Food intake inhibition by melatonin in goldfish (*Carassius auratus*)


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Abstract

Feeding regulation by monoamines, neuropeptides and certain hormones has been studied in fish, but a possible role of melatonin is unknown. The purpose of the present study was to investigate the effects of melatonin on food intake in goldfish. Fishes were housed in 12L:12D and injected with different doses of either melatonin or 2-iodomelatonin. Two routes of administration, intracerebroventricular and intraperitoneal injections, and two times of the daily photocycle, midday and midnight, were tested. Food intake was measured at 2, 5 and 8 h postinjection. Melatonin and its analog, 2-iodomelatonin intracerebroventricularly injected had no effect on food intake at any time. However, intraperitoneal injections of both indoleamines significantly reduced food intake at different postinjection times. The inhibitory effect of melatonin was blocked by intraperitoneal administration of its antagonist, luzindole. These results demonstrate the in vivo efficiency of luzindole as melatonin antagonist, and thus provide a useful experimental tool to investigate melatonin functions. In conclusion, both melatonin and its agonist 2-iodomelatonin administered peripherally, inhibit food intake in goldfish, and this inhibitory effect appears to be mediated via luzindole-sensitive melatonin receptors. Our results strongly suggest that melatonin is involved in the peripheral satiety mechanisms in goldfish. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Food intake; Goldfish; 2-Iodomelatonin; Luzindole; Melatonin; Satiety

1. Introduction

The regulation of food intake is a complex, and not yet well understood process, which requires an interaction among the central nervous system, the gastrointestinal tract and the environment. Although it has been well established that central monoaminergic and peptidergic systems play important roles in regulating food intake, a growing list of compounds affect food intake when given peripherally [1].

In fish, food intake is under multifactorial control, including environmental factors, chemosensory stimuli and endogenous factors, such as hormones, biogenic amines and neuropeptides. Feeding activity exhibits a rhythmic pattern, with most fish not feeding continuously [2]. Among the different environmental synchronizers, light is probably the main factor controlling the feeding rhythm in many fish [3, 4]. On the other hand, it has been reported that feeding, by itself, can entrain some other circadian rhythms [2].

Melatonin is synthesized by the pineal gland and retinal photoreceptors of almost all vertebrates studied to date, and it is related to many functions which have in common a rhythmic expression [5]. Melatonin has also been detected in the gastrointestinal tissues of many vertebrate species [6–10], and it has been speculated that the gastrointestinal tract may be a significant contributor to the daytime levels of peripheral melatonin in goldfish [11].

The high diversity in the distribution of melatonin binding sites in the gastrointestinal tract supports a variety of possible functions of the indole in the digestive system [12]. In fact, several studies have indicated that melatonin plays a role in the pathophysiology of the gastrointestinal tract. Thus, melatonin reduces the incidence of gastric ulcers in rats [13] and pigs [14], it prevents the development of colitis in mice [9], it is involved in the regulation of obesity in prehibernating garden dormouse [15], it acts as a local regulator of gastrointestinal motility in rat [16] and protects the gastric mucosa from damage by stress and ischaemia reperfusion in rat [17]. Finally, a relationship between peripheral melatonin levels and food intake has been reported [8,10,18].

Food intake regulation by neuropeptides [19–23], monoamines [24] and certain hormones [25] has been shown in...
fish, but the possible role of melatonin has not yet been shown. Keeping in mind the above-mentioned reports of different melatonin actions on gastrointestinal physiology, we performed the present study to investigate the possible effects of melatonin on food intake behavior in goldfish.

2. Materials and methods

2.1. Animals

Goldfish (*Carassius auratus*) were obtained from a commercial supplier, and were maintained at the laboratory in glass aquaria with dechlorinated water under 12L:12D photoperiod and 20 ± 2°C for 2 weeks. Animals were fed with floating pellets (Sera Biogram, Heinsberg, Germany) at a daily ration of 1% body weight (bw) and were divided into two groups. One group of fish (*n* = 165) was acclimated to feed at midnight, and the other one (*n* = 165) at midday, to test differences in melatonin effects on food intake depending on the time of the administration.

