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Influences of exogenous melatonin on the oocyte growth and oxidative status of ovary during different reproductive phases of an annual cycle in carp *Catla catla*

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ABSTRACT

The present study aimed to evaluate antioxidant role of melatonin in determining seasonality of ovarian growth in adult carp *Catla catla*. Accordingly, an identical regimen of exogenous melatonin administration (100 μg/100 g body weight per day for 15 days) was followed during the preparatory, prespawning, and spawning phases of an annual reproductive cycle. The study did not include postspawning phase, when the ovaries were completely regressed and devoid of any healthy growing follicles. The ovarian response was evaluated by determining relative number of developing oocytes as well as measuring the levels of melatonin, oxidative stress (using malondialdehyde [MDA] as the marker), both enzymatic (superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GPx], and glutathione *S*-transferase [GST]) and nonenzymatic (reduced glutathione [GSH]) antioxidants in the ovarian homogenates. Due to melatonin treatment, oocyte growth was accelerated in the preparatory phase but retarded in the prespawning and spawning phases of annual cycle. Conversely, melatonin administration in each reproductive phase led to a significant reduction of MDA and elevations of SOD, CAT, GPx, GST, GSH, as well as melatonin levels in the ovary. As a result, melatonin titers in the ovary always reported a negative correlation with MDA and a positive correlation with SOD, CAT, GST, GPx, as well as GSH levels. However, melatonin content of ovary and the values of gonosomatic index in melatonin-treated carp displayed a positive correlation in the preparatory phase and a negative correlation in the remaining parts of reproductive cycle. Thus, it seems likely that melatonin by acting as an antioxidant reduces intraovarian oxidative stress throughout the seasons of follicular growth, whereas exogenous melatonin administration exerts progonadal influences during the preparatory phase, but antigonadal effects during the prespawning and spawning phases of reproductive cycle.

1. Introduction

The pineal hormone melatonin (5-methoxy-*N*-acetyltryptamine) acts as a physiological signal of environmental light–dark cycles to synchronize a number of periodic body functions, especially reproduction, in a wide range of vertebrates, including fish [1–3]. Under natural photothermal conditions, circulating melatonin levels in carp [4,5] and in several other fish species [2] exhibit significant seasonal variations in relation to the reproductive status of concerned fish. Separate study on female [4] and male [6] carp reveals that an identical regimen of exogenous melatonin treatment may lead to progonadal or antigonadal or no changes *per se* in gonads depending on the reproductive phase in an annual cycle. Melatonin is mostly known to perform the functions of a hormone by interacting with the

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cascade mechanisms at a number of key steps in the hypothalamo–pituitary–gonadal (HPG) axis to regulate gametogenic and steroidogenic functions of the fish gonads [2,7]. However, the underlying mechanisms of varied actions of exogenous melatonin on the fish gonads remain largely speculative [3,8].

One of the unique features of melatonin is that, due to its lipophilic nature, it can cross plasma membrane of any cell and may act as a direct scavenger of different free radicals [9]. Moreover, this tryptophan derivative is known to play a critical role in oxidative defense by stimulating different antioxidant enzymes [10,11] in different tissues, including those present in the reproductive system [1]. Several mammalian studies found that melatonin and even its metabolites [12] may act as a potent antioxidant [13] in the regulation of ovarian functions [14]. It is evident further that excessive free-radical generation in the oocytes during ovulation results in an elevation of oxidative stress and thereby affects ovarian functions [15]. Follicular growth dependent increase in intraovarian melatonin titers also underlines its physiological significance during the complex process of ovulation [16]. Melatonin may minimize free-radical damage in the ovary by acting as a free-radical scavenger and ultimately to improve the quality of oocytes [15]. More specifically, melatonin acts on various cells of ovarian follicles as an antioxidant to activate major antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which metabolize free radicals to reduce oxidative stress *in vivo* [17]. It seems likely that melatonin detected in the mammalian preovulatory follicles plays an important role in the physiological cascade of oocyte maturation by protecting oocytes against free radicals [14]. Comparable data from any fish study are unknown. However, a seasonal study on adult carp held under natural photothermal conditions provides indications that melatonin may act as an antioxidative agent in reducing oxidative stress to augment ovarian functions during spawning [18]. A more recent study on carp by determining melatonin actions on ovaprim (synthetic GnRH and domperidone)-induced oocyte maturation promotes an idea that melatonin pretreatment alleviates oxidative stress of preovulatory follicles by stimulating different antioxidants and ameliorates ovaprim actions on the process of final oocyte maturation [19]. Thus, it appears necessary to document experimentally that carp treated with the same dose of melatonin in different parts of reproductive cycle exhibits a correlation between the profiles of ovarian melatonin, different antioxidants, and different stages of growing oocytes to ensure that melatonin is indeed a potent candidate involved in the regulation of seasonal growth and development of ovary by reducing oxidative stress of the ovary itself.

