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The arylalkylamine-N-acetyltransferase (AANAT) acetylates dopamine in the digestive tract of goldfish: A role in intestinal motility

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ABSTRACT

Melatonin has been found in the digestive tract of many vertebrates. However, the enzymatic activity of the arylalkylamine-N-acetyltransferase (AANAT) and the hydroxindole-*O*-methyltransferase (HIOMT), the last two enzymes of melatonin biosynthesis, have been only measured in rat liver. Therefore, the first objective of the present study is to investigate the functionality of these enzymes in the liver and gut of goldfish, analyzing its possible daily changes and comparing its catalytic properties with those from the retina isoforms. The daily rhythms with nocturnal acrophases in retinal AANAT and HIOMT activities support their role in melatonin biosynthesis. In foregut AANAT activity also show a daily rhythm while in liver and hindgut significant but not rhythmic levels of AANAT activity are found. HIOMT activity is not detected in any of these peripheral tissues suggesting an alternative role for AANAT besides melatonin synthesis. The failure to detect functional HIOMT activity in both, liver and gut, led us to investigate other physiological substrates for the AANAT, as dopamine, searching alternative roles for this enzyme in the goldfish gut. Dopamine competes with tryptamine and inhibits retinal, intestinal and hepatic N-acetyltryptamine production, suggesting that the active isoform in gut is AANAT1. Besides, gut and liver produces N-acetyldopamine in presence of acetyl coenzyme-A and dopamine. This production is not abolished by the presence of folic acid (arylamine N-acetyltransferase inhibitor) in any studied tissue, but a total inhibition occurs in the presence of CoA-S-N-acetyltryptamine (AANAT inhibitor) in liver. Therefore, AANAT1 seems to be an important enzyme in the regulation of dopamine and N-acetyldopamine content in liver. Finally, for the first time in fish we found that dopamine, but not N-acetyldopamine, regulates the gut motility, underlying the broad physiological role of AANAT in the gut.

1. Introduction

Melatonin is a well known hormone mainly produced by the pineal and the retina that regulates many rhythmic physiological functions, including gastrointestinal functions (Velarde et al., 2009, 2010b). The biosynthesis of melatonin is under the sequential control of four enzymes. First, tryptophan is transformed into 5-hydroxytryptophan by the tryptophan hydroxylase (E.C. 1.14.16.4). Then, the L-aromatic amino acid decarboxylase (E.C. 4.1.1.28) produces serotonin. The arylalkylamine N-acetyltransferase (AANAT: E.C. 2.3.1.87) converts serotonin into N-acetylserotonin, and finally, the hydroxindole-*O*-methyltransferase (HIOMT: E.C.2.1.1.4) transforms N-acetylserotonin into melatonin (Falcón et al., 2009).

The AANAT is broadly considered as the melatonin rhythm generating enzyme and exhibits a daily rhythm with high nocturnal levels in the pineal and retina of many vertebrates (Klein, 2007). A single AANAT gene has been found in mammals and birds, but the whole genome duplication of ancestral teleosts generated at least two AANAT genes encoding for two different enzymes. The AANAT1 is mostly expressed in the retina, where acetylates several amines, and is involved in multiple local functions. The AANAT2, mainly expressed in the pineal, appears to have acquired a higher selectivity for serotonin to enhance the efficiency of melatonin production (Zilberman-Peled et al., 2011). The enzymatic activity and circadian rhythmic profile of HIOMT was early described in the pineal gland, retina and Harderian gland of some mammals (Nowak et al., 1993) and in the retina of fish (Gern et al., 1984). Later, its mRNA expression has been reported in pineal and retina

Abbreviations: AANAT, arylalkylamine-N-acetyltransferase; ANAT, arylamine-N-acetyltransferase; DA, dopamine; NADA, N-acetyldopamine; HIOMT, hydroxindole-*O*-methyltransferase.

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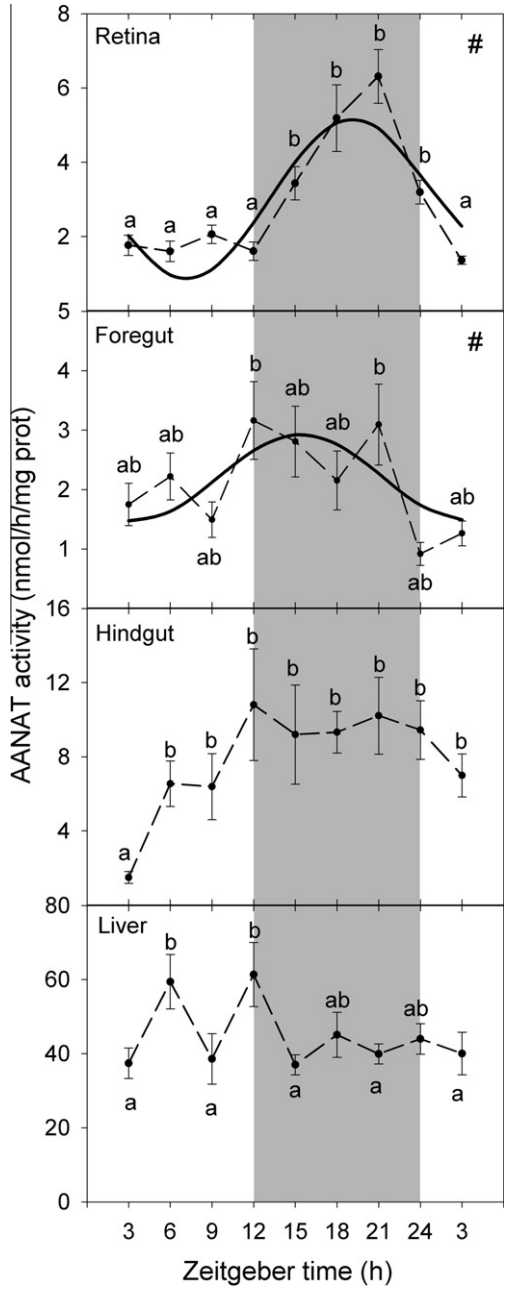

