

## EFFECT OF MELATONIN ON DOPAMINE SECRETION IN THE HYPOTHALAMUS OF MATURE FEMALE COMMON CARP, *CYPRINUS CARPIO* L.

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**Background.** The work was the continuation of previous study, which indicated that melatonin influences the inhibition of hypothalamic dopamine secretion in vitro. In the presently reported study, the influence of melatonin injection into the third cerebral ventricle on the release of dopamine was tested. The aim of this study was to investigate if and how melatonin affects dopamine secretion in the hypothalamus of mature female carp.

**Materials and Methods.** The concentration of dopamine in hypothalamic tissue was determined radioenzymatically. The experimental groups were fish deprived of melatonin by pinealectomy, fish that received intraventricular injections of melatonin, fish that received intraventricular injections of physiological saline, and intact fish (control treatment). The study was carried out in the summer during spawning and in the winter when fish gonads were regressed.

**Results.** Melatonin injection resulted in an increase in dopamine concentration in the hypothalamus, which indicates that melatonin influenced aminergic nuclei inhibiting secretion of dopamine. Dopamine probably remained unreleased in the hypothalamic tissue, whereas in pinealectomized fish the lack of melatonin influenced continuous dopamine secretion, what was shown as the low dopamine level in the hypothalamus. The results confirmed the previous study effect of melatonin in mature female carp, and point to an inhibitory effect of intraventricularly injected melatonin on the release of dopamine from the hypothalamus. This effect occurred only in the summer experiment, indicating a seasonal impact of melatonin on the dopaminergic system.

**Conclusion.** By inhibiting the release of hypothalamic dopamine, which blocks gonadotropin secretion, melatonin has a potential stimulatory effect on the release of LH and in this way may be involved in control of reproduction in carp.

**Keywords:** fish, carp, *Cyprinus carpio*, dopamine, hormonal control of reproduction, melatonin

### INTRODUCTION

In fish, just as in higher vertebrates, melatonin has a chronobiological function. In addition, this hormone may affect pituitary activity (Fenwick 1970a) and regulate gonadal function (Peter 1968, 1970, Fenwick 1970b, Urasaki 1972, de Vlaming 1974, Popek et al. 1991, 2002, Bayarri et al. 2004).

Studies on the effect of melatonin on reproductive processes in fish, most often, point to its stimulatory or inhibitory effect depending on the species, season of the year, time of the day, and degree of sexual maturity, but fail to show how and where melatonin affects the reproductive system. Ghosh and Nath (2005) showed that melatonin is effective in inhibiting ovarian vitellogenesis, plasma GtH

II levels, and gonadosomatic index (GSI) in a catfish (*Clarias batrachus*). So melatonin may control the reproduction by blocking maturation gonadotropin (LH) release from the pituitary. Opposite effect showed Breton et al. (1993) who observed increase of LH level in blood, after melatonin injection conducted during the night (dark phase) to mature female carp. During the long day the pineal gland and melatonin stimulate the final stages of sexual maturation by synchronizing full oocyte maturity with the optimal spawning period and stimulate vitellogenesis in the post-spawning period in carp (Popek et al. 1991). It was also reported that melatonin modulates the hypothalamic dopaminergic system rather (Popek 1991, Popek et al. 1994) than directly affecting gonads (Popek et

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al. 1996) or the pituitary gland (Popek et al. 1994, 2000), although in other fish species direct melatonin action on the pituitary was observed (Khan and Thomas 1996, Gaildrat and Falcón 2000). It seems that melatonin can affect reproductive functions most likely by a dopaminergic pathway, which is supported by Zisapel and Laudon (1983) and Zisapel et al. (1985) who proved that melatonin is able to inhibit the hypothalamic secretion of dopamine in rats. Dopamine is one of catecholamines produced by hypothalamic aminergic nuclei NRL (nucleus recessus lateralis) and NRP (nucleus recessus posterioris). Dopamine is the most dominant gonadotropin-releasing inhibiting factor (GRIF) (Chang and Peter 1983). The inhibiting effect of dopamine was also proved on LHRH synthesis and secretion (Yu and Peter 1990).

Previous studies on the effect of melatonin on the activity of hypothalamic aminergic nuclei in mature female carp, using the Falck-Hillarp histochemical fluorescence technique, also confirmed the ability of melatonin to inhibit the secretion of catecholamines produced by the hypothalamus (Popek and Epler 1999). Given that dopamine accounts for 70% of hypothalamic catecholamines (le Bras 1979), these results support the earlier hypotheses concerning the inhibitory role of melatonin on the dopaminergic system in the carp.

