

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

László KŐHIDAI<sup>1</sup>, Olli VAKKURI<sup>3</sup>, Márk KERESZTESI<sup>1</sup>, Éva PÁLLINGER<sup>2</sup>, Juhani LEPPÄLUOTO<sup>3</sup>, György CSABA<sup>1</sup>

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

**Summary.** Melatonin is produced, stored and secreted by *Tetrahymena*. In the present experiments the effects of exogenously 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

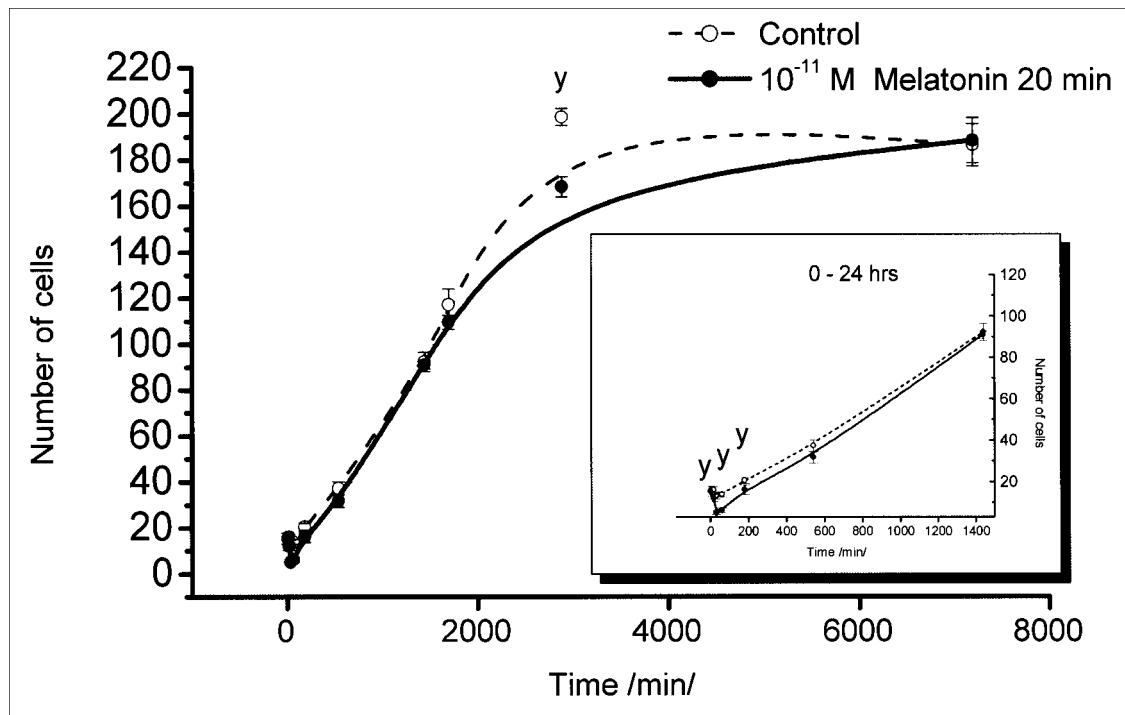
The unicellular *Tetrahymena* express hormone receptors (Csaba 1980, 1984, 2000; Kovács and Csaba 1990a, Christopher and Sundermann 1992,1995) and produces, stores and secretes vertebrate hormone-like molecules (LeRoith *et al.* 1980, 1983). If vertebrate hormones are given to *Tetrahymena* its receptors 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 exogenously administered melatonin on the basic physiological indices of *Tetrahymena* was investigated in an attempt to identify a possible functional role of this agent.

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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  $5 \times 10^2$  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  $\mu$ l), *Tetrahymena* cells (100  $\mu$ l) 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 (Köhidai and Csaba 1998, Köhidai 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

