Acta Protozool. (2002) 41: 85 - 89

ACTA
PROTOZOOLOGICA

Short communication

Impact of Melatonin on the Cell Division, Phagocytosis and Chemotaxis of *Tetrahymena pyriformis*

László KŐHIDAI[1], Olli VAKKURI[3], Márk KERESZTESI[1], Éva PÁLLINGER[2], Juhani LEPPÄLUOTO[3], György CSABA[1]

[1]Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest, Hungary, [2]Molecular Immunological Research Group of the Hungarian Academy of Sciences, Budapest, and [3]Department of Physiology, University of Oulu, Finland

Summary. Melatonin is produced, stored and secreted by *Tetrahymena*. In the present experiments the effects of exogeneously given melatonin to *Tetrahymena pyriformis* was studied. Melatonin, between 10^{-6} and 10^{-10} M concentrations, significantly stimulated the *E. coli* phagocytosis of *Tetrahymena*. Melatonin also suppressed the multiplication of *Tetrahymena* cultures. Melatonin had chemotactic effect depending on illumination: it was chemoattractant in light and chemorepellent in darkness at the concentration of 10^{-11} M. Functional and evolutionary conclusions are discussed.

Key words: cell division, chemotaxis, evolution, melatonin, phagocytosis, *Tetrahymena*.

INTRODUCTION

The unicellular *Tetrahymena* express hormone receptors (Csaba 1980, 1984, 2000; Kovács and Csaba 1990a, Christopher and Sundermann 1992,1995) and produces, stores and secretes vertebrate hormone-like molecules (LeRoith *et al.* 1980, 1983). If vertebrate hormones are given to *Tetrahymena* its receptors can bind it and - possessing signal transduction system (Kuno *et al*. 1979, Kovács and Csaba 1990b) - the cell can react to them. This reaction is specific in many cases, namely insulin influences glucose metabolism, thyroxin effects cell division, histamine stimulates phagocytosis etc (Csaba 1994).

In previous experiments (Kőhidai *et al.,* in press) melatonin - a hormone, ubiquitous in the living world (Hardeland 1999) - was found in *Tetrahymena*, the production of which was influenced by light conditions, as in vertebrates. Considering this observation, the effect of exogeneously administered melatonin on the basic physiological indices of *Tetrahymena* was investigated in an attempt to identify a possible functional role of this agent.

Address for correspondence: György Csaba, Department of Genetics, Cell and Immunobiology, Semmelweis University, H-1445 Budapest, POB 370 Hungary; Fax: (36-1) 210-2950; E-mail: csagyor@dgci.sote.hu

