Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 57; Numer/Number 1/2006
ISSN 0423–104X

Wpływ melatoniny, N-acetyloserotoniny i 6-hydroksymelatoniny na ultrastrukturę pinealocytów chomika syryjskiego (*Mesocricetus auratus*)

Anna Zielińska[1], Jacek Świętosławski[1], Russel J. Reiter[2], Michał Karasek[1]

[1]Zakład Neuroendokrynologii, Katedra Endokrynologii, Uniwersytet Medyczny, Łódź
[2]Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Streszczenie

Wstęp: Celem niniejszego badania była ocena wpływu melatoniny, a także jej prekursora (N-acetyloserotoniny) i metabolitu (6-hydroksymelatoniny) na ultrastrukturę pinealocytów chomika syryjskiego.

Materiał i metody: Badano szyszynki 2-miesięcznych samców chomików syryjskich. Zwierzęta podzielono na następujące grupy, liczące po 4 zwierzęta każda: grupa 1. — otrzymująca melatoninę; grupa 2. — otrzymująca N-acetyloserotoninę; grupa 3. — otrzymująca 6-hydroksymelatoninę (wszystkie substancje podawano podskórnie przez 7 tygodni, w dawce 25 μg/zwierzę, między godz. 16. a 17.). Zwierzęta z grupy 4. otrzymujące jedynie rozpuszczalnik stanowiły grupę kontrolną. Zwierzęta zabijano przez dekapitację między godziną 9. a 10. W celu uzyskania ilościowych danych dotyczących ultrastruktury pinealocytów stosowano rutynowe techniki mikroskopowo-elektronowe.

Wyniki: Podawanie melatoniny nie wpłynęło na wielkość pinealocytów, podczas gdy wstrzyknięcia N-acetyloserotoniny i 6-hydroksymelatoniny spowodowały znaczne zmniejszenie rozmiarów tych komórek w porównaniu z grupą kontrolną i zwierzętami otrzymującymi melatoninę. Stwierdzono istotne zmiany we względnych objętościach mitochondriów, aparatu Golgiego i lizosomów między badanymi grupami, natomiast objętości ziarnistej siateczki śródplazmatycznej i kropli lipidów nie wykazywały różnic między badanymi grupami. Pęcherzyki ziarniste były liczniejsze w pinealocytach chomików otrzymujących melatoninę lub 6-hydroksymelatoninę niż u zwierząt kontrolnych lub otrzymujących N-acetyloserotoninę.

Wnioski: Obserwowane zmiany w ultrastrukturze pinealocytów chomika syryjskiego świadczą, że podawanie zarówno melatoniny, jak i jej prekursora lub metabolitu wpływa na morfologię tych komórek i prawdopodobnie także na ich aktywność wydzielniczą.

(Endokrynol Pol 2006; 1 (57): 2–6)

Słowa kluczowe: *szyszynka, pinealocyt, chomik syryjski, melatonina, N-acetyloserotonina, 6-hydroxymelatonina, ultrastruktura, analiza ilościowa*

Prof. dr hab. med. Michał Karasek
Zakład Neuroendokrynologii, Katedra Endokrynologii,
Uniwersytet Medyczny w Łodzi
ul. Czechosłowacka 8/10, 92–216 Łódź
tel./faks: (042) 675 76 13
e-mail: karasek@csk.umed.lodz.pl

Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 57; Numer/Number 1/2006
ISSN 0423–104X

The effects of melatonin, N-acetylserotonin, and 6-hydroxymelatonin on the ultrastructure of the pinealocytes of the Syrian hamster (*Mesocricetus auratus*)

Anna Zielińska[1], Jacek Świętosławski[1], Russel J. Reiter[2], Michał Karasek[1]

[1]*Department of Neuroendocrinology, Chair of Endocrinology, Medical University of Łódź, Łódź*
[2]*Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA*

Abstract

Objectives: The aim of this study was to examine the effects of melatonin as well as of its precursor (N-acetylserotonin) and metabolite (6-hydroxymelatonin) on the ultrastructure of the pinealocytes of the Syrian hamster.

Material and methods: The pineal glands of 2-month-old male Syrian hamsters were examined. The animals were divided into the following groups of four animals each: group 1 — melatonin treatment; group 2 — N-acetylserotonin treatment; group 3 — 6-hydroxymelatonin treatment (all substances given subcutaneously at doses of 25 μg per animal between 16.00 and 17.00 h daily for seven weeks). Group 4 was given solvent treatment only and served as controls. The animals were killed by decapitation between 09:00 and 10.00 h. Routine electron microscopical techniques were used to obtain quantitative data on pinealocyte ultrastructure.

