

INDUCTION OF MELATONIN SYNTHESIS IN *TETRAHYMENA PYRIFORMIS* BY HORMONAL IMPRINTING – A UNICELLULAR "FACTORY" OF THE INDOLEAMINE

L. KÖHIDAI¹, O. VAKKURI², M. KERESZTESI¹, J. LEPPÄLUOTO² and G. CSABA¹✉

¹✉ Department of Genetics, Cell and Immunobiology, Semmelweis University of Medicine, Budapest, Hungary

Fax: +36 1 210 2950; E-mail: csagyor@dgci.sote.hu

²Department of Physiology, University of Oulu, Finland

Received April 8, 2003; Accepted April 30, 2003

Abstract - Melatonin is present in *Tetrahymena* and its synthesis can be enhanced by pretreatment (imprinting) with melatonin. Two days after imprinting melatonin level is elevated in the cells and more elevated in the supernatant. Such a minute quantity, as 10^{-12} M melatonin for 1 hour is able to provoke imprinting, however the effect is more expressed using 10^{-6} M. Maintenance in light conditions further elevated the amount of melatonin in the cells and supernatant alike, related to the melatonin content of cells kept in darkness. The experiments call attention to the light-sensitivity of imprinting-provoked melatonin production in *Tetrahymena* and to the possibility of using this property for important physiological functions in higher grades of phylogeny.

Key words: Melatonin synthesis, photoadaptation, hormonal imprinting, *Tetrahymena*

INTRODUCTION

Melatonin (N-acetyl 5-methoxytryptamine) belongs into the group of indoleamines possessing a conserved phylogenetical significance (27). Its endogenous forms were endogenous form has been described in almost all main groups of organisms including bacteria (32), protozoa e.g. *Trypanosoma cruzi* (31), *Gonylaux polyedra* (17), *Tetrahymena pyriformis* (25); fungi *Saccharomyces cerevisiae* (15) and in edible plants *Lycopersicon esculentum* or *Brassica hirta* (36) even as in invertebrate and vertebrate animals. The main and phylogenetically considerable biological role of this universal bioactive substance is the mediation of photoperiodic signals which determines circadian rhythms in unicellular and multicellular organisms as well (16). Among the above mentioned function, melatonin works as an intracellular antioxidant (1) and scavenger of free radicals which ability is promoted by its lipophilic character which provides an excellent intracellular permeability to this molecule in all levels of phylogeny. There is a list of other activities regulated or modulated by melatonin including calcium

influx and phospholipase C activity (33), microtubule based processes (3), cell division (2,24), chemotaxis or phagocytosis (24).

Tetrahymena is a widely used model cell of investigations in cell-physiology cell physiology and biochemistry (6,7). Membrane receptors (4), second messenger systems (20,26) and cell-physiological and metabolic processes (6) described in *Tetrahymena* show close homologies to vertebrates. Several studies have proved that vertebrate-type hormones and hormone-like substances e.g. insulin (29), ACTH (30), histamine (18), serotonin (19), cytokines, e.g. IL-6 (22) are endogenous substances of this cell. At the first encounter between a mammalian hormone and its receptor in *Tetrahymena*, hormonal imprinting takes place, which dramatically influences the hormone binding capacity of the progeny generations (5,6,10). Recently it was also demonstrated that this pretreatment (imprinting) durably enhances the production of the imprinter hormone by the cells, if they are able to do this at all (11,12,13). As it was mentioned above melatonin is also present in these cells and melatonin treatment can induce essential cell-physiological activities

Dedicated:
to the 80th birthday of Professor Raymond Wegmann

in them (8,24). However, though presence and activity of melatonin was described in a range of models including the ciliated protozoan *Tetrahymena pyriformis*, backgrounds of regulation are still obscure at this level. For this reason in the present study our objective was to characterize, whether (a) pretreatments (imprinting) with melatonin have the ability to modify synthetic activity of melatonin in *Tetrahymena* and (b) whether lighting conditions - the most fundamental environmental factor – are in synergy or in controversy with the effect of melatonin pretreatments.

MATERIALS AND METHODS

Tetrahymena pyriformis GL cells were maintained in axenic cultures containing 1% tryptone and 0.1% yeast extract (Difco, Michigan, USA). According to the certificate analysis, the two basic components of media were free of melatonin. However, the potential of interference of melatonin with the media composing peptides is given, the non-synthetic type of media was used as reference works in our earlier publications (21,23,25) and in the literature (28). The starting density of cultures was 5×10^2 cell/ml.