The results of experiments *in vitro*, where carp females hypothalami were perfused with, or without melatonin addition, also indicated that melatonin inhibited hypothalamic dopamine release (Popek et al. 2005).

The aim of the present study was to investigate the effect of melatonin on dopamine release in the hypothalamus of mature female carp. Experiments were conducted in summer during the spawning period of carp and in winter when fish gonads were regressed, to indicate possible seasonal effects.

## MATERIALS AND METHODS

Eighty 5-year old mature females of common carp, *Cyprinus carpio* L., from an Experimental Fish Farm of the Division of Ichthyobiology and Fisheries, Agricultural University in Kraków were used in the experiment.

After harvesting from breeding ponds, fish were moved to a facility with concrete tanks, each having a volume of 2 m<sup>3</sup>. Stock density was of 14 kg · m<sup>-3</sup>. The intensity of artificial lighting, placed above each tank, measured over the water surface, was at least 2000 lx. The time the light was on corresponded with the current environmental conditions. During spawning in the summer (June), the ratio of light : dark (L : D) was 16 : 8 and the light was turned on at 0400 h. In the winter (December) an 8 : 16 light regime (L : D) was used and the light was turned on at 0800 h. Water flow in the tanks was adjusted to four complete tank changes each day. Water in the tanks was artificially aerated and the level of dissolved oxygen was at least 6 mg O<sub>2</sub> · L<sup>-1</sup>. The water temperature in tanks also corresponded with the current environmental conditions. The water temperature in tanks was adjusted to 18°C (the average water temperature in ponds in June). In the win-

ter, the average water temperature as in ponds as in tanks was 7°C. Carp were acclimated to the artificial conditions for seven days prior to the commencement of the experiments.

Prior to all manipulations, fish harvested from the tanks were anaesthetized with 1% ethylene glycol mono-phenyl ether (Merck).

**Summer period** (water temperature 22°C, L : D = 16 : 8). Forty carp weighing an average of 2.8 kg were divided into 4 groups (each having 10 fish):

Four days before the experiment began, in Group 1, all the fish were pinealectomized (Px), as described by Popek et al. (1994), in groups 2 (ivM) and 3 (ivC), injection needle conduits leading to the third cerebral ventricle were implanted as described by Popek (1991).

After that period, midway through the dark phase (2400 h; midnight), fish from group 2 received melatonin microinjections (1 µg MT · µL<sup>-1</sup> NaCl 0.6% per 1 kg body weight) into the third cerebral ventricle. Melatonin was predissolved in 5 µL of 96% ethanol and then in a solution of physiological saline. Experimental melatonin treatment occurred during the night when melatonin levels would have been high in intact fish, in order to minimize potential circadian variation in melatonin receptor levels (Zisapel et al. 1998, Brooks and Cassone 1992, Masson-Pévet et al. 2000, Schuster et al. 2001).

Fish from group 3 received intraventricular injections of physiological solution supplemented with 5 µL of 96% ethanol.

Fish from group 4 remained intact (C).

Fifteen min after intraventricular injection, all the fish were killed and decapitated as soon as possible (about 30 s). The collected hypothalami were stored at -60°C until the level of dopamine was determined.

**Winter period** (water temperature 5°C, L : D = 8 : 16). Forty carp weighing an average of 2.6 kg were divided into 4 groups (each having 10 fish). Further treatment and injection type and method were the same as in the summer period.

The collected hypothalami were analysed radioenzymatically to determine the level of dopamine using commercial kit (Immunotech, a Coulter Company) (Johnson et al. 1980), commonly used in fish studies (le Bras 1984, Senthilkumaran and Joy 1995).

The results were analyzed using one-way analysis of variance. Student's t-test was used to determine statistical differences between the groups.

The experimental protocol was approved by the Local Ethical Committee for Experiments on Animals, Jagiellonian University in Kraków.