Results: Melatonin administration did not influence the size of the hamster pinealocytes, whereas administration of N-acetylserotonin and 6-hydroxymelatonin caused a significant reduction in cell size in comparison to the melatonin-treated and control groups. There were changes in the relative volumes of the mitochondria, Golgi apparatus and lysosomes in the pinealocytes of the studied groups, while the volumes of granular endoplasmic reticulum and lipid droplets were unchanged. The dense-core vesicles were more numerous in the pinealocytes of the melatonin and 6-hydroxymelatonin-treated groups in comparison to those of animals treated with N-acetylserotonin or the controls.

Conclusions: The changes observed in the ultrastructure of hamster pinealocytes indicate that administration of melatonin as well as of its precursor or metabolite influences the morphology of these cells and also, perhaps, their secretory activity.

(Endokrynol Pol 2006; 1 (57): 2–6)

Key words: *pineal gland, pinealocyte, Syrian hamster, melatonin, N-acetylserotonin, 6-hydroxymelatonin, ultrastructure, quantitative analysis*

Michał Karasek, M.D., Ph.D.
Department of Neuroendocrinology,
Chair of Endocrinology, Medical University of Łódź
ul. Czechosłowacka 8/10, 92–216 Łódź
tel./fax: (042) 675 76 13
e-mail: karasek@csk.umed.lodz.pl

Introduction

The number of papers devoted to pineal morphology, and especially its ultrastructure, is very high. The ultrastructure of the pinealocyte has been examined in various species in a variety of natural and experimental conditions [see e.g. 1 – 7]. However, studies related to the quantitative ultrastructural features of mammalian pinealocytes are relatively rare. Melatonin is a pineal secretory product with a multiple action. The action of exogenous melatonin on pinealocyte morphology has seldom been investigated. Therefore, the aim of this study was to examine the effect of melatonin itself as well as of its precursor (N-acetylserotonin) and metabolite (6-hydroxymelatonin) on the ultrastructure of the pinealocyte of the Syrian hamster.

Material and methods

The pineal glands of 2-month-old male Syrian hamsters (*Mesocricetus auratus)* were examined. The animals were divided into the following groups of four animals each: group 1 — melatonin treatment; group 2 — N-acetylserotonin treatment; group 3 — 6-hydroxymelatonin treatment (all substances given subcutaneously in of doses 25 μg per animal between 16.00 and 17.00 h daily for seven weeks). Group 4 received solvent treatment only and served as controls. The animals were housed in a room with controlled illumination (LD 12:12; light on at 073:00 h) and temperature (22 ± 2°C) and received standard laboratory food and tap water *ad libitum*. The animals were killed by decapitation between 09.00 and 10.00 h.

The pineal gland of each animal was immersion-fixed in 3.5% glutaraldehyde in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide, and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate and examined using a JEM 100B electron microscope.

For quantitative estimation 5 to 7 micrographs at a magnification of × 3,000 and 30 to 35 micrographs at a magnification of × 10,000 were taken from each gland using a slightly modified systematic random sampling method [8]. Every upper right corner of the grid aperture in which pinealocytes were present was photographed. Altogether 623 prints were used for a quantitative study. A digital analyser connected online to an IBM-PC computer (Logitex, Poland) was used to obtain the morphometric data. For estimation of the cross-sectional areas of the pinealocyte and its nucleus the prints were enlarged photographically to × 7500, whereas for estimation of the relative volume of cell organelles the prints were enlarged photographically to × 25,000. The relative volumes of the following cytoplasmic organelles were analysed: the mitochondria, lysosomes, Golgi apparatus, granular endoplasmic reticulum and lipid droplets. In addition, the numerical density of dense-core vesicles (expressed as a number per 50 μm^2 of cell body cytoplasm) was estimated.

Statistical analysis of the data was performed by ANOVA followed by the LSD test.

Results

There were no significant differences in the size of the pinealocytes, their nuclei or the cytoplasm between the melatonin-treated animals and the control animals (Fig. 1). However, the sizes of the pinealocytes and their nuclei were significantly less in animals given either N-acetylserotonin or 6-hydroxymelatonin in comparison to melatonin-treated and control glands (Fig. 1).