2.2. Drugs

Melatonin (Sigma, Spain) and luzindole (Tocris, UK) were dissolved in a small amount of ethanol and then diluted with teleost saline (20 mg Na₂CO₃/100 ml of 0.6% NaCl) until the desired final concentrations were obtained. 2-Iodomelatonin (Tocris) was dissolved in teleost saline with 10% dimethyl sulphoxide (DMSO). Teleost saline with an equivalent amount of ethanol or DMSO was used for control injections.

2.3. Experimental procedure

Fish were anesthetized at the midnight or the midday in water containing tricaine methanesulfonate (MS-222, 1/10000) and the injections were performed when loss of equilibrium was observed. The intracerebroventricular and intraperitoneal injection procedures were previously described [19]. In brief, the intracerebroventricular injections (1 μl) were carried out with a 0.3-mm Microlance needle connected to a 5 μl Hamilton microsyringe with an 18-Venocath cannula. The intraperitoneal injections (10 μl/g bw) were performed using a 1-ml syringe and a 0.3-mm Microlance needle, close to the ventral midline posterior to the pelvic fins. The injections at midnight were done under dim red light conditions.

2.3.1. Experiment 1: Effect of melatonin intracerebroventricular injection on food intake in goldfish

Six groups of goldfish (6.1 ± 1.3 g, *n* = 8 fish/group) were injected intracerebroventricularly with 1 μl saline or melatonin at the following doses: 1, 10, 100, 1000 and 5000 ng/μl. The experiment was replicated at midday and at midnight.

2.3.2. Experiment 2: Effect of 2-iodomelatonin intracerebroventricular injection on food intake in goldfish

Four groups of goldfish (7.8 ± 0.3 g, *n* = 8 fish/group) were intracerebroventricularly injected with 1 μl saline or 2-iodomelatonin at the following doses: 1, 10 and 100 ng/μl. The experiment was replicated at midday and at midnight.

2.3.3. Experiment 3: Effect of melatonin intraperitoneal injection on food intake in goldfish

Four groups of goldfish (6.6 ± 1.7 g, *n* = 8 fish/group) were intraperitoneally injected with 10 μl saline/g bw or melatonin at the following doses: 2, 20 and 200 μg/g bw. The experiment was replicated at midday and at midnight.

![Fig. 1. Food intake (mg) after intracerebroventricular administration of 1 μl saline alone or containing different doses of melatonin, at 0–2, 2–5 and 5–8 h postinjection, in goldfish (*C. auratus*). Fish were injected either at midnight (upper graph) or at midday (lower graph). Data are expressed as mean ± S.E.M. (*n* = 8/group).](image)

Fig. 1. Food intake (mg) after intracerebroventricular administration of 1 μl saline alone or containing different doses of melatonin, at 0–2, 2–5 and 5–8 h postinjection, in goldfish (*C. auratus*). Fish were injected either at midnight (upper graph) or at midday (lower graph). Data are expressed as mean ± S.E.M. (*n* = 8/group).
2.3.4. Experiment 4: Effect of 2-iodomelatonin intraperitoneal injection on food intake in goldfish

Four groups of goldfish (7.3 ± 2.0 g, n = 8 goldfish/group) were intraperitoneally injected with 10 μl saline/g bw or 2-iodomelatonin at the following doses: 0.2, 2, 20 and 200 μg/g bw. The experiment was replicated at midday and at midnight.

2.3.5. Experiment 5: Effect of luzindole on melatonin-induced feeding reduction

Four groups of goldfish (7.2 ± 0.3 g, n = 8 fish/group) received two sequential intraperitoneal injections: (a) control group: two injections of 10 μl saline/g bw; (b) melatonin group: saline injection followed by melatonin injection (200 μg/g bw); (c) luzindole group: luzindole injection (0.1 μg/g bw followed by saline injection; and (d) luzindole + melatonin group: luzindole injection (0.1 μg/g bw) followed by melatonin injection (200 μg/g bw).

