

Regulation of hydroxyindole-O-methyltransferase gene expression in the pineal gland and retina

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Abstract. Hydroxyindole-O-methyltransferase (HIOMT) catalyzes the final step of melatonin biosynthesis and appears to be specifically expressed in the pineal gland and in the retina. This review deals with the regulation of HIOMT by environmental light and with the developmental aspects of HIOMT expression in chicken and rat. Early studies based on HIOMT activity measurements and more recent studies involving cDNA hybridization to HIOMT mRNA are taken into consideration. Together, the data reveal that long term regulation of HIOMT by light would rely on a day/night rhythm of HIOMT gene transcription, coupled to a slow turnover of the protein. Rapid changes in HIOMT mRNA levels and early expression during embryonic development suggest that further studies on this gene may shed light on the molecular mechanisms involved in the differentiation of the melatonergic function and in its regulation by light, both in the pineal gland and in the retina.

Key words: HIOMT, gene expression, pineal gland, retina, melatonin

INTRODUCTION

The 24 hour light/dark cycle acts as a synchronizer for a number of biological rhythms organized on a daily basis (i.e. locomotor activity, body temperature, skin pigmentation) or on a seasonal basis (i.e. reproduction, thermoadaptation). In all vertebrates, the 24 hour light/dark cycle is converted into a synchronous rhythm of melatonin, a hormone synthesized almost exclusively at night, through the metabolic pathway : tryptophan --- 5-hydroxytryptophan --- serotonin --- N-acetylserotonin --- melatonin (Klein et al. 1981). In non-mammals, melatonin is produced in the pineal gland and in the retina, and both organs are directly sensitive to light (Binkley et al. 1979, Gern and Ralph 1979, Besharse and Iuvone 1983, Falcon et al. 1987). As a result of evolution, the pineal gland and the retina of mammals appear more specialized as neuroendocrine and visual organs, respectively. However, melatonin production has been demonstrated in the retina of some mammalian species and some proteins characterized as components of the phototransduction cascade are still expressed in mammalian pinealocytes (Cardinali and Rosner 1971, Pang et al. 1980, Lolley et al. 1992). Therefore, melatonin synthesis may be viewed as an endocrine function of photoreceptors and photoreceptor-derived cells, that is specifically involved in translating photoperiodic information. Experimental evidence indicates that the photoperiodic control of melatonin synthesis is exerted principally on the last two enzymes of the pathway : serotonin-N-acetyltransferase and hydroxyindole-O-methyltransferase (Klein et al. 1981). A better understanding of the melatoninergic function in the pineal gland and retina would be achieved with the identification of the molecular mechanisms involved in the tissue-specific expression of these enzymes and in their regulation by light. The present review will focus on hydroxyindole-O-methyltransferase (HIOMT), the enzyme that catalyzes the final step of melatonin synthesis.

REGULATION OF HIOMT BY ENVIRONMENTAL LIGHT

Pineal gland

The regulation of HIOMT activity by light has been studied primarily in the pineal gland of rat and chicken. In contrast to the large nighttime increase in N-acetyltransferase activity (the penultimate enzyme in the melatonin pathway), daily changes in HIOMT activity are of small amplitude. In the rat pineal, a 10-20% increase in HIOMT activity can be observed at night. However, long-term exposure to light causes significant changes in HIOMT activity, with a 70% decrease observed after 2 weeks of constant light (Axelrod et al. 1965). Detailed studies have shown that this effect of constant light is due to permanent interruption of the noradrenergic input which is normally delivered to the pineal gland during the night and plays a prominent role in maintaining constitutive HIOMT levels (Sugden and Klein 1983).

Regulation of HIOMT activity in the chicken pineal is the opposite of that observed in the rat: the daily change consists of a 20% increase during the day-time and constant light causes a 2-fold increase in HIOMT activity after 5-15 days (Axelrod et al. 1964, Binkley et al. 1973). The mechanism of this regulation is not entirely clarified. However, some studies have indicated that the effect of constant light on HIOMT activity is not interrupted by enucleation or superior cervical sympathectomy and it was suggested that it might involve direct light-sensitivity of the chicken pineal gland (Lauber et al. 1968).

