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Melatonin in the unicellular *Tetrahymena pyriformis*: effects of different lighting conditions

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Melatonin content in the cellular fraction and medium of *Tetrahymena pyriformis* GL cultures was measured at different time points of light and dark exposures. Tetrahymena produced, stored and secreted immunoreactive melatonin, which in displacement and HPLC studies, behaved like synthetic melatonin. There was not a continuous secretion of melatonin produced by the cells. In contrast to this, storage of melatonin was observed, which was more expressed in dark conditions. Prolonged light exposure suppressed melatonin production and secretion alike, however it did not block it completely.

KEY WORDS — melatonin; evolution; Tetrahymena; light–dark exposure

INTRODUCTION

Unicellular animals such as the ciliated Tetrahymena produce, store and secrete materials, immunologically and functionally similar to mammalian hormones.[1–3] They also have membrane receptors[4–7] which can bind vertebrate hormones and transmit, through signal transduction systems,[8,9] the information provided by the hormone thereby provoking a response by the cell.[10,11] A number of hormones (including insulin, ACTH, relaxin, endothelin, histamine, serotonin) have been found in Tetrahymena. Many of these hormones influence physiological mechanisms similar to mammalian target cells, e.g. insulin-regulated sugar metabolism, histamine and serotonin-stimulated phagocytosis.

In mammals melatonin is produced by the pineal gland and it has many roles including in particular the function of the biological clock.[12,13] However, it seems to be an ubiquitous hormone. In addition to vertebrates, it can also be found in invertebrates, plants, fungi and multicellular algae and in certain unicellular organisms such as the bioluminescent dinoflagellates.[14–16] Melatonin secretion from the pineal gland is dependent on the rhythmicity of light and darkness and this was also found in several unicellular organisms. However its presence has not been systematically studied in a protozoan such as Tetrahymena.

In the present experiments we studied the presence and secretion of melatonin in Tetrahymena and the effects of different lighting conditions on these parameters were also investigated.

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 5×10^2 cell ml^{-1}.

Schedules of lighting

Melatonin synthesis and other melatonin-related physiological responses were observed 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 aluminium foil. The duration of incubations were of 24, 48

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or 96 h; the two kinds of cultures were labelled as 24L, 48L, 96L and 24D, 48D, 96D, respectively.

Isolation and measurement of melatonin

The supernatant samples were assayed for melatonin as described previously.[17] Samples (1 ml) were first extracted with chloroform (4 ml), washed with distilled water (2 ml) and the residue after evaporation of the chloroform phase was diluted into radioimmuno assay (RIA) buffer (250 µl phosphate-buffered saline) and assayed for melatonin using-MT-K1 antiserum and ^{125}I-melatonin tracer.[18] The cell pellets were first sonicated for 3–4 s in 0.5 ml of RIA buffer and further diluted into 1.2 ml of RIA buffer. After centrifugation for 10 min ($4000 \times g$) the1-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 were run in reverse-phase HPLC using a Vydac C_{18} 218TP54 column and a methanol gradient of 12 to 32% in 0.05% trifluoroacetic acid with a rate of increase of 0.5% min^{-1}.

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 analysed with an ANOVA test. Standard deviations (SD) and levels of significance are shown in the figures.

RESULTS

Melatonin was present in the cellular fractions of Tetrahymena cultures (Figure 1), as well as in the cell medium. Its presence was confirmed in parallel HPLC studies. Different amounts of medium extracts displaced tracer from antibody in parallel with a melatonin standard (Figure 1). Additionally the immunoreactive material of these extracts eluted in reverse-phase HPLC at the position of synthetic melatonin (Figure 2).

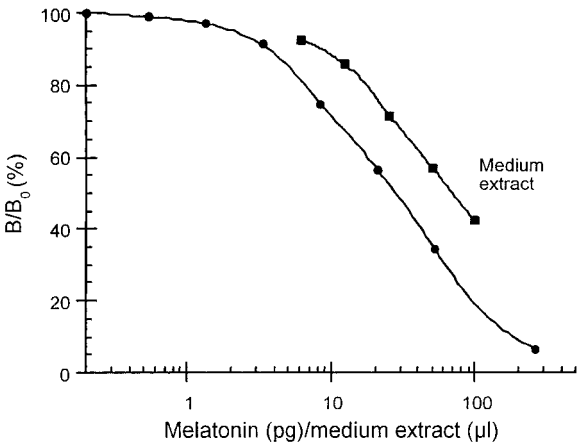

Figure 1. Displacement curves of different amounts of a medium extract of Tetrahymena culture and melatonin standard. B/B_0, binding/maximal binding

A continuous increase of melatonin both in cellular and supernatant fractions as a function of time (Figure 3) was observed which ran in parallel with the protein content of the cellular fraction, i.e. with the number of cells (Figure 4). After the first 24 h there was no significant difference between the cultures (cells and supernatants) kept in darkness or light; however, after incubation for 48 h significantly more melatonin was secreted in the cultures kept in the light ($p < 0.05$). After incubation for 96 h in darkness a three-fold larger amount of melatonin was present in the cells ($p < 0.05$) and a two-fold larger amount in the medium ($p < 0.05$), relative to the 48-h values. After 96 h incubation in the light there was no significant elevation of melatonin content in the cellular

