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Oral administration of melatonin counteracts several of the effects of chronic stress in rainbow trout

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ABSTRACT

To assess a possible antistress role of melatonin in fish, we orally administered melatonin to rainbow trout for 10 d and then kept the fish under normal or high stocking density conditions during the last 4 d. Food intake; biochemical parameters in plasma (cortisol, glucose, and lactate concentrations); liver (glucose and glycogen concentrations, and glycogen synthase activity); enzyme activities of amylase, lipase, and protease in foregut and midgut; and content of the hypothalamic neurotransmitters dopamine and serotonin, as well as their oxidized metabolites, 3,4-dihydroxyphenylacetic acid and 5-hydroxy-3-indoleacetic acid, were evaluated under those conditions. High stocking density conditions alone induced changes indicative of stress conditions in plasma cortisol concentrations, liver glycogenolytic potential, the activities of some digestive enzymes, and the 3,4-dihydroxyphenylacetic acid-to-dopamine and 5-hydroxy-3-indoleacetic acid-to-serotonin ratios in the hypothalamus. Melatonin treatment in nonstressed fish induced an increase in liver glycogenolytic potential, increased the activity of some digestive enzymes, and enhanced serotoninergic and dopaminergic metabolism in hypothalamus. The presence of melatonin in stressed fish resulted in a significant interaction with cortisol concentrations in plasma, glycogen content, and glycogen synthase activity in liver and dopaminergic and serotoninergic metabolism in the hypothalamus. In general, the presence of melatonin mitigated several of the effects induced by stress, supporting an antistress role for melatonin in rainbow trout.

1. Introduction

Stress effects are usual causes of undesirable damages in modern intensive fish cultures. High circulating concentrations of catecholamines and cortisol are involved in the first line of the integrated response to stress in fish [1]. Hormonal changes induced by stress trigger immediate tissue alterations to restore the effect of the stressor and to satisfy the increased energy demand. Thus, although catabolic actions in metabolic processes are considered the main secondary responses to stress, the effects at organism level such as alterations in food intake (FI), growth, and reproduction are considered as tertiary responses to stress [1,2].

Besides the complexity of neuroendocrine mechanisms of stress, a characteristic behavioral response to intensive stress in fish is a reduction in appetite [2]. Several central and peripheral peptides and hormones have been investigated in relation to their effects to induce or suppress fish appetite (reviewed in Volkoff et al [3]). Among them, a particular focus has been placed on corticotropin-releasing hormone, which initiates the activation of the hypothalamus-pituitary-interrenal (HPI) axis and seems to have a predominant role in the feeding inhibition under stress conditions [4]. Several other peptides, and also brain monoamine neurotransmitters such as serotonin (5HT) and dopamine (DA), which are important in triggering the initial steps of stress, are candidates to mediate some of the physiological and behavioral stress effects on FI [5,6].

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The hormone melatonin is mainly synthesized in the pineal organ and plays a crucial role in the regulation of seasonal and circadian rhythms of physiology and behavior in all vertebrates, including fish [7]. Melatonin is also likely to be synthesized in other fish organs such as the gastrointestinal tract (GIT) where it could be involved in the regulation of gut motility and other digestive- and metabolism-related processes [8–10]. As a multifunctional molecule, melatonin is believed to affect several neural and endocrine mechanisms, mediating relevant functions, such as feeding, osmoregulation, reproduction, and stress response [7]. An antistress function of melatonin at both central and peripheral levels has been proposed in vertebrates, including mammals [11,12] and birds [13]. In fish, there is some evidence about a suppressor effect of melatonin treatment on the HPI axis, such us reduction in glucocorticoid secretion and locomotor activity [14,15]. Other precursors of melatonin synthesis, such as 5HT and the amino acid L-tryptophan also have been reported to induce mitigating effects on stress responsiveness in fish [16].

The presumptive role of melatonin in FI of fish regulation is also far from fully understood. For instance, studies in goldfish (*Carassius auratus*) found that melatonin induced an anorexigenic effect when injected peripherally but not after central intravenous treatment [17]. An inhibitory effect of oral melatonin treatment on feeding was also described in European sea bass, *Dicentrarchus labrax* [18], and tench, *Tinca tinca* [19], suggesting that some melatonin effects on FI might not be centrally but peripherally mediated. However, a central action of melatonin is also possible because treatments with the hormone alter gene expression of some brain neuropeptides involved in feeding regulation in zebrafish, *Danio rerio* [20], and rainbow trout, *Oncorhynchus mykiss* [21]. Moreover, the brain monoaminergic systems that are believed to participate in the regulation of feeding behavior in fish [22,23] are also altered by melatonin treatments, which in addition mitigated some effects of stress [15].