In order to study the regulation of HIOMT at the molecular level, the enzyme has been purified from bovine, chicken and rat pineal glands (Jackson and Lovenberg 1971, Nakane et al. 1983, Sugden et al. 1986, Voisin et al. 1988). In all three species, HIOMT was characterized as a 37-39 kDa protein. Antibodies have been produced against HIOMT purified from bovine and chicken pineals (Kuwano and Takahashi 1978, Nakane et al. 1983, Voisin et al. 1988). More recently, antibodies have been

raised against synthetic peptides derived from the predicted aminoacid sequence of human HIOMT (Donohue et al. 1993). Using anti-HIOMT antibodies, studies performed in rat and chicken demonstrated that the long-term effects of light on HIOMT activity reflect changes in HIOMT protein concentration (Yang and Neff 1976, Guerlotté et al. 1992). This observation strongly suggested that HIOMT gene expression in the pineal could be regulated by environmental light.

The changes in HIOMT protein concentration observed in rat and chicken pineals after constant light exposure could be produced either at transcriptional level or at translational level. Direct measurements of HIOMT mRNA levels with cDNA probes were required to elucidate this point. The cloning of cDNA encoding HIOMT has now been reported for bovine (Ishida et al. 1987), chicken (Voisin et al. 1992) and human (Donohue et al. 1993). Up to now, the regulation of HIOMT mRNA levels by light has been reported only in chicken (Bernard et al. 1993). Indeed, Northern blot analysis allowed us to show that HIOMT mRNA concentration is 3- to 4-fold higher at midday than at midnight in the chicken pineal gland. This rhythm does not appear to be a mere response to light, as dark exposure did not prevent the day-time rise in HIOMT mRNA levels. In contrast, light exposure efficiently increased HIOMT mRNA concentration at midnight (Bernard et al. 1993). The presence of a photorefractory period strongly suggests that a circadian oscillator is involved in controlling HIOMT mRNA levels in the chicken pineal gland. Whether this oscillator is located in the pineal itself or in the hypothalamus remains an open question (Takahashi et al. 1989).

The observed day/night changes in HIOMT mRNA concentration were quite unexpected, in view of the slow changes in HIOMT protein. This situation may be explained by a slow turnover of the HIOMT protein. Indeed, we have observed that 99% inhibition of protein synthesis by cycloheximide in cultured chick pineal cells did not affect HIOMT activity over 24 h (Bernard et al., unpublished).

At the cellular level, immunocytochemistry and *in situ* hybridization agreed on the expression of

HIOMT in modified photoreceptors and parafollicular pinealocytes of the chicken pineal gland (Guerlotté et al. 1988, Grève et al. 1993). Immunocytochemical localization of HIOMT has also been demonstrated in the pineal photoreceptors of several species of fish (Falcon et al. 1994) and in bovine pinealocytes (Kuwano et al. 1983).

Retina

Little is known about the regulation of HIOMT in the retina. A decrease in HIOMT activity has been described in the rat retina, after 2 to 4 days of continuous light (Cardinali et al. 1972). Whether this was due to specific regulation of HIOMT gene expression or to photoreceptor degeneration remains an open question.

Our preliminary studies on the chicken retina would indicate that HIOMT gene regulation in this tissue is exerted in an opposite way to that observed in the pineal gland. Indeed, HIOMT mRNA concentration in the retina increased during the dark phase and decreased during the light phase of a 24 h light/dark cycle (Guerlotté et al., unpublished). Further analysis of this difference should provide an insight into tissue-specific mechanisms of HIOMT gene regulation.

At the cellular level, *in situ* hybridization has been used to localize HIOMT mRNA in the visual cell layer of the chicken retina (Wiechmann and Craft 1993).