The relative volumes of the Golgi apparatus were significantly higher in the pinealocytes of animals treated with melatonin, N-acetylserotonin, and 6-hydroxymelatonin as compared to the control animals (Fig. 2), whereas the relative volumes of the mitochondria were significantly higher in the pinealocytes of animals given N-acetylserotonin and 6-hydroxymelatonin in comparison to those in melatonin-treated and control pineals (Fig. 2). The relative volumes of lysosomes were significantly higher in the pinealocytes of animals treated with melatonin and 6-hydroxymelatonin in comparison to those of the control animals (Fig. 2).

The dense-core vesicles were more numerous in the pinealocytes of melatonin and 6-hydroxymelatonin-treated groups in comparison with animals treated with N-acetylserotonin or the controls (Fig. 2).

There were no significant differences between the groups in terms of the relative volume of granular endoplasmic reticulum and lipid droplets (Fig. 2).

Discussion

There are some data indicating that melatonin exerts a modulatory effect on other pineal indoles. A single injection of melatonin at evening or at night reduced the content of serotonin and 5-hydroxyindoleacetic acid and increased the content of N-acetylserotonin when rat pinealocytes were cultured [9]. Moreover, in the rat pineal gland, melatonin administration for two weeks resulted in a dose-dependent increase in the activity of

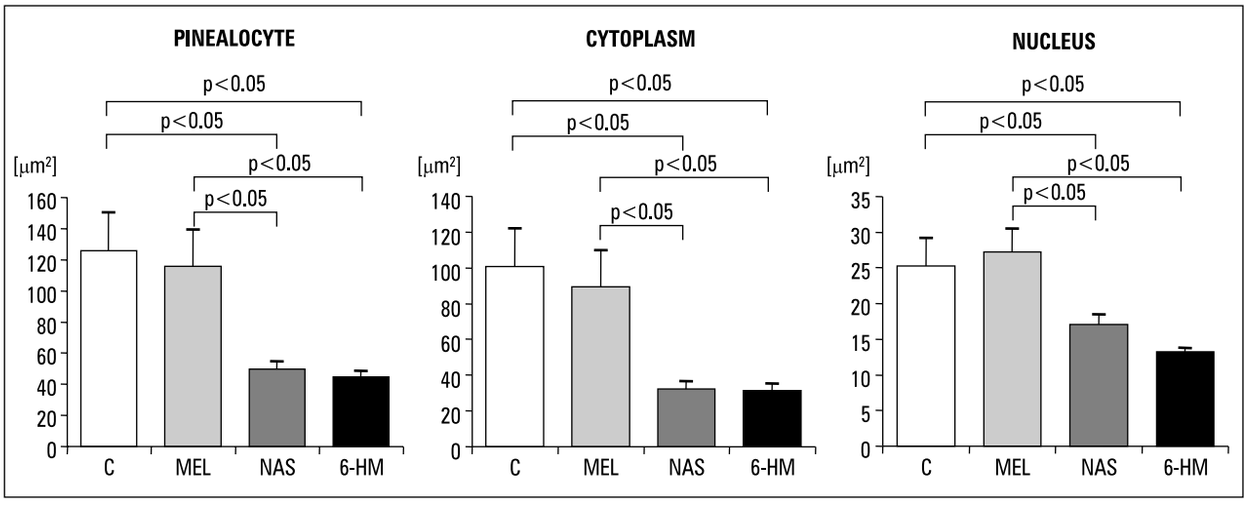

Figure 1. *Cross-sectional areas of pinealocyte, pinealocyte cytoplasm, and pinealocyte nucleus in the Syrian hamsters treated with melatonin (MEL), N-acetylserotonin (NAS), and 6-hydroxymelatonin (6-HM); C — controls*

Rycina 1. *Powierzchnie przekroju pinealocyta, jego jądra i cytoplazmy u chomika syryjskiego po podaniu melatoniny (MEL), N-acetyloserotoniny (NAS) i 6-hydroksymelatoniny (6-HM); C – kontrola*