In this investigation, an identical schedule of exogenous melatonin administration has been followed during the preparatory, prespawning, and spawning phases (corresponding to the seasons of ovarian follicular growth and development) of an annual reproductive cycle to demonstrate the effects on the profiles of growing oocytes, the levels of melatonin, oxidative stress, as well as different enzymatic and nonenzymatic antioxidants in the ovaries of adult carp (*Catla catla*). No study has been performed during the postspawning phase, when the ovaries undergo complete regression and do not contain any healthy growing follicles.

2. Materials and methods

2.1. Chemicals and reagents

The primary antibody of melatonin raised in sheep and tritiated melatonin (O-methyl-^{3}H melatonin, specific activity 84.0 Ci/mmol) were procured from Stockgrand Ltd., Surrey, UK and GE Healthcare Life Sciences, UK, respectively. All other chemicals of the highest commercially purity were purchased from Sigma–Aldrich Chemical Co., St. Louis, MO, USA.

2.2. Animals: collection and care

A total number of 36 adult female carp (*Catla catla*, Cyprinidae, and Cypriniformes) weighing between 1000 and 1200 g were used covering three distinct reproductive (the preparatory, prespawning, and spawning) phases of an annual cycle. On each occasion, the fish were captured from large water bodies in and around Santiniketan (latitude 23°39′ N, longitude 87°42′ E) in India by local fishermen 1 week before the start of each experiment. The live fish were quickly (within 1 hour) transported to the laboratory and transferred to large open-air tanks measuring ~4 m (L) × 2 m (W) × 1 m (D) for acclimatization for a week under ambient photothermal conditions, which varied with the reproductive seasons. During preparatory phase (January–February), the duration of natural photoperiod (NP) was ~11 hours 30 minutes, while water temperature (WT) varied in between 21.5 °C and 23.5 °C. During prespawning phase (May–June): NP was ~13 hours 15 minutes, and WT varied in between 30.0 °C and 31.5 °C; and during spawning phase (July–August): NP was ~13 hours 30 minutes, and WT varied in between 27.5 °C and 28.5 °C. Each tank had artificial aeration and continuous flow of running water. The fish were held under natural light–dark conditions and fed once daily (at ~10:00 hours) with artificially prepared balanced food (~200 g/tank/day equivalent to 6% body weight) comprising 35% fish meal, 28% mustard oil cake, 28% rice bran, 2% each sunflower and cod liver oils, 5% carboxy methyl cellulose, and multivitamin–multimineral tablets. Laboratory care of fish and adopted study schedules were in agreement with international standards [20].

2.3. Experimental regimens followed

An identical dosage regimen of exogenous melatonin administration was followed during the preparatory, prespawning, and spawning phases of an annual reproductive cycle in carp [21]. In each reproductive phase, 12 randomly selected fish were equally divided into 2 groups, maintained in separate open-air tanks but under identical light–dark conditions (without any artificial lights) as experienced during acclimatization. A uniform ambient physical condition including temperature, dissolved O_2 and