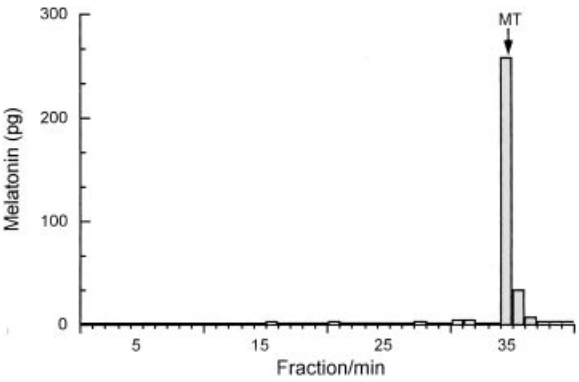

Figure 2. Reverse-phase HPLC elution of melatonin immunoreactivity of a Tetrahymena culture medium sample. Column: Vydac C_{18} 218TP54 (250×4.6 mm per 5 µm); methanol gradient: 12–32% over 40 min in 0.05% trifluoroacetic acid. MT, elution position of synthetic melatonin

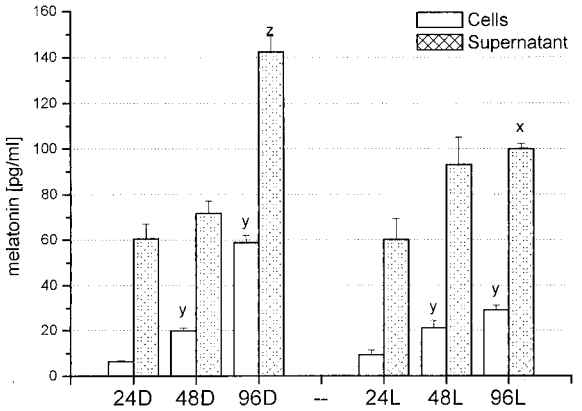

Figure 3. Endogenous melatonin content of cellular and supernatant fractions of Tetrahymena cultures maintained for 24, 48 and 96 h in darkness (D) or in constant light (L). ($x = p < 0.05$; $y = p < 0.01$, $z = p < 0.001$)

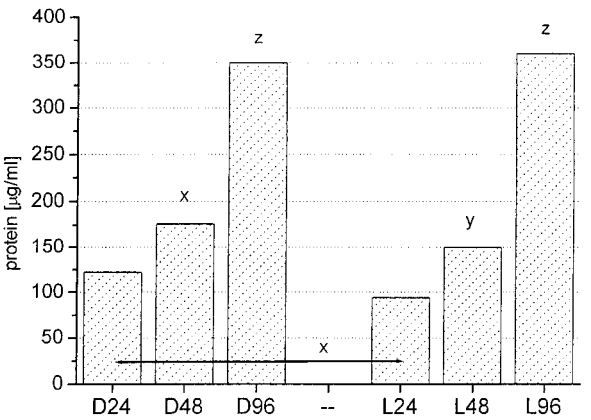

Figure 4. Protein content as an index of cell density of cellular fractions of 24-, 48- and 96-h cultured Tetrahymena maintained in darkness (D) or constant light (L). ($x = p < 0.05$; $y = p < 0.01$; $z = p < 0.001$)

fraction or in the medium, relative to the 48-h incubations.

The protein content of the cellular fractions increased steadily as a function of time (Figure 4). However, a more pronounced increase was observed between 48 and 96 h. After 48 h incubations in the light, less protein was present than after 48-h dark incubations (the difference was significant in the case of 24 h). This difference disappeared by the 96-h time point.

DISCUSSION

Previous studies showed melatonin to be ubiquitously distributed in animals and plants.[14] On the basis of our present experiments, melatonin is also present in Tetrahymena, a ciliated unicellular organism. Melatonin, produced by Tetrahymena is stored and secreted into the medium of the cells. The greater the number of cells present in a culture, the greater was the melatonin secretion; however this correlation depended on the light conditions.

In darkness, the cell number increased by about 40% between 24 and 48 h, while the melatonin content of cells tripled. Between 48 and 96 h the cell number doubled and their melatonin content tripled again. On the other hand, the melatonin content of the medium was almost the same at 24 and 48 h but doubled by 96 h ($p < 0.05$). This means that melatonin is stored in the cells and there is not a continuous secretion of the melatonin produced. This storage is not so obvious under light conditions. In addition, during light exposure between 48 and 96 h a block of melatonin production must occur, or a decomposition process begins since neither the melatonin content of the cells nor secretion increased significantly, while the number of cells (protein content of the supernatant) reached the value measured in darkness. The results indicate that melatonin content (production and storage) and secretion are influenced by light exposure. A similar light dependent decrease in melatonin production was observed earlier in *Trypanosoma cruzi*.[19]

The light-induced inhibition of melatonin synthesis is well known in all animal species studied and this is an important mechanism that determines its rhythmicity. The light intensity used in this study (7500 lux) is very high compared with previous animal and human studies. Despite this, melatonin synthesis was not totally blocked in Tetrahymena cells since the amount of melatonin increased as a function of incubation time both in cells and the medium. Therefore, Tetrahymena seem to be relatively insensitive to light and light-induced inhibition of melatonin synthesis. This interesting finding requires further study.

ACKNOWLEDGEMENTS

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