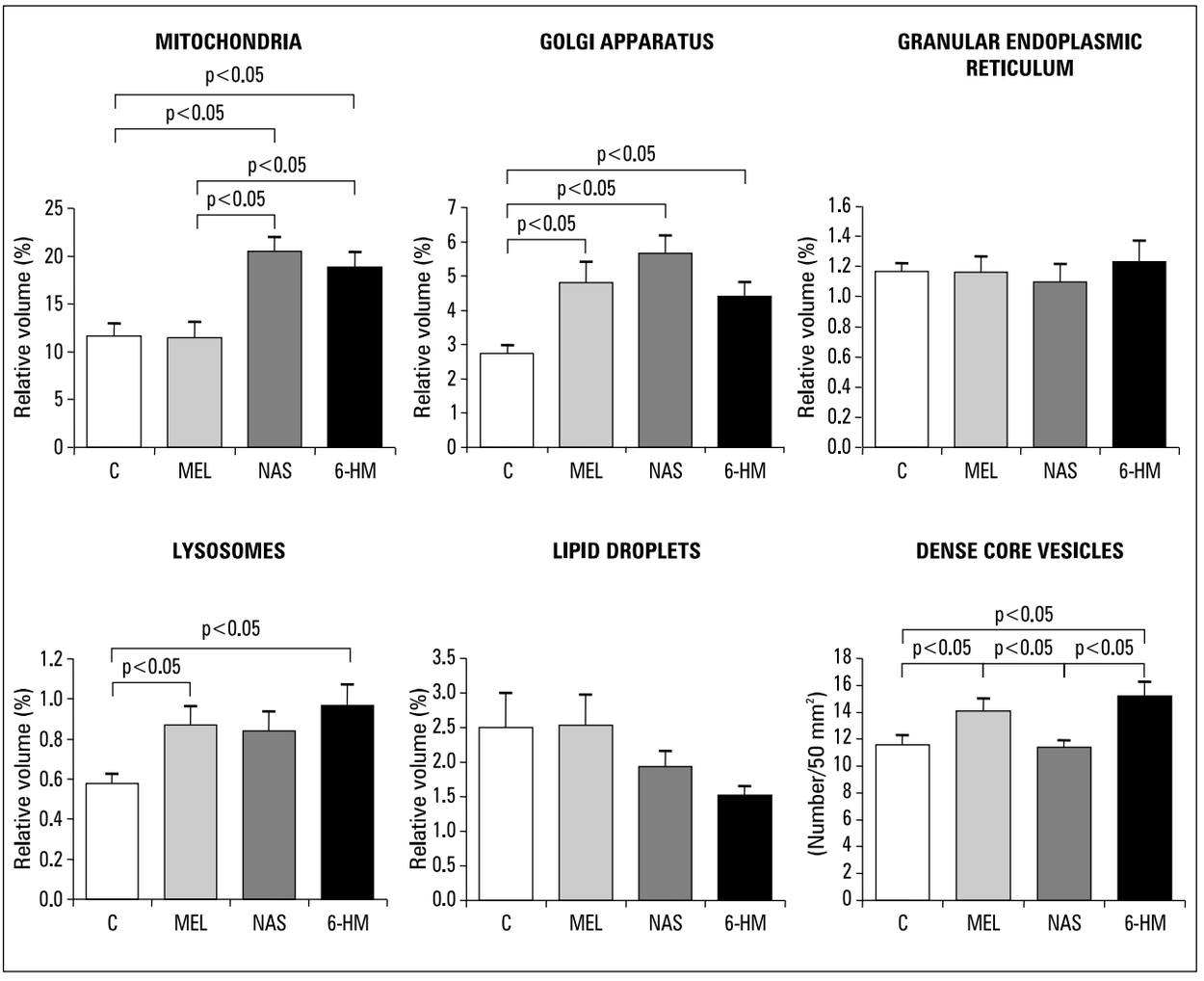

Figure 2. *The relative volumes of the mitochondria, Golgi apparatus, lysosomes, granular endoplasmic reticulum and lipid droplets, and the number of dense-core vesicles in the pinealocytes of the Syrian hamsters treated with melatonin (MEL), N-acetylserotonin (NAS), and 6-hydroxymelatonin (6-HM); C — controls*

Rycina 2. *Względne objętości mitochondriów, aparatu Golgiego, lizosomów i kropli lipidów oraz liczba pęcherzyków ziarnistych w pinealocytach chomika syryjskiego po podaniu melatoniny (MEL), N-acetyloserotoniny (NAS) i 6-hydroksymelatoniny (6-HM); C — kontrola*

the two enzymes involved in melatonin synthesis, namely serotonin N-acetyltransferase and hydroxyindole-O-methyl transferase [10].