Integrative studies in fish about the possible mitigating effect of melatonin on stress response are still lacking. The aim of this study was to explore a putative antistress role of melatonin in the rainbow trout by evaluating changes in some neuroendocrine and metabolic parameters related to the stress response. Food intake and the activity of some digestive enzymes were also measured. Melatonin was administered as a dietary supplementation to avoid any negative effect of fish handling and to better define changes in the assessment of the stress response.

2. Material and methods

2.1. Fish

The experiments described comply with the Guidelines of the European Union Council (2010/63/EU) and of the Spanish Government (RD 1201/2005) for the use of animals in research. Female rainbow trout (*Oncorhynchus mykiss* Walbaum) were obtained from a local fish farm (Soutorredondo, Noia, Spain). Fish were maintained for 1 mo in 100-L tanks under laboratory conditions and a fixed 12:12 light/dark photoperiod (lights on at 8 AM; light intensity of 300 lux) in dechlorinated tap water at 15°C. Fish mass was 145 ± 3 g. Commercial dry pellets (Dibaq-Diproteg SA, Segovia, Spain; proximate food analysis was 48% crude protein, 14% carbohydrates, 25% crude fat, and 11.5% ash; 20.2 MJ/kg of feed) were used to fed fish once daily (10 AM) to satiety.

2.2. Experimental protocol

2.2.1. Preliminary experiment: Time course of plasma melatonin concentrations after oral administration

Food pellets were submerged in a solution with melatonin (Sigma, Indianapolis, IN, USA) at 2 different concentrations (0.04 and 0.2 g/kg food) and then dried at 37°C for 24 h. After acclimation, fish were separated into 2 experimental groups and fed with pellets supplemented with the 2 doses of melatonin. After anesthesia with MS-222 (50 mg/L) buffered to pH 7.4 with sodium bicarbonate, blood samples were taken before feeding and at 0.5, 1, 2, 4, and 6 h after feeding. Plasma was obtained after centrifuging blood samples for 10 min at 9,000 × *g* and stored at −80°C for melatonin analysis.

2.2.2. Effects of oral administration of melatonin on stress response

After acclimation, fish were distributed into 6 tanks and fed once daily (10 AM) for 10 d with commercial pellets alone (control) or supplemented with a low (0.04 g/kg food) or a high (0.2 g/kg food) concentration of melatonin (2 tanks per treatment). The last 4 d of the experiment, half of the tanks (1 tank per treatment) were kept at 10 kg fish mass/m^3 and denoted as nonstressed groups. In the remaining tanks (1 tank per treatment) a quantity of water was removed until reaching stressful high stocking density conditions (70 kg fish mass/m^3) and denoted as stressed groups. Therefore, 6 experimental treatments were used: a) nonstressed fish fed with commercial pellets, b) nonstressed fish fed with pellets supplemented with a low concentration of melatonin (0.04 g/kg food), c) nonstressed fish fed with pellets supplemented with a high concentration of melatonin (0.2 g/kg food), d) stressed fish fed with commercial pellets, e) stressed fish fed with pellets supplemented with a low concentration of melatonin (0.04 g/kg food), and f) stressed fish fed with pellets supplemented with a high concentration of melatonin (0.2 g/kg food).

On the last day, 4 h after feeding, fish were anesthetized with MS-222 (50 mg/L) buffered to pH 7.4 with sodium bicarbonate. Blood was collected by caudal puncture with ammonium-heparinized syringes, and plasma samples were obtained after blood centrifugation and divided into 2 aliquots. One aliquot was immediately frozen on liquid nitrogen for the assessment of plasma cortisol concentrations, whereas the other aliquot, for the assessment of plasma metabolites, was deproteinized immediately (using 0.6 *M* perchloric acid) and neutralized (using 1 mol/L potassium bicarbonate) before freezing on liquid nitrogen and storage at −80°C until further assay. Fish were sacrificed rapidly by decapitation, and the liver was removed, snap-frozen in liquid nitrogen, and stored at −80°C until assayed. Foregut and hindgut were removed, cleaned from