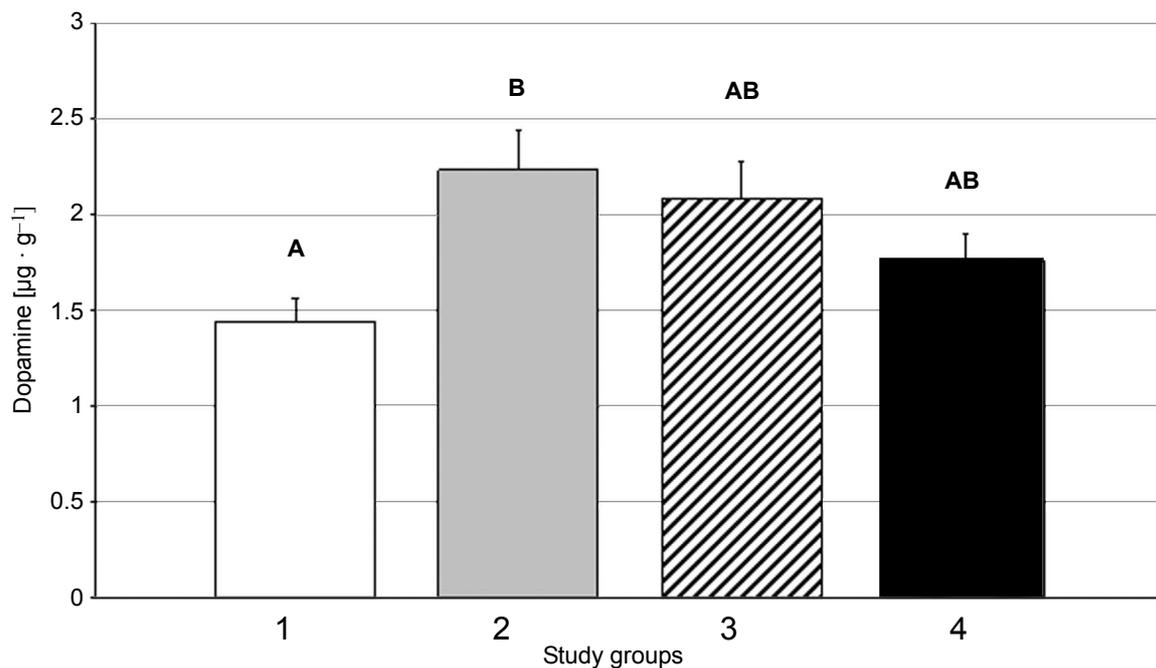
## RESULTS

**Summer period.** In the summer, the level of dopamine (DA) in the hypothalamus of pinealectomized fish (group 1, Px) was the lowest, averaging 1.44 ± 0.12 µg · g<sup>-1</sup> tissue. In the hypothalamus of females that received intraventricular injection of melatonin (group 2, ivMT), the

level of dopamine was the highest, averaging  $2.23 \pm 0.21 \mu\text{g} \cdot \text{g}^{-1}$  tissue. In females microinjected with a physiological saline solution (group 3, ivC), the hypothalamic level of dopamine averaged  $2.09 \pm 0.19 \mu\text{g} \cdot \text{g}^{-1}$  tissue. In the hypothalamus of control females (group 4, C), the average level of dopamine was  $1.75 \pm 0.15 \mu\text{g} \cdot \text{g}^{-1}$  tissue.

Statistical analysis showed that the difference between DA concentrations in groups 1 and 2 was statistically significant ( $P < 0.01$ ).

Fig. 1 shows the pattern of changes in the hypothalamic dopamine level in mature female carp in particular groups during the summer.



**Fig. 1.** Mean ( $\pm s$ ) dopamine concentrations in the hypothalamus of female carp during the summer in pinealectomized fish (group 1; Px), intraventricular melatonin injected fish (group 2; ivMT), intraventricular NaCl injected fish (group 3; ivC) and intact fish (group 4; C); different letters indicate significant difference ( $P < 0.01$ )

**Winter period.** The winter experiment showed that the level of dopamine in the hypothalamus of pinealectomized fish (group 1, Px) was the lowest, averaging  $1.07 \pm 0.12 \mu\text{g} \cdot \text{g}^{-1}$  tissue. In the hypothalamus of females, which received intraventricular melatonin infusions (group 2, ivMT) the level of dopamine was the highest, averaging  $1.44 \pm 0.17 \mu\text{g} \cdot \text{g}^{-1}$  tissue. In females microinjected with a physiological saline solution (group 3, ivC) the average level of dopamine in the hypothalamus was  $1.08 \pm 0.08 \mu\text{g} \cdot \text{g}^{-1}$  tissue. In the hypothalamus of control females (group 4, C), the average dopamine level was  $1.15 \pm 0.09 \mu\text{g} \cdot \text{g}^{-1}$  tissue. Statistical analysis did not show any significant ( $P < 0.05$ ) differences between the groups.

Fig. 2 shows changes in the hypothalamic level of dopamine in mature female carp in particular groups during the winter.