2.4. Feeding quantification

Fish recovered equilibrium and normal swimming activity in anesthetic-free water within 1–2 min after the injections. Individual goldfish were transferred to 5 l aquaria and 10 min after the last injection received preweighed food in excess (7% bw). Food intake (FI) was measured at 2, 5 and 8 h (Experiments 1, 2, 3 and 4) and at 2 h (Experiment 5) postinjection, and calculated as follows, FI = W_i - (W_f × F), where W_i = initial dry food weight and W_f = remaining dry food weight. F was previously calculated (F = W_f/W_i) to determine the reduction in the weight of food pellets due to water dissolution after remaining 2, 5 and 8 h in the aquaria [24].

2.5. Statistical analysis

Data are expressed as mean ± S.E.M. and were analyzed by an ANOVA test followed by Student–Newman–Keuls (SNK) multiple range test for multigroup comparisons. A probability level of $P < .05$ was considered statistically significant.

3. Results

Fig. 1 shows the effect of an intracerebroventricular injection of melatonin given at midnight (upper graph) and midday (lower graph) on feeding in goldfish. There were no significant differences in food intake either at any...
of the melatonin tested doses or at any of the studied time intervals (0–2, 2–5 and 5–8 h).

The intracerebroventricular administration of 2-iodomelatonin did not significantly modify feeding in goldfish either at midnight (Fig. 2, upper) or at midday (Fig. 2, lower) and at any of the studied discrete time intervals.

Fig. 3 summarizes the effect of an intraperitoneal injection of melatonin given at midnight (upper graph) and midday (lower graph) on feeding in goldfish. All melatonin tested doses significantly reduced food intake with respect to the control group at 2 h postinjection at midnight. The highest doses (20 and 200 μg/g bw) of melatonin also significantly reduced feeding during the 5–8 h interval. The melatonin-induced feeding reduction during the 2–5-h interval was not statistically significant. The cumulative food intake at 8 h postinjection indicates that intraperitoneal-administered melatonin (20 and 200 μg/g bw) significantly reduced feeding at any of the studied time intervals at midday (lower graph). The reduction in food intake evoked by intraperitoneal-administered melatonin (2 μg/g bw) was only observed during the 2–5-h interval postinjection.

Food consumption after intraperitoneal injections of 2-iodomelatonin at midnight (upper graph) and at midday (lower graph) is summarized in Fig. 4. As it occurred with intraperitoneal melatonin administration, the highest doses of the agonist (20 and 200 μg/g bw) significantly reduced food intake with respect to the saline-injected fish at midnight. The cumulative feeding at 8 h postinjection indicates an anorectic effect similar to that observed for melatonin. At midday, only the highest 2-iodomelatonin doses (200 μg/g bw) significantly reduces feeding at any of the studied time intervals (lower graph).

Food intake of saline-injected fish did not show significant differences between midnight and midday in any of the experiments carried out.

The intraperitoneal administration of luzindole (0.1 μg/g bw) by itself did not significantly alter food intake in goldfish (Fig. 5). However, luzindole administered before melatonin (200 μg/g bw), totally blocked the melatonin-induced feeding reduction in goldfish, being the food ingestion values similar to those observed in the control group.