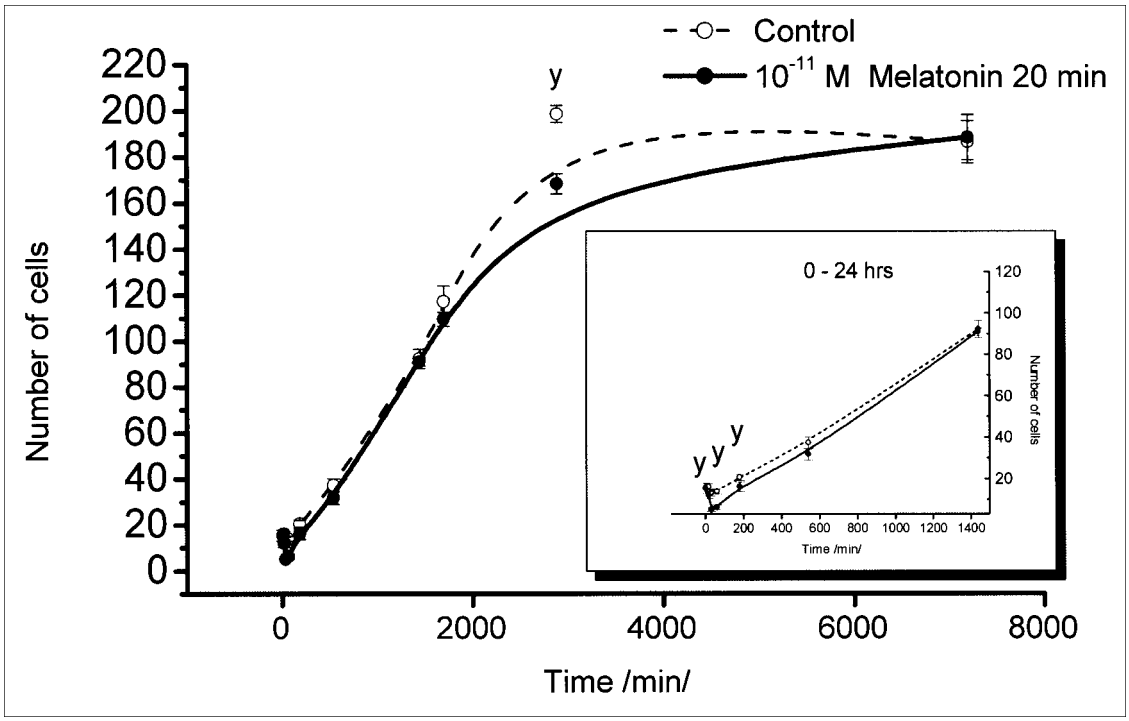

Fig. 1. Effect of exogenous melatonin on the growth of *Tetrahymena* cultures. Treatment with 10^{-11} M melatonin results a suppressed multiplication of cells in early phase (0-200 min. in the insert); and in the late phase (2-3 days) of growing. (y - $p<0.01$)

MATERIALS AND METHODS

Tetrahymena pyriformis GL cells were maintained in axenic cultures containing 1% tryptone and 0.1% yeast extract (Difco, Michigan, USA). The starting density of cultures was $5x10^2$ cell/ml.

Cell division. Low-density cultures of *Tetrahymena* (10^1 cell/ml) were treated with 10^{-11} M melatonin for 20 min. Our pilot experiments had shown that the applied concentration of exogenous melatonin is neutral on phagocytosis, and equal with the concentration of endogenous melatonin of *Tetrahymena* released to the medium. The density of control and melatonin -treated samples was counted in Neubauer haemocytometer after 0, 15, 30, 60, 180, 540, 1440, 1700, 2880 and 7200 min. Each data set of the experiment represented the average of counts of 10 individual parallels.

Phagocytotic activity. Phagocytotic activity of cells treated with 10^{-10}, 10^{-8} and 10^{-6} M melatonin was evaluated with FITC-labelled *E. coli* particles (Phagotest; Orpegen Pharma) (Bassone 1984). Bacteria (20 µl), *Tetrahymena* cells (100 µl) with different concentrations of melatonin were incubated for 10 min. Then the samples were fixed with 4% formaldehyde in PBS. The extracellular fluorescent activity was neutralized with quenching solution. The samples were washed with PBS trice. The number of fluorescent particles taken up by cells was measured with fluorescent-activated cell sorter (FACS-Calibur, Becton-Dickinson). The number of evaluated cells was 10000/sample.

Chemotaxis assay. Two-chamber capillary chemotaxis assay of Leick and Helle (1983) was modified as previously published (Kőhidai and Csaba 1998, Kőhidai 1999). In this assay we used an 8-channel micropipette, where the tips of pipette filled with test substance served as inner chambers, while 96-well microtiter plates, filled with *Tetrahymena* cultures (cell density 10^4 cell/ml), served as outer chambers. In the assay the concentration course of chemotactic responsiveness (10^{-12} -10^{-6} M) was tested in light- and darkness-stressed cultures. The incubation time was 20 min. Based on pilot experiments with several other ligands this is the optimal incubation time when the concentration gradient required for chemotaxis is still present in the chamber. The shorter times provided an insufficient number of cells in the sample, while at times longer than 20 min it was not possible to distinguish chemotactic-responder cells from chemokinetic-responder cells. The samples were fixed in 4% formaldehyde containing PBS. The number of cells was counted in a Neubauer cytometer by light microscopy.

Light and darkness effect. Chemotaxis was observed in cultures kept in light or darkness. For light stress, an intensity of 7500 lux was applied, while cultures kept in darkness were wrapped in special aluminium foil.

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

RESULTS AND DISCUSSION

In an earlier experiment the presence, storage and secretion of melatonin in *Tetrahymena* were demonstrated. The aim of the present experiments was to study

