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Effects of intracerebroventricular administered fluoxetine on cardio-ventilatory functions in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Fluoxetine (FLX) is a selective serotonin (5-HT) reuptake inhibitor present in the aquatic environment which is known to bioconcentrate in the brains of exposed fish. FLX acts as a disruptor of various neuroendocrine functions in the brain, but nothing is known about the possible consequence of FLX exposure on the cardio-ventilatory system in fish. Here we undertook to investigate the central actions of FLX on ventilatory and cardiovascular function in unanesthetized rainbow trout (*Oncorhynchus mykiss*). Intracerebroventricular (ICV) injection of FLX (dosed between 5 and 25 μg) resulted in a significantly elevated total ventilation (V_{TOT}), with a maximum hyperventilation of +176% (at a dose of 25 μg) compared with vehicle injected controls. This increase was due to an increase in ventilatory amplitude (V_{AMP}: +126%) with minor effects on ventilatory frequency. The highest dose of FLX (25 μg) produced a significant increase in mean dorsal aortic blood pressure (P_{DA}: +20%) without effects on heart rate (fH). In comparison, intra-arterial injections of FLX (500–2500 μg) had no effect on ventilation but the highest doses increased both P_{DA} and fH. The ICV and IA cardio-ventilatory effects of FLX were very similar to those previously observed following injections of 5-HT, indicating that FLX probably acts via stimulating endogenous 5-HT activity through inhibition of 5-HT transporter(s).

Our results demonstrate for the first time in fish that FLX administered within the brain exerts potent stimulatory effects on ventilation and blood pressure increase. The doses of FLX given to fish in our study are higher than the brain concentrations of FLX in fish that result from acute exposure to FLX through the water. Nonetheless, our results indicate possible disrupting action of long term exposure to FLX discharged into the environment on central target sites sensitive to 5-HT involved in cardio-ventilatory control.

1. Introduction

Fluoxetine (FLX) is a selective serotonin (5-HT) reuptake inhibitor (SSRI) and the active ingredient of the largely prescribed antidepressive drug Prozac™. FLX leads to an accumulation of 5-HT in the extracellular space of synapses, increasing the magnitude and duration of the activity of 5-HT on pre- and post-synaptic receptors. Although FLX is generally considered to lack the peripheral cardiovascular effects well documented for the tricyclic antidepressants (Roose et al., 1998), evidence has shown that patients taking FLX may exhibit some cardiovascular side effects (Pacher and Kecskemeti, 2004). Very few studies have been performed in experimental models to determine the possible central effects of FLX on cardiovascular and respiratory functions, and the results of these studies are rather conflicting. In unanesthetized rats, central acute administration of FLX induces an increase in blood pressure without affecting heart rate (fH). The hypertensive effect of FLX appears to be mediated by an increase in both sympathetic outflow and vasopressin release without the involvement of the 5-HT_2 receptor (Lazartigues et al., 1999, 2000). In anesthetized and chemodenervated rabbits, acute intracerebroventricular (ICV) administration of FLX stimulates ventilation, and enhances ventilatory response to hypercapnia and these effects are abolished after

Abbreviations: a.u., arbitrary unit; 5-HT, 5-hydroxytryptamine (serotonin); CNS, central nervous system; ECG, electrocardiographic; fH, heart rate; fV, ventilation rate; IA, intra-arterial; ICV, intracerebroventricular; P_{DA}, dorsal aortic blood pressure; PwO_2, partial oxygen pressure in water; V_{AMP}, ventilation amplitude; V_{TOT}, total ventilation.

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pre-treatment with ketanserin, a 5-HT_2 receptor antagonist (Sahin et al., 2011). Intra-peritoneal (IP) injection of FLX evokes changes in the baroreflex response that may result from diffusion of the drug to the central nervous system (CNS) (Crestani et al., 2011).

Due to its excretion by patients and the direct disposal into wastewaters, FLX and its metabolite norfluoxetine enter into the aquatic environment and these compounds bioconcentrate in fish tissues and notably in the brain (Brooks et al., 2005; Schultz et al., 2011). Concentrations of FLX in wastewater effluents have been measured in range of low μg/L (Brooks et al., 2005). Effects of FLX in fish have mainly been examined in the context of reproduction (Uphouse et al., 2006; Mennigen et al., 2011), and behavior (Clements et al., 2003; Semsar et al., 2004; Airhart et al., 2007) and surprisingly there is no report on the effects of FLX on the cardio-ventilatory system. In the teleost fish brain, 5-HT-immunoreactive perikarya and fibers are localized in the diencephalon at the level of the hypothalamic nuclei, within the isthmus (an area homologous to the raphe nuclei of terrestrial vertebrates), the reticular formation of the medulla oblongata and within the spinal cord (Kah and Chambolle, 1983; Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; Ekström and Ebbesson, 1989; Batten et al., 1993; Kaslin and Panula, 2001; Lillesaar, 2011). The 5-HT transporter(s) and 5-HT receptor genes have been identified in various species of fish (Yamaguchi and Brenner, 1997; Wang et al., 2006; Wang and Tsai, 2006; Lim et al., 2013), and their expression is widely distributed within their brains (Norton et al., 2008). Moreover, in our previous work we have demonstrated that in unanesthetized rainbow trout, *Oncorhynchus mykiss*, ICV administration of exogenous 5-HT increased ventilation and dorsal aortic blood pressure (P_{DA}) without changing fH, suggesting that the endogenous indoleamine might be involved in the physiological regulation of these vital regulations (Kermorgant et al., 2014). Therefore, we hypothesised that brain cardio-ventilatory pathways sensitive to 5-HT might be affected by FLX treatment. To test this hypothesis we analyzed the effects of ICV administration of FLX on ventilation rate (fV), ventilation amplitude (V_{AMP}), total ventilation (V_{TOT}), P_{DA} and fH in the unanesthetized rainbow trout, *O. mykiss* and compared these central actions of FLX with its effects after intra-arterial (IA) injections.