4. Discussion

Our results strongly suggest that melatonin is involved in regulation of feeding in goldfish. Both melatonin and its agonist 2-iodomelatonin peripherally administered inhibit food intake at different postinjection times. To our knowledge, this is the first report concerning melatonin and feeding in fish. Feeding-dependent alterations of peripheral melatonin have been described in different mammalian species, including humans [10,26,27], thus suggesting a physiological function of melatonin on nutritional status. Little information, however, is available as to melatonin’s actions on food intake in mammals [18]. A body mass gain in the garden dormouse (Eliomys quercinus) treated with a melatonin agonist (10 mg/kg) has been reported [15], which mainly corresponds to a rapid increase in energy intake associated to a decrease in energy expenditure. The authors suggest that the melatonin agonist trigger the prehibernation mechanisms. The well-documented melatonin actions on mammalian thermogenesis [28], which strongly determines nutritional requirements makes a comparison of the present results in fish with above-described melatonin effects on feeding in mammals highly difficult. To date, there are no data on the possible role of melatonin on feeding in ectotherm vertebrates.
The intraperitoneal melatonin-induced food intake reduction in goldfish is observed at 20 μg/g bw. This melatonin dose is clearly higher than endogenous melatonin levels so far reported in goldfish in in vivo [11,29] and in vitro [30] experiments. Nevertheless, the melatonin doses used in the present study are similar to those employed in previous reports studying melatonin effects in poikilotherm species, e.g. are in the same range as melatonin concentration which enhances horizontal cell sensitivity in salamander retina [31]. Significant changes in retina ganglionic cell activity of the trout at 100 μM–5 mM melatonin range have been reported [32], and 10 μg of melatonin daily injected for 30 days entrains the circadian locomotor activity rhythm in the Japanese newt [33]. We suggest that high melatonin doses must be used in exogenous administration in in vivo experiments due to the rapid metabolism of this hormone in the organism. Although melatonin metabolism has not been studied in all vertebrates, it has been estimated that the apparent half-life of melatonin in circulation is 7.5 min; the melatonin injection initially produced pharmacological concentrations that are followed by low serum melatonin levels within 2 h in the hamster [34]. The low luzindole dose (0.1 μg/g bw) needed to block the food intake inhibition by 200 μg melatonin/g bw supports such a rapid metabolism of the indoleamine also in the goldfish.

The 2-iodomelatonin is a selective, high-affinity agonist broadly used for the identification and characterization of melatonin binding sites. The inhibitory effect of 2-iodomelatonin feeding demonstrates the efficiency of the agonist to reproduce the melatonin effect. Similar reductions in food intake induced by the endogenous ligand and its agonist reinforce the anorectic effect of melatonin in goldfish.

One question remains to be solved, whether the anorectic effect observed could be due to a melatonin unspecific action, e.g. a sedative effect. No alteration in the locomotor pattern and exploratory activity has been observed in melatonin-injected fish. Similarly, peripheral administration of a high melatonin dose (30 mg/kg) did not alter locomotor activity in the mouse [35], and daily injections of 12.5 μg did not modify locomotor activity in the hamster [36].

The present results demonstrate the in vivo inhibition of melatonin by its antagonist, luzindole. This supports the specificity of the food intake inhibition by melatonin in goldfish. Luzindole is a competitive melatonin receptor antagonist with high potency and selectivity [37]. In our study, the peripheral administration of luzindole did not modify food intake by itself, but totally blocked the anorectic effect of melatonin. From the present results, at least two facts are outcoming. On one hand, the in vivo efficiency of a melatonin antagonist provides a useful experimental tool to investigate different melatonin functions. On the other hand, the specificity of the in vivo anorectic effect observed after melatonin administration suggests that it is mediated by peripheral melatonin receptors.

It is generally assumed that in the central nervous system, melatonin acts upon the hypothalamus–pituitary axis. In fact, melatonin binding sites have been located in the brain and pituitary of different fish species [38–41]. However, central administration of both melatonin and its agonist did not significantly alter food intake in goldfish, despite the use of a broad range of doses. Thus, this lack of effect of intracerebroventricularly injected melatonin precludes the possible central role on feeding, and supports a peripheral role of this indoleamine in the control of feeding in fish. In fact, melatonin binding sites in peripheral organs, including the gastrointestinal tract of several avian and mammalian species, have been reported [42–45].

The mechanism of action of melatonin on gastrointestinal tract has been investigated by Bubenik and Pang [18,46]. It was hypothesized that via mutual feedback, melatonin and serotonin keeps equilibrium in gastrointestinal levels. In our study, at least two possibilities can be suggested. Melatonin can modify directly or indirectly secretions of other hormones involved in food intake control, as it has been previously reported [25]. As serotonin has an anorectic effect in goldfish [24], it could be speculated that the anorexic effect of melatonin might be mediated by serotonin. On the other hand, melatonin can be acting as a local regulator of gastrointestinal tissue, e.g. reducing gut tone [16]. This last hypothesis would justify the lack of melatonin effect centrally administered and points to melatonin as a peripheral satiety signal.

In summary, from our results, we suggest that melatonin is involved in the peripheral regulation of feeding in goldfish, being the food intake inhibitory effect mediated via luzindole-sensitive melatonin receptors.

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