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Comparative Biochemistry and Physiology, Part C 145 (2007) 245–255
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Peripheral serotonin dynamics in the rainbow trout (*Oncorhynchus mykiss*)

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Received 24 July 2006; received in revised form 14 December 2006; accepted 15 December 2006
Available online 16 January 2007

Abstract

Serotonin (5-hydroxytryptamine, 5-HT) occurs in a wide range of tissues throughout the body of the rainbow trout. Results reported here indicate that the main peripheral sources of serotonin are the intestinal tract and the gill epithelium (levels above 1500 ng/g). The high intestinal serotonin concentration is mostly due to serotoninergic nerve fibres, which are present at high density in the intestinal wall. Only about 2% of serotonin is associated with mucosal enterochromaffin cells. In the remaining tissues studied serotonin concentration was below 160 ng/g: the highest concentrations were seen in the anterior and posterior kidneys, followed by the liver, heart, and spleen. 5-Hydroxyindolacetic acid (5-HIAA) levels, except in plasma, were generally lower than serotonin levels, and were below our detection limits in heart, spleen and posterior kidney. Acute *d*-fenfluramine treatment (5 or 15 mg/kg i.p.) significantly increased 5-HIAA/5-HT ratio in the anterior intestine, pyloric caeca and plasma. Serotonin released from intestinal serotoninergic fibres in response to *d*-fenfluramine treatment is metabolized locally, and only a small part reaches the blood, from where it can be taken up and metabolized by other peripheral tissues, such as the liver and gill epithelium. The non-metabolized serotonin pool in the blood appears to be located extracellularly, not intracellularly as in mammals. In view of these findings, we present an overview of peripheral serotonin dynamics in rainbow trout.

Keywords: Serotonin; 5-Hydroxyindolacetic acid; Peripheral tissues; Plasma; Blood; Enteroendocrine cells; Fenfluramine; *Oncorhynchus mykiss*; Rainbow trout

1. Introduction

A considerable body of biochemical, physiological and histochemical evidence suggests a transmitter function for serotonin (5-hydroxytryptamine, 5-HT) in the animal kingdom, and in vertebrates this molecule is a well-established central neurotransmitter. Its levels in different brain regions have been widely studied in higher vertebrates, mainly mammals (Essman, 1978; Durán et al., 1985; Míguez et al., 1994, 1999), though also to a lesser extent in fish (Pouliot et al., 1988; Nilsson, 1989; Rozas et al., 1990).

Central and peripheral 5-HT synthesis takes place from tryptophan in a two-step process that is strongly conserved in the animal kingdom (Aldegunde, 1998). In the first step, 5-hydroxytryptophan (5-HTP) is formed from trytophan by the action of tryptophan hydroxylase, and the 5-HTP is then converted to 5-HT by the action of 5-HTP decarboxylase. 5-HT is converted to its main metabolite, 5-HIAA, by monoamine oxidase (Senatori et al., 2003), and 5-HIAA is excreted in urine.

In mammals the distribution of 5-HT in tissues outside the central nervous system is well known (Fozard, 1989; Martín and Aldegunde, 1995). In other vertebrates, however, there have been fewer studies of its distribution and concentration in tissues other than brain. Studies in some fish species have determined 5-HT levels in various tissues (for a review see Essman, 1978), including intestine, gills and kidney of *Conger conger* and *Scyliorhinus stellaris* (Piomelli and Tota, 1983). All these studies have mainly used bioassays for 5-HT determination. Very few studies have used high performance liquid chromatography with electrochemical detection, which offers very high sensitivity and specificity. The few published studies of this type include studies of 5-HT and 5-HIAA levels in the blood of *Anguilla anguilla* (Caroff et al., 1986) and in the intestine of *Platycephalus bassensis* (Anderson et al., 1989).

In mammals, peripheral 5-HT shows a wide range of biological activities. It is known to modulate the activity of neurons of the peripheral nervous system (the sympathetic, parasympathetic and

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1532-0456/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2006.12.017

enteral system), and thus mediates numerous physiological functions; however, its main peripheral function remains to be elucidated (Fozard, 1989; Doggreu, 2003; Côte et al., 2004). In fish, there is likewise evidence implicating 5-HT in the modulation of diverse physiological functions, such as nervous control of gastrointestinal function (Burka et al., 1989; Kiliaan et al., 1989; Mori and Ando, 1991; Venugopalan et al., 1995; Buddington and Krogdahl, 2004), the secretion of inter-renal catecholamines (Fritsche et al., 1993; Bernier and Perry, 1996; Tubío et al., 2002), branchial function (Sundin, 1995; Sundin et al., 1995; Forster et al., 1998; Sundin and Nilsson, 1998), cardiovascular function (Meghji and Burnstock, 1984; Janvier et al., 1996; Pellegrino et al., 2003) and lymphocyte proliferation (Ferriere et al., 1996).

