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Central ventilatory and cardiovascular actions of serotonin in trout

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ABSTRACT

This study was undertaken to investigate the central actions of 5-HT on ventilatory and cardiovascular variables in the unanesthetized trout. Compared to vehicle, intracerebroventricular injection (ICV) of 5-HT elevated the total ventilation. This elevation was due to its stimulatory action on ventilatory amplitude. Moreover, 5-HT produced a dose-dependent increase in mean dorsal aortic blood pressure (P_{DA}) without change in heart rate (f_H). Methysergide, a 5-HT_1/5-HT_2 receptor antagonist, reduced the hyperventilatory and hypertensive actions of 5-HT. 8-OH-2-(di-*n*-propylamino) tetralin, a 5-HT_{1A} receptor agonist, increased P_{DA} while α-methyl-5-HT, a 5-HT_2 receptor agonist, elevated all ventilatory variables and increased P_{DA} without changing f_H. Intra-arterial injection of 5-HT was without effect on ventilation, but 5-HT initially produced hypotension followed by hypertension. These changes were accompanied by tachycardia. It remains to be determined whether endogenous 5-HT within the brain of trout may act as a potent neuroregulator causing stimulatory effects on cardio-ventilatory functions. In the periphery, 5-HT may act as local modulator involved in vasoregulatory mechanisms.

1. Introduction

The serotonergic system is one of the most phylogenetically ancient and highly conserved neurotransmitter systems within the brains of vertebrates (Parent, 1981). In the brains of terrestrial vertebrates, the indoleamine serotonin (or 5-hydroxytryptamine, 5-HT), is mainly synthesized within the neurons of the raphe nuclei which innervate many areas of the brain and the spinal cord. The central serotonergic system is involved in multiple neuroregulatory processes, including stress, mood, aggression, pain, neuronal differentiation and synaptogenesis, sleep, appetite, reproduction, circadian rhythm, volume regulation, cardiovascular and respiratory functions (Gillis et al., 1989; Ramage, 2001; Nalivaiko and Sgoifo, 2009; Hilaire et al., 2010). In mammals, the central cardiorespiratory action of 5-HT is complex depending on the species used, the presence or absence of anesthesia, the doses used, the brain areas involved and the subtypes of central 5-HT receptors that mediate positive or negative effects on the autonomic nervous system (Ramage, 2001; Pilowsky et al., 2009). In the periphery, 5-HT is released from platelets and is present within the gastrointestinal tract. 5-HT can act as a local vasoactive agent or systemic hormone that evokes complex changes in blood pressure and heart rate (f_H) according also to the type of 5-HT receptors that mediate its action (Ramage and Villalón, 2008).

In the brains of teleost fish, 5-HT-immunoreactive perikarya and fibers are mainly 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 transporters and 5-HT receptor genes have been identified in several species of fish (Yamaguchi and Brenner, 1997; Wang et al., 2006; Wang and Tsai, 2006), and their expression is widespread within the brains of fishes (Norton et al., 2008). Taken collectively, these neuroanatomical data suggest that the serotonergic system in fish also plays diversified and important functions within the teleost brain. The central actions of 5-HT in fish have been reviewed (Kreke and Dietrich, 2008; Lillesaar, 2011). 5-HT is involved in sensory responsiveness during arousal (Yokogawa et al., 2012), stress response (Winberg et al., 1997), locomotion (Clements et al., 2003; Gabriel et al., 2008), fear and anxiety (Maximino et al., 2010), control of feeding (De Pedro et al., 1998; Ruibal et al., 2002), neuroendocrine reproductive processes and various behaviors, including aggression (Winberg and Lepage, 1998; Larson et al., 2003; Summers et al., 2005; Mennigen et al., 2011), circadian rhythm (Ceinos et al., 2005), possible adaptation to various osmotic conditions (Mazeaud et al., 1985), and glycogenolysis (Pérez-Maceira et al., 2012). However,

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the potential role of 5-HT in mediating central cardio-respiratory functions in fish has never been explored.