Pretreatment of cultures

Tetrahymena cultures were pretreated with 10^{-12} and 10^{-6} M melatonin (Sigma, St. Louis, USA) for 1 hr. Following the treatments the samples were washed twice with fresh culture medium and the cells were transferred into fresh culture media. Control samples were treated with the solvent (fresh culture medium) of melatonin. The cultures were obtained for 48 hr, then cellular and supernatant samples were fractionated by high pressure lipid chromatography (HPLC) and assayed radioimmunologically for melatonin.

Schedules of lighting

Melatonin synthesis was monitored in light- and darkness-stressed cultures. For light stress, an intensity of 7500 lux was applied, while flasks containing the darkness-stressed cultures were wrapped in special aluminium foil. Duration of incubation was 48 hr.

Isolation and measurement of melatonin

Melatonin was studied in the supernatants and in the cells. The supernatant samples were assayed for melatonin as described previously (35). Briefly, samples (1 ml) were first extracted with chloroform (4 ml), washed with distilled water (2 ml) and the evaporation residue of the chloroform phase was diluted into RIA buffer (250 μ l phosphate-buffered saline) and assayed for melatonin using a-MT-K1 antiserum and 125 I-melatonin tracer (34). The cell pellets were first sonicated for 3-4 sec in 0.5 ml of RIA buffer and further diluted into 1.2 ml of RIA buffer. After centrifugation of 10 min (4,000 g) the 1 ml samples were extracted with chloroform and assayed for melatonin as described above.

Identity studies of immunoreactive melatonin

Melatonin immunoreactivity was studied by comparing the displacement of different amounts of a culture medium sample with that of synthetic melatonin. Furthermore, medium samples of high melatonin immunoreactivity was run in reverse-phase HPLC using Vydac C₁₈ 218TP54 column and a methanol gradient of 0.5%/min from 12% to 32% in 0.05% trifluoroacetic acid.

Measurement of protein content of samples

Protein was measured with a commercial dye-binding (Coomassie Brilliant Blue G-250) assay method by Bio-Rad (Richmond, CA).

Statistical analysis of data

Data of experiments were analyzed with an ANOVA test. Standard deviations (S.D.) and levels of significance are shown in the figures (z: $p < 0.001$; y: $p < 0.01$; x: $p < 0.05$).

RESULTS AND DISCUSSION

Our melatonin measurements were validated in parallelism and HPLC studies: a) melatonin immunoreactivity of the cell and supernatant samples displaced tracer parallelly with synthetic tracer; b) additionally melatonin immunoreactivity of the cell and supernatant samples eluted in HPLC as synthetic melatonin. The results show that pretreatments (imprinting) with different concentrations (10^{-12} and 10^{-6} M) of melatonin can significantly induce melatonin synthesis of *Tetrahymena* cells (Fig. 1).

This synthesis is embodied in two distinct and additive compartments: endogenous melatonin content of cellular fraction and the detectable melatonin of supernatant which is a result of the release from the cells. Pretreatments both with the low and high concentrations of melatonin could positively modulate melatonin content of cultures, in both cases an increased release of endogenous melatonin was the dominant over the increased melatonin content of cellular fraction (average ratios: 1.55 in light-stressed and 1.83 in dark-stressed cultures).

As light conditions have a crucial role in the case of melatonin-associated biochemical and physiological mechanisms we intended to study the effect of these environmental stresses on the synthesis and release of melatonin following a single pretreatment with the homologue substance. Our present data –in good correlation with our previous results (25)– showed that constant 48 hr lighting can induce melatonin synthesis and especially its release. However, pretreatments could result to a 300-500% enhance of endogenous melatonin, the detected amplitude was also influenced by the lighting: constant light-stress could duplicate the concentration of the released melatonin in the supernatant.

The measurement of melatonin was done 48 hr after treatment, and this means that in the 14th generation. This time and generation change seems to be enough for the emptying the melatonin pools possibly formed during treatment. However, theoretically it can be imagined that the exogenously given melatonin elevated the melatonin content of cells as well, as that of the supernatant. Nevertheless, the facts oppose the theoretical possibility: imprinting (pretreatment) with the smaller concentration (10^{-12}) gives such a minute amount of melatonin, which is not enough for the elevation of this material after 48 hr. However, it is understandable that imprinting with the higher (10^{-6}) concentration produced higher increase, as this concentration had the optimal imprinting effect also in case of other hormones (9).