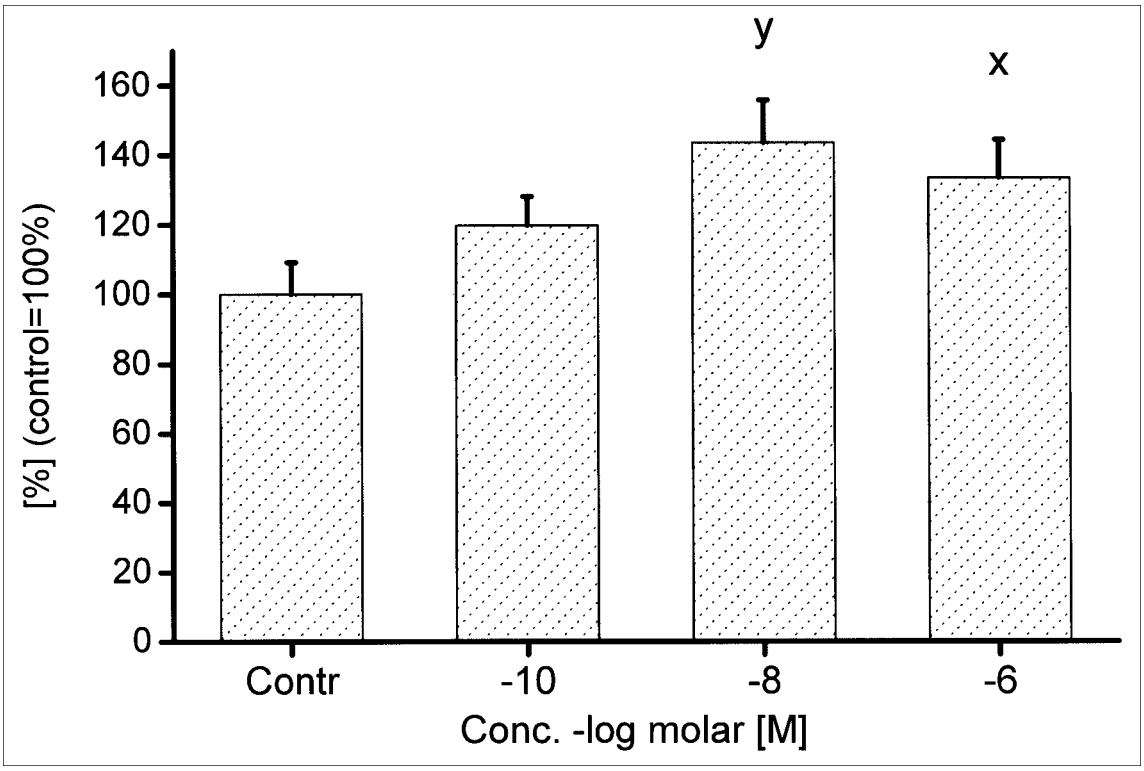

Fig. 2. Flow-cytometric evaluation of the uptake of FITC labelled *E. coli* particles by *Tetrahymena* cells. The phagocytic activity of cells is induced with 10^{-10}, 10^{-8} and 10^{-6} M melatonin. (x - $p<0.05$; y - $p<0.01$)