2. Materials and methods

2.1. Chemicals

FLX was purchased from TCI Europe (Tokyo, Japan). On the day of experimentation, FLX was dissolved in Ringer's solution (vehicle) at appropriate concentration for working solutions. The composition of the Ringer's solution was (in mM): NaCl 124, KCl 3, $CaCl_2$ 0.75, $MgSO_4$ 1.30, KH_2PO_4 1.24, $NaHCO_3$ 12, glucose 10 (pH: 7.8). All solutions were sterilized by filtration through 0.22 μm filters (Millipore, Molsheim, France) before injection.

2.2. Animals

Adult rainbow trout (body wt 256 ± 4 g; mean ± S.E.M., $N = 42$) were purchased locally (Pisciculture du Moulin de Lescoat, 29260 Lesneven, France) and transferred into a well-oxygenated and thermostatically controlled water tank in the laboratory. All the fish were kept in a 1000 L tank containing circulating dechlorinated and aerated tap water held at 11–12 °C under a photoperiod of 12 h light/12 h dark (lights on 08:00 h). The fish were allowed at least 3 weeks to acclimate under these conditions before the experiments were conducted. Experimental protocols were approved by the Regional Ethics Committee in Animal Experiments of Brittany, France.

2.3. Experimental procedures

All surgical procedures were made under tricaine methane sulfonate (3-amino-benzoic acid ethyl ester; 60 mg/L in tap water buffered with $NaHCO_3$ to pH = 7.3–7.5) anesthesia. The techniques used for placements of the electrocardiographic (ECG) electrodes and the buccal catheter, cannulation of the dorsal aorta and insertion of the ICV microguide have been described previously (Le Mével et al., 1993; Lancien et al., 2004). Briefly, two ECG AgCl electrodes (Comepa, 93541 Bagnolet, France) were subcutaneously implanted ventrally and longitudinally at the level of the pectoral fins. The incision was sutured across the electrodes and the leads were sutured to the skin. The dorsal aorta was cannulated with a PE-50 catheter (Clay Adams, Le Pont De Claix, France) (Soivio et al., 1972). A flared cannula (PE-160) was inserted into a hole drilled between the nares such that its flared end was resting against the roof of the mouth. This cannula was used to record any changes in buccal ventilatory pressure (Holeton and Randall, 1967). The absence of a neocortex in fish allows placement of the ICV microguide accurately under stereomicroscopic guidance. A 25-gauge needle fitted with a PE-10 polyethylene catheter was inserted between the two habenular ganglia and descended into the third ventricle until the tip lay between the two preoptic nuclei (Le Mével et al., 2009). An obturator was placed at the end of the PE-10 tubing and the cranial surface was covered with hemostatic tissue followed by light quick-curing resin. The entire surgical procedures took around 35 min. After surgery, the animals were force-ventilated with dechlorinated tap water and, following recovery of opercular movements, were transferred to a 6 L blackened chamber supplied with dechlorinated and aerated tap water (10–11 °C) that was both re-circulating and through-flowing. Oxygen partial pressure (PwO_2) within the water tank and pH were continuously recorded and maintained at constant levels ($PwO_2 = 20$ kPa; pH = 7.4–7.6). A small horizontal aperture was made along the upper edge of the chamber in order to connect the ECG leads to an amplifier and to connect the dorsal aorta and the buccal cannula to pressure transducers. This aperture permitted ICV and IA injections of peptides without disturbing the trout.

The trout were allowed to recover from surgery and to become accustomed to their new environment for between 48 and 72 h. Each day, the general condition of the animals was assessed by observing their behavior, checking the ventilatory and the cardiovascular variables, and measuring their hematocrit. Animals that did not appear healthy, according to the range of values detailed in our previous studies (Le Mével et al., 2009, 2013) were sacrificed by decapitation, a procedure approved by the Animal Care Committee (Regional Ethics Committee in Animal Experiments of Brittany, France).

After stable fV, V_{AMP}, P_{DA} and fH were maintained for at least 90 min, parameters were recorded for 30 min without any manipulation, ICV or IA injection in control experiments. To minimize the use of experimental animals, some trout received both ICV and IA injections. In this later case, the delay between the two injections was 1 day, and the order of the injections was randomized.

2.4. Intracerebroventricular administration of fluoxetine

For 34 trout, the injector was introduced within the ICV guide prior to the start of a recording session which lasted 30 min. All injections were made at the 5th min of the test but the injector was left in place for a further 5 min to allow for complete diffusion of the agent and to minimize the spread of substances upwards in the cannula tract.

The vehicle was tested to make sure that it did not induce any effect on cardiovascular and ventilatory variables. Furthermore, control experiments using two ICV injections 30 min apart have shown

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