Fig. 1 Melatonin content (pg/ml) of *Tetrahymena* cultures pretreated with 10^{-12} and 10^{-6} M melatonin for 1 hr. Cell: cellular; Medium: supernatant fraction of cultures. z: $p < 0.001$; y: $p < 0.01$; x: $p < 0.05$

As our data show, we can not consider melatonin synthesized by *Tetrahymena* as an example of "trials" of early phylogeny with the later repertoire of hormones or as an endogenous "side product". However, the reaction-ability of melatonin level in this very low phylogenetic level establishes the possibility of using this system in

higher animals, when it is necessary from physiological point of view. This imprinting effect of pretreatments with this indoleamine verifies our previous data gained on insulin (21), histamine (14) and other hormones. Concentration dependence of induction as well as active and illumination-dependent melatonin release points to that

melatonin is joined into a fine-adjusted signalling mechanism of the unicellular ciliate studied.

Acknowledgment – This work was supported by grant from the Hungarian Research Fund (OTKA, T032533 and T037303).

REFERENCES

- Antolin, I., Obst, B., Burkhardt, S. and Hardeland, R., Antioxidative protection in a high-melatonin organism: the dinoflagellate *Gonyaulax polyedra* is rescued from lethal oxidative stress by strongly elevated, but physiologically possible concentrations of melatonin. *J. Pineal Res.* 1997, **23**: 182-190.
- Banerjee, S. and Margulis, L., Mitotic arrest by melatonin. *Exp. Cell Res.* 1973, **78**: 314-318.
- Banerjee, S., Kerr, V., Winston, M., Kelleher, J.K. and Margulis, L., Melatonin: inhibition of microtubule-based oral morphogenesis in *Stentor coeruleus*. *J. Protozool.* 1972, **19**: 108-113.
- Christensen, S.T., Guerra, C.F., Awan, A., Wheatley, D.N. and Satir, P., Insulin receptor-like proteins in *Tetrahymena thermophila* ciliary membranes. *Curr. Biol.* 2003, **13**: R50-52.
- Csaba, G., Phylogeny and ontogeny of hormone receptors: the selection theory of receptor formation and hormonal imprinting. *Biol. Rev.* 1980, **55**: 47-63.
- Csaba, G., The unicellular *Tetrahymena* as a model cell for receptor research. *Int. Rev. Cytol.* 1985, **95**: 327-377.
- Csaba, G., Presence and development of hormone receptors in unicellular organisms. *Prog. Clin. Biol. Res.* 1990, **342**: 116-126.
- Csaba, G., Presence in and effects of pineal indoleamines at very low level of phylogeny. *Experientia* 1993, **49**: 627-634.
- Csaba, G., Phylogeny and ontogeny of chemical signaling: origin and development of hormone receptors. *Int. Rev. Cytol.* 1994, **155**: 1-48.
- Csaba, G., Hormonal imprinting: its role during the evolution and development of hormones and receptors. *Cell Biol. Intn.* 2000, **24**: 407-414.
- Csaba, G. and Kovács, P., Insulin treatment (hormonal imprinting) increases the insulin production of the unicellular *Tetrahymena* long term. Is there a simultaneous formation of hormone receptor and hormone? *Cell Biol. Intn.* 1995, **19**: 1011-1014.
- Csaba, G. and Kovács, P., Localization of β -endorphin in *Tetrahymena* by confocal microscopy. Induction of the prolonged production of the hormone by hormonal imprinting. *Cell Biol. Intn.* 1999, **23**: 695-702.
- Csaba, G. and Kovács, P., Human chorionic gonadotropin (HCG)-like hormones (FSH, LH, TSH) in *Tetrahymena*. A confocal microscopic analysis. *Acta Protozool.* 2000, **39**: 191-198.
- Darvas, Z. and Csaba, G., Dose-dependent impact of pretreatment (imprinting) with histamine and serotonin on the phagocytic activity of *Tetrahymena*. *Acta Microbiol. Hung.* 1990, **37**: 285-287.
- Hardeland, R., Melatonin and 5-methoxytryptamine in non-metazoans. *Reprod. Nutr. Dev.* 1999, **39**: 399-408.
- Hardeland, R., Balzer, I., Poeggeler, B., Fuhrberg, B., Urias, H., Behrmann, G., Wolf, R., Meyer, T.J. and Reiter, R.J., On the primary functions of melatonin in evolution: mediation of photoperiodic signals in a unicell, photooxidation, and scavenging of free radicals. *J. Pineal Res.* 1995, **18**: 104-111.
