Effects of melatonin, p-chlorophenylalanine, and \( \alpha \)-methylparatyrosine on plasma gonadotropin level and ovarian activity in the catfish, *Heteropneustes fossilis*: A study correlating changes in hypothalamic monoamines

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Abstract

The effects of (ip, 10 injections over 20 days) of melatonin (75 \( \mu \)g 100 g\(^{-1}\) BW), the serotonin (5-HT)-synthesis blocker, para-chlorophenylalanine (p-CPA, 10 mg 100g\(^{-1}\) BW) and the catecholamine-synthesis blocker, \( \alpha \)-methylparatyrosine (a-MPT, 10 mg 100 g\(^{-1}\) BW) on gonadotropin (GTH) secretion and ovarian activity were studied in *Heteropneustes fossilis* during late preparatory to early prespawning (April–May). The treatments resulted in significant reductions of plasma GTH and estradiol-17\( \beta \) levels, the gonadosomatic index, frequency distribution of vitellogenic and postvitellogenic oocytes, and ovarian and serum \( ^{32p} \)-labelled alkali-labile phosphoprotein (a marker of vitellogenic activity). Most of the oocytes were nonvitellogenic or had undergone atretic changes. The hepatic \( ^{32p} \)-phosphoprotein content increased significantly over the saline control value. The effects were similar and pronounced in the p-CPA and melatonin-treated groups but were moderate in the a-MPT-treated group. Hypothalamic 5-HT content and turnover were significantly inhibited in the p-CPA and melatonin-treated groups but the content and turnover of catecholamines were not. The a-MPT treatment decreased significantly the content and turnover of dopamine (DA), noradrenaline (NA), and adrenaline (A) but did not influence the 5-HT content or turnover. These results suggest that 5-HT, NA and A are stimulatory to GTH secretion and that melatonin may act on the serotonergic system to inhibit the pituitary-gonadal axis.

Introduction

Central monoamines (MA) play an important role in the regulation of gonadotropin (GTH) secretion and modulate basal and/or gonadotropin releasing hormone (GnRH)-induced GTH release in goldfish (Peter *et al.* 1991), African catfish (de Leeuw *et al.* 1987), Atlantic croaker (Khan and Thomas 1994), and *Heteropneustes fossilis* (Senthilkumaran and Joy 1994a, 1995). These studies have demonstrated that serotonin (5-HT) and noradrenaline (NA) are stimulatory, and dopamine (DA) is inhibitory to GTH secretion. The central MA-ergic system is suggested to act as a common pathway for mediation of both extrinsic (photoperiod and temperature) and intrinsic (steroid feedback) factors in the neuroendocrine control of GTH secretion (de Vlaming and Olcese 1981; Khan and Joy 1990; Senthilkumara-

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1 A part of the results was presented at the International Workshop on Pineal gland: Its molecular signals and published as an abstract in Neuroendocrinol. Lett. 14: 399 pp., 1992.
ran and Joy 1994a,b, 1995). The hypothalamic serotonergic system is considered an important component of photosexual mechanism in teleosts (de Vlaming and Olcese 1981; Joy and Khan 1991). Pinealectomy and melatonin treatment are known to influence hypothalamic 5-HT in mammals (Kachi 1987; Glass 1988) and teleosts (de Vlaming and Olcese 1981; Joy and Khan 1991).

Most investigations in vertebrates show that the endocrine role of the pineal organ is mediated through melatonin, the secretion of which is influenced by changes in external photoperiod (Reiter 1982; Ralph 1983). The teleost pineal organ secretes melatonin with a nocturnal surge (Gern et al. 1978; Falcon et al. 1987). The administration of melatonin is reported to have varied effects on gonadal activity depending on season, photoperiod, temperature, and the dose administered (see de Vlaming and Olcese 1981; Joy and Khan 1991). A previous morphological study in the catfish, \textit{H. fossilis} has indicated an inhibitory effect of melatonin on the gonadal activity (Joy and Agha 1991); however, although reports describing effects of pinealectomy and melatonin treatment on gonads of teleosts are numerous, studies elucidating the neuroendocrine mechanisms associated with these morphological effects are scarce.

Previous investigations in teleosts have reported short-term effects of MA-synthesis blockers or inhibitors on pituitary or blood levels of GTH (Peter et al. 1991) but were not extended to monitor their long-term effects on gonadal activity. Such long-term studies will be useful to understand the neuroendocrine mechanisms controlling seasonal reproduction. In \textit{H. fossilis}, hypothalamic 5-HT and catecholamines (CA except DA) levels are high in the gonadal recrudescent phase and low in the quiescent phase (Senthilkumaran and Joy 1993, 1995). However, the significance of these high levels to gonadal activity have not been examined in any teleost. In the present study, therefore, both 5-HT and CA activities were blocked by parchlorophenylalanine (p-CPA) and \( \alpha \)-methylparatyrosine (\( \alpha \)-MPT), their respective synthesis blockers, at the peak period of gonadal activity and changes in GTH and ovarian activity were monitored to understand the specific role of the MA system on the pituitary-gonadal axis. Secondly, effects of administration of melatonin on GTH secretion and ovarian activity in the catfish were also studied and correlated with changes in hypothalamic monoamines, such as 5-HT, DA, NA and adrenaline (A) to elucidate the possible mechanism of action of melatonin on the hypothalamo-hypophyseal-gonadal system.

**Materials and methods**

The reproductive cycle of \textit{H. fossilis}, an airbreathing catfish, can be divided into four phases (Senthilkumaran and Joy 1993). Adult female catfish (35–45g) were collected from local fishermen in Varanasi during late preparatory phase (April 10, 1992). They were treated with benzanthine penicillin (16,000 IU l-1) daily for three days to prevent skin infection and were maintained in aquarium tanks under ambient photoperiod and temperature conditions (13.2\,L: 10.3\,D; 24 \( \pm \) 2\,C). They were acclimated for 14d and fed goat liver daily.

**Experimental design**

After acclimation, the fish were divided into 5 groups of 50 each. Group I served as initial control and the fish were killed at the beginning of the experiment (last week of April). Group II was injected with p-CPA (Sigma) (10 \( \text{mg} \, 100 \, \text{g}^{-1} \, \text{BW} \)), Group III with \( \alpha \)-MPT (Sigma) (10 \( \text{mg} \, 100 \, \text{g}^{-1} \, \text{BW} \)), and Group IV with melatonin (Sigma) (75 \( \mu \text{g} \, 100 \, \text{g}^{-1} \, \text{BW} \)). The doses of p-CPA and \( \alpha \)-MPT used in the study were low compared to those used in rats for depletion of the MA (Koe and Weissman 1966; Ojeda and McCann 1973). The compounds were dissolved in acidic saline (dissolved in half the required volume of saline (0.65\% NaCl) at pH 10 with 5N NaOH, rapid precipitation by acidifying to pH 1.5 with 5N HCl and diluted with remaining amount of saline to give a final pH of 1.8) and were given 10 intraperitoneal injections (0.1 ml per injection) in the evening of every second day. The Group 5 fish were injected with the same volume of the vehicle (acidified saline) in a similar manner. Two fish