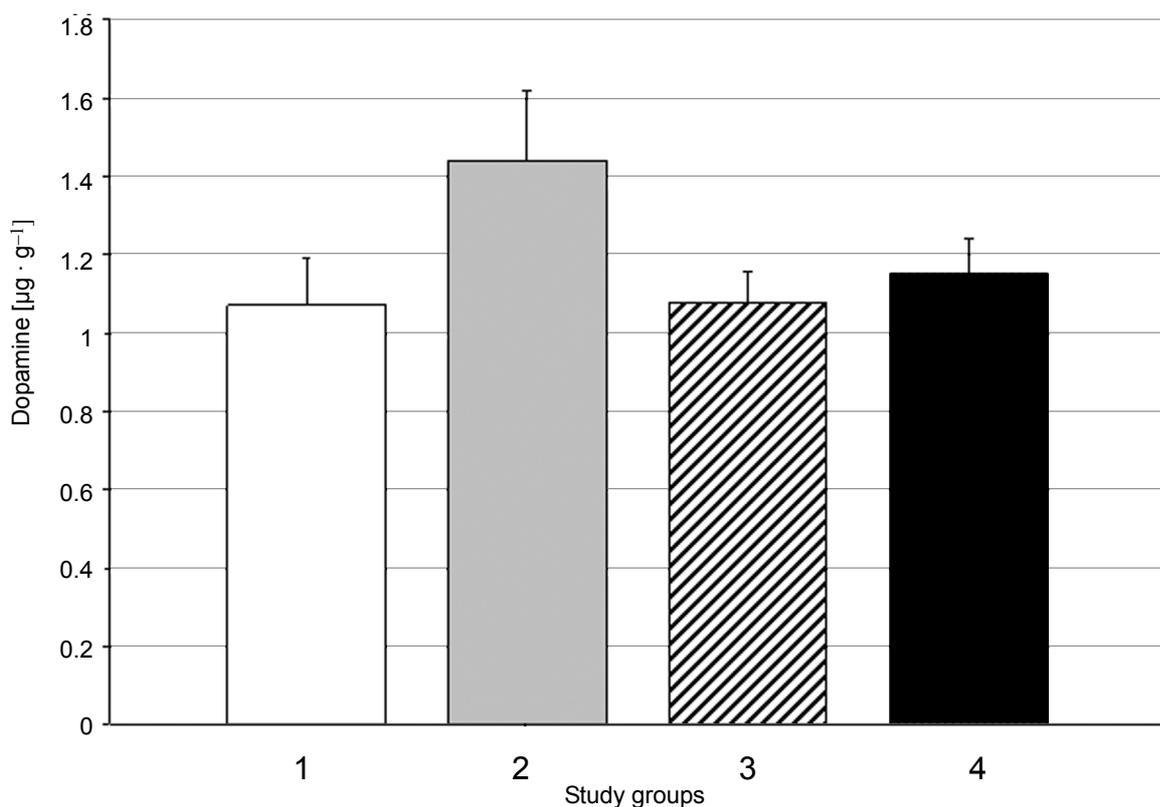
Statistical analysis revealed that the level of dopamine in the hypothalamus of fish in particular groups was sig-

nificantly ( $P < 0.01$ ) lower in the winter than the level of dopamine in corresponding groups during the summer.

## DISCUSSION

In the present study, hypothalami taken from fish, after the injection, were analysed radioenzymatically, which allowed the actual dopamine concentration to be determined. During the summer, the highest level of dopamine ( $2.23 \mu\text{g} \cdot \text{g}^{-1}$  tissue, on average) was observed in the group of females, which received intraventricular melatonin injections (group 2). Dopamine concentration was significantly ( $P < 0.01$ ) higher than that in the group of

pinealectomized fish ( $1.44 \mu\text{g} \cdot \text{g}^{-1}$  tissue, on average; group 1) (Fig. 1). Low concentration of dopamine, found in the hypothalami of fish after pinealectomy treatment indicates the release of this hormone from aminergic nuclei while a lack of melatonin. Endogenous melatonin present in fish of control groups (NaCl-injected—group 3 and intact—group 4) insignificantly inhibited dopamine secretion from aminergic nuclei. Stronger inhibiting effect was observed after intraventricular injection of exogenous melatonin in fish of group 2. The same results were observed in in vitro experiments (Popek et al. 2005), where hypothalami were perfused in the medium containing melatonin or pineal glands were implanted into hypothalamic tissue. But then, dopamine concentration was analyzed in samples of the effluent perfusate, not in the hypothalamic tissue. The highest dopamine levels were secreted by control hypothalami perfused without melatonin addition, and the lowest dopamine levels were found in group with hypothalami perfused in the medium con-