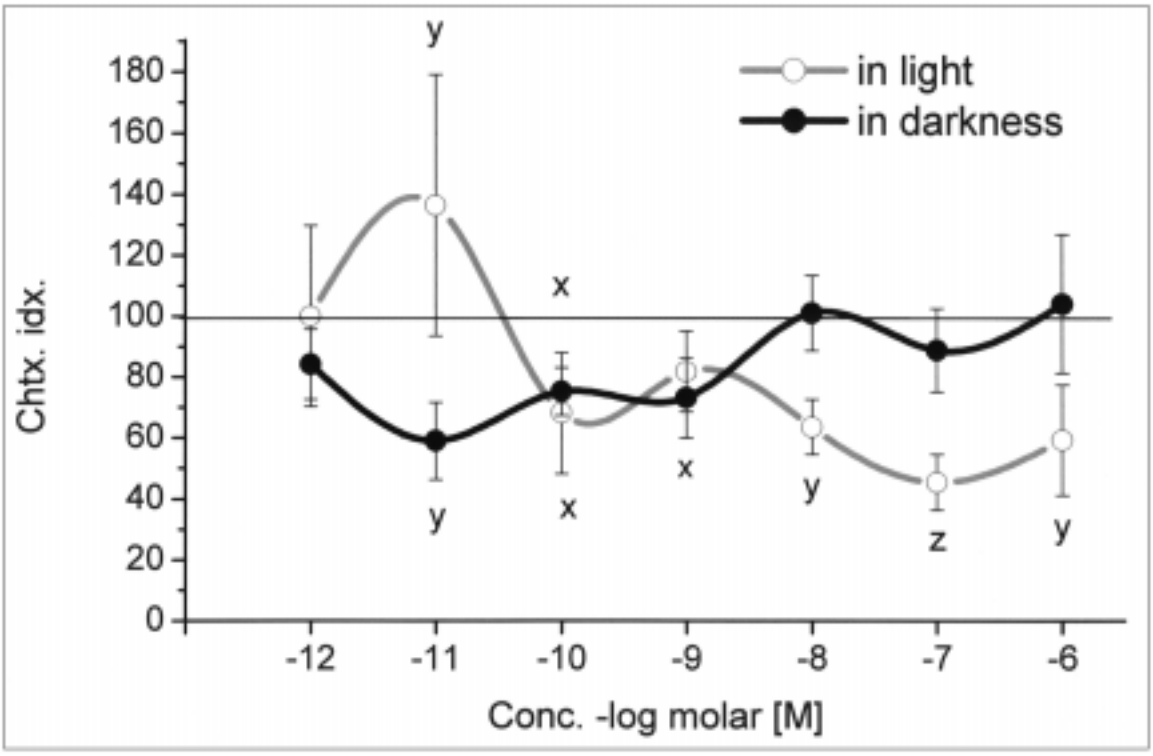

Fig. 3. Chemotactic effects of exogenous melatonin in light- and darkness-stressed *Tetrahymena* cultures. In light 10^{-11} M melatonin acts as a chemoattractant, while the higher concentrations 10^{-10}-10^{-6} M possess chemorepellent effect (open circules). In darkness-stressed cultures melatonin acts as strong chemorepellent even in low concentrations 10^{-11}-10^{-9} M (filled circules). (x - $p<0.05$; y - $p<0.01$; z - $p<0.001$)

the possible effects of melatonin at this low level of phylogeny.

Exogeneously administered melatonin at the concentration of 10^{-11} M suppressed the growth (cell division) of *Tetrahymena* (Fig. 1). The inhibition was significant during the first 3 h ($p<0.01$) also later during the second and third day ($p<0.01$). The phagocytic activity of *Tetrahymena* was increased by 10^{-6} and 10^{-8} M melatonin (133.41% ±10.86 $p<0.05$; 143.35% ±12.3 $p<0.01$ respectively) as shown in Fig. 2. Melatonin had a strong chemoattractant effect at very low concentration (10^{-11} M) in light ($p<0.01$), while at higher concentrations it had the opposite effect (Fig. 3). In darkness melatonin at the concentration of 10^{-11} M showed the most intensive chemorepellent effect which was not seen at 10^{-8} M, or at higher melatonin concentrations.

Melatonin prevents oxidative damage at cellular, tissue, organ and organismic levels (Tan *et al*. 2000). This antioxidant effect has been demonstrated in plants (Tan *et al.* 2000), a dinoflagellate (Antolin *et al.* 1997) and *Trypanosoma* (Macias *et al.* 1999). This allows to suppose that it has the same function in *Tetrahymena*. However, based on our present observations, melatonin in *Tetrahymena* could have also other functions.

Phagocytic activity for a unicellular organism is very important, and in our experiments it was elevated using a low (10^{-10} M) concentration of melatonin, with a peak at 10^{-8} M. The lower concentrations were easily reached in the 96 h cultures of our previous experiments, by secreted melatonin (Kõhidai *et al.,* in press). The cell number was suppressed as a consequence of melatonin (10^{-11} M). On the basis of the present experiments the total amount of exogenous plus endogeneous melatonin in the cultures was unknown, however the data suggest an autocrine regulation by melatonin in both physiological processes. In addition, the chemotactic effect, which is important at this level of phylogeny (Kõhidai and Csaba 1998, Kõhidai 1999), secreted melatonin also could have a regulatory role in a colony of unicells. Considering the presence of melatonin in bacteria (Manchester *et al.* 1995), which during phagocytosis is the main food for *Tetrahymena* under natural conditions, the strong chemoattractant effect in light is also understandable.

In previous experiments serotonin, a precursor molecule of melatonin, was found to have a significant phagocytosis promoting effect in *Tetrahymena* (Csaba and Lantos 1973, Csaba 1993). Melatonin had a similar action in the present experiments. Melatonin metabolites are free radical scavengers, like melatonin itself (Tan *et al.* 2000). These data suggest that this group of indoleamines may have an important role in *Tetrahymena*, which are shared by a broad spectrum of similar molecules.

Acknowledgements. This work was supported by the National Research Fund (OTKA-T-022754 and T- 037303), Hungary.