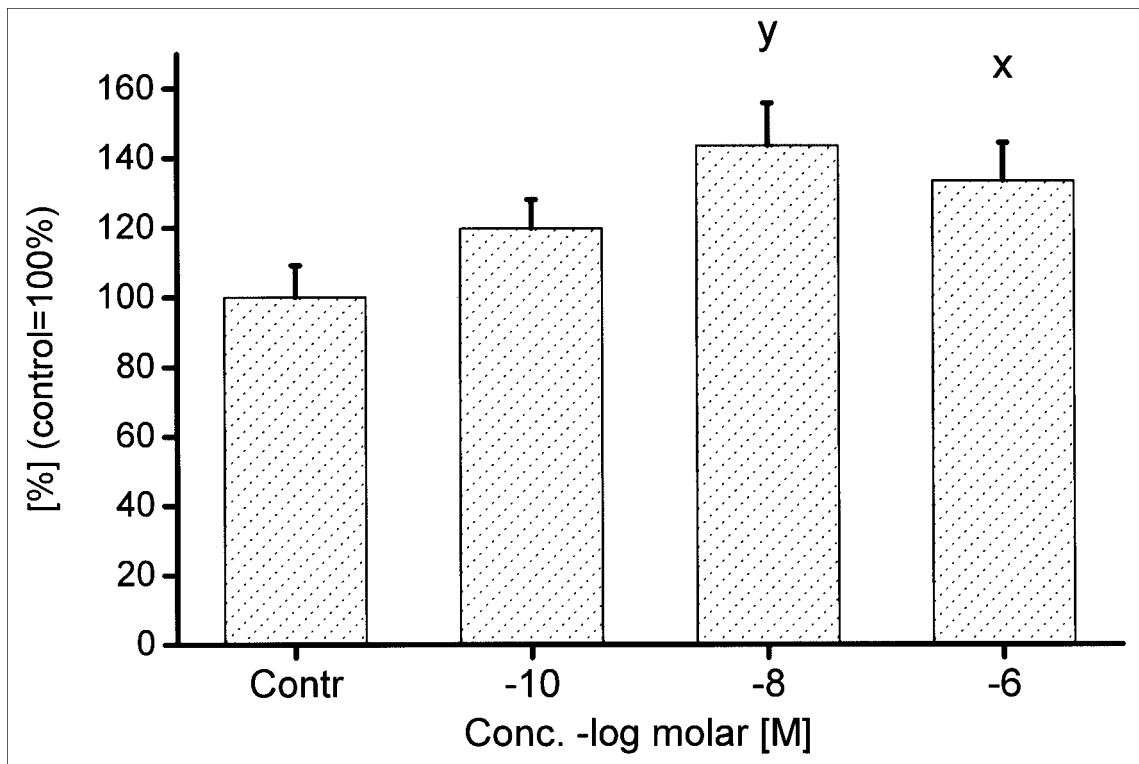


Fig. 2. Flow-cytometric evaluation of the uptake of FITC labelled *E. coli* particles by *Tetrahymena* cells. The phagocytic activity of cells is induced with  $10^{-10}$ ,  $10^{-8}$  and  $10^{-6}$  M melatonin. (x -  $p < 0.05$ ; y -  $p < 0.01$ )

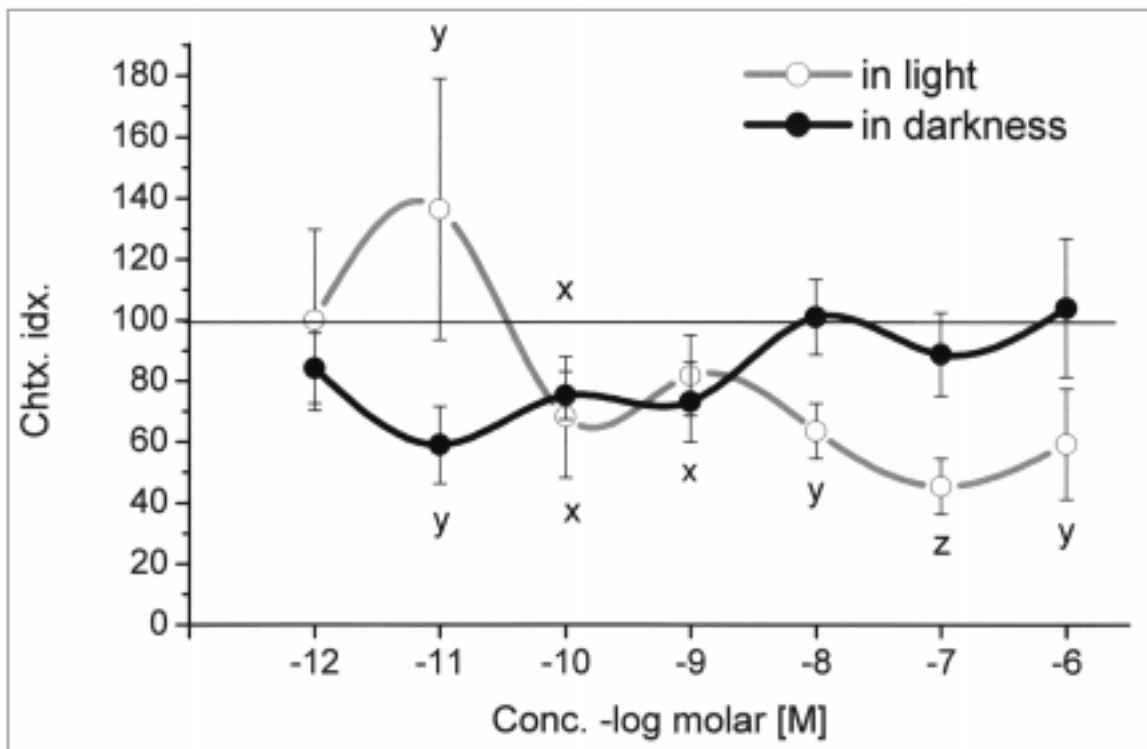


Fig. 3. Chemotactic effects of exogenous melatonin in light- and darkness-stressed *Tetrahymena* cultures. In light  $10^{-11}$  M melatonin acts as a chemoattractant, while the higher concentrations  $10^{-10}$ - $10^{-6}$  M possess chemorepellent effect (open circles). In darkness-stressed cultures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

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

Exogenously 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 ( $133.41\% \pm 10.86$   $p < 0.05$ ;  $143.35\% \pm 12.3$   $p < 0.01$  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 (Kőhidai *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 endogenous 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 (Kőhidai and Csaba 1998, Kőhidai 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.

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