than the highest fish plasma level measured here. A limited uptake could therefore explain the lack of effects. Although lipophilicity often is a good predictor, bioconcentration factors from water to fish plasma appear to vary between drugs, sites and experimental setups dues to factors we do not yet understand [14,28,29]. Particularly for ionisable drugs, such as citalopram, the uptake may also depend on the environmental pH [23].

In conclusion the present study does not support the hypothesis that citalopram alone affects aggressive or reproductive behaviour in fish at water concentrations regularly found in the environment. Of course, negative findings such as those presented here, never exclude the possibility of effects on other endpoints, in other species or experimental settings. Furthermore, in the environment, fish may be exposed for longer times to several SSRIs acting additively and in concert with other contaminants and other abiotic or biotic factors.

Conflict of interest statement and role of funding agencies

The authors declare that there are no conflicts of interest. The funding agencies had no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Acknowledgements

We thank Christina Olsson for assistance with guppy experiments. We also thank the Swedish Foundation for Strategic Environmental Research (MISTRA), the Swedish Research Council (Medicine) and the Swedish Research Council for Environment Agriculture and Spatial Planning (FORMAS) for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.01.055.

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