**Fig. 2.** Mean ( $\pm s$ ) dopamine concentrations in the hypothalamus of female carp during the winter in pinealectomized fish (group 1; Px), intraventricularly melatonin injected fish (group 2; ivMT), intraventricularly NaCl injected fish (group 3; ivC) and intact fish (group 4; C)

taining exogenous melatonin, whereas in the present study the lowest concentration of dopamine was determined in the hypothalamic tissue of pinealectomized fish and the highest dopamine level was determined in the hypothalamic tissue after exogenous melatonin injection. In the present work no significant differences were determined between control group (intact or ivC) and the group of fish, which received the intraventricular melatonin injection (group 2). It should be noted that the dose of melatonin used in the experiment was physiological not pharmacological. On the other hand, the pinealectomy treatment totally eliminates blood level of melatonin in fish (Popek et al 1997). This may be the reason why significant differences were observed only in comparison to pinealectomized fish. Maybe it would be possible to observe higher or lower dopamine levels in the hypothalamus in case of use another dose of melatonin. But the aim of the study was to examine if melatonin is able to influence dopamine secretion. These results support our earlier hypothesis that melatonin, by leading to increased dopamine concentration in aminergic nuclei of the hypothalamus, may inhibit the release of hypothalamic dopamine during the summer. These data confirm the results obtained by Hernández-Rauda et al. (2000), who was studying the influence of melatonin on dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) metabolism in the hypothalamus and the pituitary of rainbow trout. The

increase in circulating melatonin was accompanied by a reduction in the amount of DOPAC in the hypothalamus and the pituitary.

During the winter, dopamine concentration in hypothalamic aminergic nuclei of female carp was lower than that in the summer and ranged from 1.07 to 1.44  $\mu\text{g} \cdot \text{g}^{-1}$  tissue (Fig. 2). Analysis of these results shows that the dopamine level in the hypothalamus of fish with regressed gonads in the winter is by 30 percentage points lower than that observed during the summer ( $P < 0.01$ ). It is also evident (as during the summer period) that in the winter melatonin exerted a certain effect on the hypothalamic concentration of dopamine, but differences between particular groups of fish were not significant.

It should also be added that the findings of our previous research (Popek and Epler 1999, Popek et al. 2005) and our present findings are convergent, showing a similar response of the aminergic system to melatonin. What is also evident is the seasonal activity of the hypothalamic-pineal system, which reflects the sexual cycle of the whole carp family. The physiological activity of fish increases in the summer, when gonads become able to produce mature gametes, and reaches a peak during the spawning period. Spawning (second half of spring) is followed by the regeneration of gonads and its end in the autumn coincides with winter regression that lasts for several months. Melatonin, by acting as hormonal hands of the biological clock (pin-

eal gland) synchronizes the fish with the changing environment. This seasonal (summer–winter) influence of melatonin on the hypothalamic dopamine secretion of the carp serves as a good example.

Discussing the role of melatonin in carp reproduction it is worth to mention that melatonin also stimulates hypothalamic noradrenaline secretion in spawning time, what was showed in experiments in vivo and in vitro in maturing carp female (Drag-Kozak et al. 2004). Melatonin affects not only the level of catecholamines in the carp hypothalamus by stimulating or inhibiting their release during the summer, but can also take part in the indirect stimulation of LH release by stimulating and inhibiting the activity of the aminergic system. This hypothesis is possible because in the family Cyprinidae noradrenaline stimulates GnRH release and LH secretion in mature fish female (Chang et al. 1991, Peter et al. 1991) and dopamine acts as a hypothalamic factor that inhibits LH secretion. Dopamine is the most dominant inhibitory mechanism that inhibits basal or GnRH-induced LH release in several species (Chang and Peter 1983, Chang et al. 1984, Senthikumar and Joy 1996). Therefore the stimulation of noradrenaline and inhibition of dopamine activity may lead to increased secretion of LH, which during the spawning period most often leads to oocyte ovulation and spawning of fish. Enhancing spawning in fish by removal of dopaminergic inhibition became a popular treatment (Kumakura et al. 2003, Aizen et al. 2005). The use of GnRH and its superactive analogues in combination with dopamine receptor antagonists (pimozide, domperidone, or metaclopramide) has become the basis of a simple and reliable method of influenced breeding in teleosts (Peter et al. 1991, Yaron 1995). This provides very good results, but can evoke side effects as mortality (Kucharczyk et al. 1999). So the replacement of synthetic drugs by natural melatonin seems to be serious alternative for fisheries. Further research is required to examine the dose and time as well the way of administration dependence of melatonin treatment, on LH secretion, ovulation and spawning efficiency in carp.

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