DEVELOPMENTAL ASPECTS

Pineal gland

The developmental appearance of HIOMT activity in the chicken pineal gland has been observed between embryonic day 16 (E16) and E18 (Wainwright 1974). Enzyme activity increases from that stage on, to reach adult value (100-fold increase) around day 20 posthatch (Wainwright 1974). Using an anti-HIOMT antibody, we have shown that the developmental increase in HIOMT activity reflects an 80-fold increase in the rate of HIOMT neosynthesis

(Bernard et al. 1991). At the cellular level, HIOMT immunocytochemistry revealed that the developmental increase in HIOMT activity reflects both a progressive recruitment of HIOMT-positive cells and an increase in the intensity of the immunoreactions. Between E18 and day 5 posthatch, a striking increase in the number of HIOMT-positive cells could be observed. From day 5 on, the number of HIOMT-positive cells stabilized and the major phenomenon was an increase in the intensity of immunoreaction (Bernard et al. 1991).

While different reports agree on the appearance of HIOMT protein in the chicken pineal between E16 and E18, we have recently observed that HIOMT mRNA is present as early as E12 (Grechez-Cassiau et al., unpublished). This observation suggests that the signals that trigger tissue-specific expression of the HIOMT gene are active during the second week of embryonic life. Therefore, further studies performed at this developmental stage may provide valuable informations on the early events of melatoninergic differentiation.

Developmental studies in the rat pineal gland have been limited to HIOMT activity measurements, for want of molecular probes directed against the rat enzyme. The appearance of HIOMT activity in the rat pineal gland has been reported at day 5 after birth. Depending on studies, this activity increased progressively until day 30 or showed a sharp rise between day 10 and day 13 (Klein and Lines 1972, Sugden and Klein 1983). The sympathetic innervation of the rat pineal apparently is not essential for the initial appearance of HIOMT activity. However, the rate of increase of HIOMT activity during postnatal development is greatly dependent upon sympathetic stimulation (Sugden and Klein 1983).

Retina

The course of HIOMT activity development in the chicken retina has been reported between E15 and adult age (Wainwright 1979). In the same report, it was mentioned that low levels of HIOMT activity could be detected as early as E11. Based on

these observations, we have recently attempted to detect HIOMT mRNA in embryonic chicken retina, by polymerase chain reaction. Preliminary data revealed the presence of HIOMT transcripts at E18 but not at E14 or earlier (Grechez-Cassiau et al., unpublished).

CONCLUSIONS

Similarities between the pineal gland and the retina were first recognized at the cellular level, with the identification of photoreceptors in the pineal of anamniotes (Studnicka 1905) and with the description of a phylogenetic link between these pineal photoreceptors and the pinealocytes of mammals (Collin 1971). Functional similarities have also been described, aspecially concerning melatonin synthesis (Binkley 1986). All available evidence suggest that melatonin constitutes a specific signal of photoreceptors and photoreceptor-derived cells, through which they convey photoperiodic information. A better understanding of the melatoninergic function in the pineal gland and the retina will be achieved with the elucidation of the tissue-specific and light-dependent regulations of the genes that specify this function. The recent development of molecular probes directed against HIOMT make it possible to tackle these questions for one of the key enzymes of the melatonin pathway. Based on both published and preliminary data, a number of interesting points can be raised concerning the regulation of this gene, that had been missed by previous studies on HIOMT activity, due to the slow turnover of the protein. Firstly, the day/night rhythm of HIOMT mRNA levels in the chicken pineal, with the presence of a circadian component, suggests that further studies on the regulation of this gene may provide informations on the molecular mechanisms of circadian rhythmicity in the melatonin pathway. Secondly, the opposite effects of light on HIOMT gene transcription in the pineal and in the retina emphasize the need for comparative studies, to fully elucidate the regulation of this gene. Such studies should provide informations on the cytosolic and nuclear signals that mediate light-induced

regulation of the HIOMT gene. Finally, the early expression of HIOMT mRNA during embryonic life should provide a way to identify tissue-specific transcription factors that control the differentiation of the melatoninergic phenotype.

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