Given that in mammals it has been demonstrated that the presence of 5-HT in tissues and organs is related to functional activity, it seems reasonable to suppose that this same relationship between location and function will also be seen in lower vertebrates. In several fish, 5-HT levels have been determined in individual tissues; however, there have been no studies of serotonin metabolism in peripheral tissues, or of relationships between different peripheral 5-HT pools. We thus consider that it is of interest to determine levels of 5-HT and its main metabolite 5-HIAA in diverse peripheral tissues in the rainbow trout (*Oncorhynchus mykiss*), which will be additionally overviewed in the context of peripheral serotonin.

2. Materials and methods

2.1. Experimental animals

Immature rainbow trout (*O. mykiss*) (86±5 g) were obtained from a commercial trout farm (Soutorredondo, Noia) and acclimated for 3 weeks in running dechlorinated tap water (temperature 14±0.5 °C, pH 6.3±0.1) with continuous aeration, in 200-L tanks. Fish were maintained under a 12-h-light and 12-h-dark photoperiod (lights on at 8:00 h), and during the acclimation period were fed once daily in the morning (at 12:00 h) with commercial dry pellets (ration equivalent to 1.5% of body weight per day), and were fasted 24 h prior to sampling or intraperitoneal injections (see below). All samples were obtained at the same time each morning to avoid possible effects of circadian variations. To minimize stress, fish were anaesthetised (50 mg L^{-1} MS-222 buffered to pH 7.4 with sodium bicarbonate) before handling, injection or decapitation. Replicate or triplicate tanks were established for each experiment.

2.2. Experimental protocols

In Experiment I we studied the levels of 5-HT and 5-HIAA in peripheral tissues of rainbow trout. Fish were distributed in 100-L tanks at 4 fish per tank. After anaesthesia, blood was obtained with ammonium-heparinized syringes from the caudal peduncle, then centrifuged to obtain plasma. Liver, heart, anterior and posterior kidney, and spleen were dissected out and weighed. The intestinal tract was removed and transferred to a chilled glass plate (2 °C), for removal of external fat followed by washing with chilled saline; it was then dissected into three regions, the pyloric caeca (CP), the anterior intestine without pyloric caeca (AI), and the posterior intestine including the middle intestine (PI). Finally, samples of gill epithelium were obtained by scraping the gill arches, again on a chilled glass plate. All samples (organs, intestinal tract, gill epithelium, plasma) were frozen on dry ice and stored at −80 °C until analysis.

In Experiment II we investigated the distribution (Experiment IIa) and origin (Experiment IIb) of 5-HT and 5-HIAA in the intestinal tract of the rainbow trout. In Experiment IIa we used 12 fish distributed in three 100-L tanks. After anaesthesia, the protocol was like that of Experiment I, but using a different dissection of the intestinal tract: the pyloric caeca (CP), the anterior intestine without pyloric caeca but with middle intestine (AIa), and the posterior intestine (PIa). Immediately, the intestinal mucosa was removed from each region by scraping, and the resulting mucosal cells and intestinal tissue without mucosa (denominated intestinal wall) were weighed and stored at −80 °C. In Experiment IIb the protocol was similar to that followed for Experiment IIa, but in this experiment trout were anaesthetised with MS-222 (0.1% solution) before fixation of intestinal tissues, by transcardial perfusion with freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (PB) at pH 7.4. The intestine was then carefully dissected out (anterior intestine, pyloric caeca and posterior intestine), cut

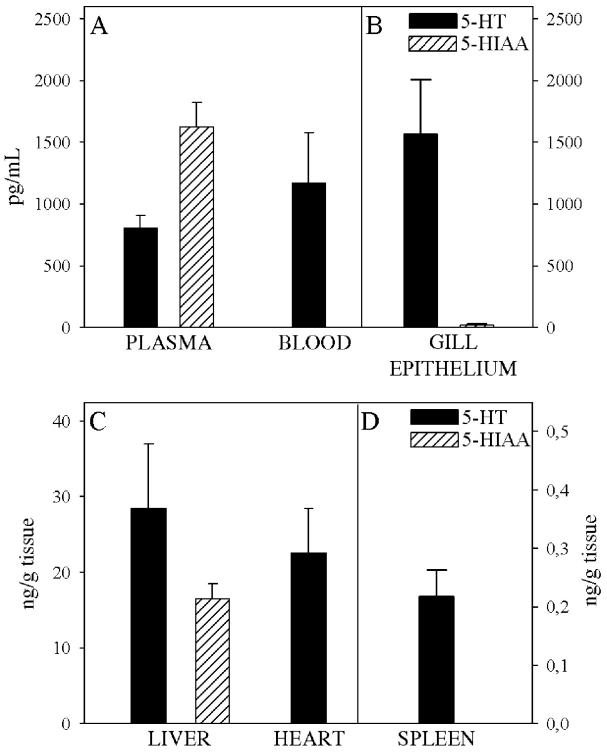

Fig. 1. 5-HT and 5-HIAA concentrations in (A) plasma and blood, (B) gill epithelium, (C) liver, heart and (D) spleen in rainbow trout. Each bar represents the mean±SEM for $n=17$ fish per group (plasma), $n=4$ fish per group (blood) and $n=6$–8 fish per group (liver, heart and spleen). 5-HIAA levels were below detection limits in heart and spleen.