Fig. 1. Daily AANAT activity during a 24 h cycle in goldfish retina, foregut, hindgut and liver. Data are represented as mean ± SEM ($n = 8$). Different letters indicate significant differences (ANOVA, $p < 0.05$). Solid lines represent the periodic sinusoidal functions adjusted to experimental data when the rhythms are significant (#). Gray area indicates darkness.

of mammals (Pévet et al., 1980), birds (Bernard et al., 1999), and fish (Velarde et al., 2010a).

Melatonin is present in the gut of many vertebrates, including fish (Bubenik and Pang, 1997; Kulczykowska et al., 2006). Some studies emphasize the local synthesis of melatonin in the gastrointestinal tract based on the existence of high expression levels of its synthesizing enzymes (Fernández-Durán et al., 2007; Stefulj et al., 2001). However, functional studies about AANAT and HIOMT activities have only been performed in rat liver (Sánchez-Hidalgo et al., 2009). In goldfish, the gene expression of AANAT1, AANAT2 and HIOMT in liver and gut has been recently reported (Velarde et al., 2010a, 2013), but the functionality of these two key enzymes producing melatonin in these tissues remains to be demonstrated. Then, our first objective was to investigate the functionality of these two key enzymes, AANAT and HIOMT, in the liver and gut of goldfish, analyzing its possible daily changes and comparing its catalytic properties with those from pineal and retinal isoforms.

The role of dopamine (DA) in the gastrointestinal tract is supported by immunohistochemical studies that demonstrate the presence of DA-containing neurons and dopaminergic receptors in the myenteric plexus of mice (Li et al., 2004, 2006), and by functional studies on dopamine effects on gastrointestinal motility in rat (Kirschstein et al., 2009). Present results showing a broad capability of AANAT in the acetylation of amines in the gut and liver of goldfish. However, we do not find functional HIOMT in these two tissues. This fact encouraged us to investigate the possible acetylation of DA by the AANAT looking for alternative roles for this enzyme in the liver and gut. Finally, we used an organ bath system to test the physiological relevance of DA in the goldfish gut.

2. Materials and methods

2.1. Animals

Goldfish (*Carassius auratus*) were purchased from a local supplier in Madrid (Spain) and maintained as previously described (Velarde et al., 2009) for at least two weeks before assays. The acclimation conditions at the laboratory were 12 h light and 12 h dark (12L:12D), lights on at 8:00 h, and daily feeding time at 10:00 h (zeitgeber time 2). The experiments were approved by the Animal Experimentation Committee of Complutense University, and were carried out in accordance with the European Communities Council Directive (2010/63/UE).

2.2. Chemicals

N-acetyltryptamine, acetyl coenzyme-A, tryptamine, tyramine, S-(5′-adenosyl)-L-methionine, N-acetylserotonin, folic acid, melatonin, DA and acetylcholine were purchased for Sigma Aldrich Chemicals (Madrid, Spain). N-acetyldopamine (NADA) was synthesized at the Department of Organic Chemistry of Autónoma University of Madrid. CoA-S-acetyltryptamine was obtained from the National Institute of Mental Health's Chemical Synthesis and Drug Supply Program. All drugs were prepared fresh before use.

2.3. In vitro enzymatic assays

2.3.1. Quantification of tryptamine acetylation

The AANAT activity was assayed *in vitro* based on the method by Alonso-Gómez et al. (1992) with some modifications. Briefly, gut and liver tissues were sonicated in five volumes per gram of wet tissue, and retinas in 300 μL of assay buffer (10 mM EGTA in 0.25 M sodium phosphate, pH 6.5). The homogenates were centrifuged at 16,000*g* during 10 min at 4 °C. Reactions were carried out during 20 min at 25 °C in a total volume of 100 μL consisting of 25 μL of supernatant from homogenates, 0.3 mM tryptamine as substrate, and 1 mM acetyl-Coenzyme-A as the acetyl donor. The reaction was stopped by adding 20 μL of 2 N perchloric acid, centrifuged at 16,000*g* for 10 min at 4 °C, and the supernatants were placed into HPLC vials. All reactions were done in duplicates. The N-acetyltryptamine formation was quantified by HPLC coupled to a fluorescence detector (HPLC-FD, Agilent 1100, Madrid, Spain) as previously described (Velarde et al., 2013). The mobile phase consisted of 0.125 mM sodium decanesulfonate, 50 mM phosphoric acid, and 12% acetonitrile (vol/vol) adjusted to pH 3.5 with 10 N NaOH.