REFERENCES

Antolin I., Obst B., Burkhardt S., Hardeland R. (1997) Antioxidative protection in a high-melatonin organism: the dinoflagellate *Gonyaulax polyedra* is rescued from lethal oxydative stress by strongly elevated, but physiologically possible concentrations of melatonin. *J. Pineal Res*. **23:** 182-190

Bassone C.-F. (1984) Flow-cytometric studies on phagocyte function in bacterial infections. *Acta Path. Microbiol. Immunol. Scand.* **92:** 167-171

Christopher G. K., Sundermann C. A. (1992) Conventional and confocal microscopic studies of insulin receptor induction in *Tetrahymena pyriformis. Exp. Cell Res.* **201:** 477-484

Christopher G. K., Sundermann C. A. (1995) Isolation and partial characterization of the insulin binding sites of *Tetrahymena pyriformis. Biochem. Biophys. Res. Com*. **212:** 515-523

Csaba G. (1980) Phylogeny and ontogeny of hormone receptors: the selection theory of receptor formation and hormonal imprinting. *Biol. Rev.* **55:** 47-63

Csaba G. (1984) The unicellular *Tetrahymena* as a model cell for receptor research. *Int. Rev. Cytol.* **95:** 327-377

Csaba G. (1993) Presence in and effects of pineal indoleamines at very low level of phylogeny. *Experientia* **49:** 627-634

Csaba G. (1994) Phylogeny and ontogeny of chemical signaling: origin and development of hormone receptors. *Int. Rev. Cytol.* **155:** 1-48

Csaba G. (2000) Hormonal imprinting: its role during the evolution and development of hormone receptors. *Cell Biol.Int.* **24:** 407-414

Csaba G., Lantos T. (1973) Effect of hormones on Protozoa. Studies on the phagocytotic effect of histamine 5-hydroxitryptamine and indeleacetic acid in *Tetrahymena pyriformis. Cytobiologie* **7:** 361-365

Hardeland R. (1999) Melatonin and 5-methoxytryptamine in non metazoans. *Reprod. Nutr. Dev.* **39:** 399-408

Kőhidai L. (1999) Chemotaxis: the proper physiological response to evaluate phylogeny of signal molecules. *Acta Biol. Hung*. **50:** 375-394

Kőhidai L., Csaba G. (1998) Chemotaxis and chemotactic selection induced with cytokines (IL-8, RANTES and TNFa) in the unicellular *Tetrahymena pyriformis. Cytokine* **10:** 481-486

Kőhidai L., Vakkuri O., Keresztesi M., Pállinger É., Leppäluoto J., Csaba G. (2002) Melatonin in the unicellular *Tetrahymena*: effects of different lighting conditions. *Cell Biochem. Funct.* (in press)

Kovács, P., Csaba G.(1990a) Evidence of the receptor nature of the binding sites induced in *Tetrahymena* by insulin treatment. A quantitative cytofluorimetric technique for the study of binding kinetics. *Cell Biochem. Funct*. **8:** 49-56

Kovács, P., Csaba G. (1990b) Involvement of phosphoinositol (PI) system in the mechanism of hormonal imprinting. *Biochem. Biophys. Res. Com.* **170:** 119-126

Kuno T., Yoshida N., Tanaka C. (1979) Immunocytochemical localization of cyclic AMP and cyclic GMP in synchronously dividing *Tetrahymena. Acta Histochem.* **12:** 563

Leick V., Helle J. (1983) A quantitative assay for ciliate chemotaxis. *Anal. Biochem.* **135:** 466-469

LeRoith D., Schiloach J., Roth J., Lesniak M.A. (1980) Evolutionary origins of vertebrate hormones: substances similar to mammalian insulin are native to unicellular eukaryotes. *Proc. Natl. Acad. Sci. USA* **77:** 6184-6186

LeRoith D, Schiloach J., Berelowitz M., Frohmann L. A., Liotta A. S., Krieger B.T., Roth J. (1983) Are messenger molecules in microbes the ancestors of the vertebrate hormones and tissue factors? *Fed. Proc.* **42:** 2602-2607

Macias M., Rodriguez-Cabesas M. N., Reiter R. J., Osuna A., Acuna-Castroviejo D. (1999) Presence and effects of melatonin in *Trypanosoma cruzi. J. Pineal Res.* **27:** 86-94

Manchester L. C., Poeggeler B., Alvares F. L., Ogden G. B., Reiter R. J. (1995) Melatonin immunoreactivity in the photosynthetic prokaryote *Rhodospirillum rubrum*: implications for an ancient antioxydant system. *Cell Molec. Biol. Res.* **41:** 391-395

Tan D. X., Manchester L. C., Reiter R. J., Qui W. B. Karbownik M., Calvo J. R. (2000) Significance of melatonin in antioxidative defense system: reaction and products. *Biol. Signal. Recept.* **9:** 137-159

Received on 20th September, 2001; accepted on 7th December, 2001