- Hardeland, R., Burkhardt, S., Antolin, I., Fuhrberg, B. and Coto-Montes, A., Melatonin and 5-methoxytryptamine in the bioluminescent dinoflagellate *Gonyaulax polyedra*. Restoration of the circadian glow peak after suppression of indoleamine biosynthesis or oxidative stress. *Adv. Exp. Med. Biol.* 1999, **460**: 387-390.
- Hegyési, H., Kovács, P., Falus, A. and Csaba, G., Presence and localization of histidine decarboxylase enzyme (HDC) and histamine in *Tetrahymena pyriformis*. *Cell Biol. Intn.* 1998, **22**: 493-497.
- Janakidevi, K., Dewey, V.C. and Kidder, G.W., Serotonin in protozoa. *Arch. Biochem. Biophys.* 1966, **113**: 758-759.
- Köhidai, L., Barsony, J., Roth, J. and Marx, S.J., Rapid effects of insulin on cyclic GMP location in an intact protozoan. *Experientia* 1992, **48**: 476-481.
- Köhidai, L., Csaba, G. and László, V., Persistence of receptor "memory" induced in *Tetrahymena* by insulin imprinting. *Acta Microbiol. Hung.* 1990, **37**: 269-275.
- Köhidai, L., Kovács, P., Lázár-Molnár, E. and Csaba, G., Presence, uptake and localization of the cytokine, interleukine 6 /IL-6/ in the unicellular *Tetrahymena pyriformis*. *Cell Biol. Intn.* 2002, **24**: 749-755.
- Köhidai, L., Lemberkovits, É. and Csaba, G., Molecule dependent chemotactic responses of *Tetrahymena pyriformis* elicited by volatile oils. *Acta Protozool.* 1995, **34**: 181-185.
- Köhidai, L., Vakkuri, O., Keresztesi, M., Pállinger, É., Leppäluoto, J. and Csaba, G., Impact of melatonin on the cell division, phagocytosis and chemotaxis of *Tetrahymena pyriformis*. *Acta Protozool.* 2002, **41**: 85-89.
- Köhidai, L., Vakkuri, O., Keresztesi, M., Leppäluoto, J. and Csaba, G., Melatonin in the unicellular *Tetrahymena pyriformis*: effects of different lighting conditions. *Cell Biochem. Funct.* 2002, **20**: 269-272.
- Kovács, P. and Csaba, G., Involvement of the phosphoinositol (PI) system in the mechanism of hormonal imprinting. *Biochem. Biophys. Res. Commun.* 1990, **170**: 119-126.
- Kumar, V., Melatonin: a master hormone and a candidate for universal panacea. *Indian J. Exp. Biol.* 1996, **34**: 391-402.
- Kuruvilla, H.G. and Hennessey, T.M., Purification and characterization of a novel chemorepellent receptor from *Tetrahymena thermophila*. *J. Membr. Biol.* 2001, **162**: 51-57.
- Le Roith, D., Shiloach, J., Roth, J. and Lesniak, M.A., Evolutionary origins of vertebrate hormones: substances similar to mammalian insulins are native to unicellular eukaryotes. *Proc. Natl. Acad. Sci. USA* 1980, **77**: 6184-6188.
- Le Roith, D., Shiloach, J., Berelowitz, M., Frohman, L.A., Liotta, A.S., Krieger, D.T. and Roth, J., Are messenger molecules in microbes the ancestors of the vertebrate hormones and tissue factors? *Fed. Proc.* 1983, **42**: 2602-2607.
- Macias, M., Rodriguez-Cabezas, M.N., Reiter, R.J., Osuna, A. and Acuna-Castroviejo, D., Presence and effects of melatonin in *Trypanosoma cruzi*. *J. Pineal Res.* 1999, **27**: 86-94.
- Tilden, A.R., Becker, M.A., Amma, L.L., Arciniega, J. and McGaw, A.K., Melatonin production in an aerobic photosynthetic bacterium: an evolutionarily early association with darkness. *J. Pineal Res.* 1997, **22**: 102-106.
- Tsim, S.T., Wong, J.T. and Wong, Y.H., Regulation of calcium influx and phospholipase C activity by indoleamines in dinoflagellate *Cryptocodinium cohnii*. *J. Pineal Res.* 1998, **24**: 152-161.
- Vakkuri, O., Lamsa, E., Rahkamaa, E., Ruotsalainen, H. and Leppäluoto, J., Iodinated melatonin: preparation and characterization of the molecular structure by mass and ^1H NMR spectroscopy. *Anal. Biochem.* 1984, **142**: 284-289.
- Vakkuri, O., Leppäluoto, J. and Vuolteenaho, O., Development and validation of a melatonin radioimmunoassay using radioiodinated melatonin as tracer. *Acta Endocrinol.* Copenhagen 1984, **106**: 152-157.
- Van Tassel D.L., Roberts N., Lewy A. and O'Neill, S.D., Melatonin in plant organs. *J. Pineal Res.* 2001, **31**: 8-15.