The main peripheral sources of 5-HT in teleost fish are the intestinal tract and the gills (Caamaño-Tubio et al., 2007). Histochemical and immunohistochemistry studies have revealed that at the level of the gills, 5-HT neurons innervate vascular tissues, notably the efferent arterial vasculature, and that 5-HT is also present within the neuroepithelial cells (Bailly et al., 1989; Sundin et al., 1998; Pelster and Schwerte, 2012; Porteus et al., 2012). The major cardiovascular action of 5-HT at the vasculature of the gills is to constrict the arterio-arterial pathway (Sundin and Nilsson, 2002) through 5-HT_1/5-HT_2 receptors (Fritsche et al., 1992; Janvier et al., 1996a; Sundin, 1995). 5-HT-immunoreactivity was also observed in chromaffin cells of teleost fish (Reid et al., 1995; Lim et al., 2013). Intra-arterial (IA) or intraperitoneal injection of 5-HT causes an increase in the plasma levels of noradrenaline, adrenaline and cortisol (Fritsche et al., 1993; Lim et al., 2013). In trout and eel, peripheral 5-HT provokes hyperventilation (Thomas et al., 1979; Fritsche et al., 1992; Janvier et al., 1996b), and affects acid-base balance (Thomas et al., 1979). It was suggested that 5-HT-induced catecholamine release and hyperventilation following IA injections could be centrally-mediated (Fritsche et al., 1992, 1993; Janvier et al., 1996b).

The main goal of the present study was to analyze the effects of intracerebroventricular (ICV) administration of 5-HT on ventilation rate (f_V), ventilation amplitude (V_{AMP}), total ventilation (V_{TOT}), dorsal aortic blood pressure (P_{DA}) and heart rate (f_H) in the unanesthetized rainbow trout, *Oncorhynchus mykiss*. Additionally, these central actions of 5-HT were compared with its effects after IA injections.

2. Materials and methods

2.1. Chemicals

5-HT (serotonin hydrochloride), methysergide (non-selective 5-HT_1/5-HT_2 receptor antagonist), 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT, specific 5-HT_{1A} receptor agonist), and α-methyl serotonin maleate (α-methyl-5-HT; specific 5-HT_2 receptor agonist) were purchased from Sigma–Aldrich (St Quentin Fallavier, France). All compounds were dissolved in Ringer's solution (vehicle) to make stock solution and were frozen at −20 °C. On the day of experimentation, working solutions were made after serial dilution of the aliquot in Ringer's solution. A fresh aliquot was used on each day of testing. 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 252 ± 4.0 g; mean + S.E.M., $N = 47$) of both sexes were purchased locally and transferred in a well-oxygenated and thermostatically controlled water tank to the laboratory. All the fish were kept in a 1000 l tank containing circulating dechlorinated and aerated tap water (11–12 °C), under a standard photoperiod (lights on 09:00–20:00 h). The fish were allowed at least three weeks to acclimate under these conditions before the experiments were started. 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 placement of the electrocardiographic (ECG) electrodes, placement of the buccal catheter, cannulation of the dorsal aorta and insertion of the ICV microguide have previously been described in detail (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 the accurate placement of the ICV microguide 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 its 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. 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 within the water tank (PwO_2) 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 entire surgical procedures took around 35 min. The trout were allowed to recover from surgery and to become accustomed to their new environment for 48–72 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, were discarded. After stable fV, V_{AMP}, V_{TOT}, P_{DA} and f_H were maintained for at least 90 min, variables 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 one day, and the order of the injections was randomized among animals.

2.4. Intracerebroventricular administration of 5-HT and 5-HT receptor antagonist and agonists

The injector was introduced within the ICV guide prior to the beginning 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. Some fish that received firstly the lowest dose of 5-HT were subsequently re-used to test the highest dose of the indoleamine. In this case the recovering time after the first injection was at least 2 h, a delay sufficient for cardio-ventilatory variables to return to baseline levels. No single fish was studied for more than 2 days.