Studies of the effect of melatonin and other pineal indoles on the ultrastructural features of mammalian pinealocytes have been rare. In an early qualitative study melatonin treatment induced some ultrastructural changes in the pinealocytes of the rat, including an increase in the number of Golgi profiles, ribosomes, microtubules and annulate lamellae [10]. Subcutaneous injections of melatonin did not influence the number of dense-core vesicles in the pinealocytes of Syrian hamsters housed in a long photoperiod (LD 14:10) [11]. Melatonin also failed to influence the number of dense-core vesicles in the pinealocytes of the Syrian hamster *in vitro*, although it increased their number in rat pinealocytes cultured under the same conditions [12]. Moreover, melatonin increased the number of "synaptic" ribbons in rat pinealocytes *in vitro* when added to the incubation medium in the first half of the night, whereas serotonin decreased their number when added in the morning [13].

It appears from the present study that long-term administration of melatonin, its precursor or its metabolite influences the ultrastructure of pinealocytes of the Syrian hamster, although there are some differences in the pinealocyte response to these compounds. The administration of melatonin did not change the size of the hamster pinealocytes but caused some ultrastructural changes in these cells, expressed by an increase in the relative volumes of the Golgi apparatus and lysosomes and in the number of dense-core vesicles; this suggests an effect of melatonin on the pinealocyte secretory processes. General cell metabolism, expressed by the relative volumes of the mitochondria, granular endoplasmic reticulum and

lipid droplets, seems not to have been influenced by melatonin administration. Following administration of the melatonin precursor (N-acetylserotonin) or its metabolite (6-hydroxymelatonin) the pinealocytes and their nuclei were smaller and the volumes of the mitochondria, Golgi apparatus and lysosomes were elevated in these cells relative to those in the control glands.

The changes observed in the ultrastructure of hamster pinealocytes indicate that the administration of melatonin as well as of its precursor or metabolite influences the morphology of these cells and may also influence their secretory activity. However, it should be mentioned that in this study the animals were killed during the daytime, when the ultrastructural and biochemical features of pinealocytes indicate that these cells are less active than at night [14].

Acknowledgements

The study was supported by grant no. 502–11–148 from the Medical University of Łódź.

References

1. Vollrath L. The pineal organ. Springer, Berlin, 1981.
2. Karasek M. Ultrastructure of the mammalian pineal gland: its comparative and functional aspects. Pineal Res Rev 1983; 1: 1–48.
3. Karasek M. Quantitative aspects of the ultrastructure of the mammalian pinealocyte. Adv Pineal Res 1986; 1: 9–18.
4. Karasek M. Ultrastructure of the mammalian pinealocyte under natural and experimental conditions: quantitative aspects. Micr Res Tech 1992; 21: 116–123.
5. Bhatnagar KP The ultrastructure of mammalian pinealocytes: a systemic investigation. Micr Res Tech 1992; 21: 85–115.
6. Karasek M, Reiter RJ. Morphofunctional aspects of the mammalian pineal gland. Micr Res Tech 1992; 21: 136–175.
7. Karasek M, Reiter RJ. Functional morphology of the mammalian pineal gland. In: Jones TC, Capen CC, Mohr U, (eds.) Monographs on the pathology of laboratory animals. Endocrine system. Second edition. Springer, Berlin, 1996; 193–204.
8. Weibel ER. Stereological methods. Vol. 1. Practical methods for biological morphometry. Academic Press, London, 1979.
9. Miguez MJ, Simonneaux V, Pevet P. Changes in pineal indoleamines in rats after single melatonin injections: evidence for diurnal sensitivity to melatonin. J Neuroendocrinol 1996; 8: 611–616.
10. Freire F, Cardinali DP. Effects of melatonin treatment and environmental lighting on the ultrastructural appearance, melatonin synthesis, norepinephrine turnover and microtubule protein content of the rat pineal gland. J Neural Transm 1975; 37: 237–257.
11. Krasovich M, Benson B. A study of the relationship between photoperiod and pinealocyte granulated vesicles in the golden Syrian hamster. Cell Tissue Res 1982; 223: 155–163.
12. Haldar-Misra C, Pevet P. Influence of melatonin on the process of protein and/or peptide secretion in the pineal gland of the rat and hamster. Cell Tissue Res 1983; 231: 73–82.
13. Vollrath L, Karasek M, Kosaras B, et al. Influence of melatonin and serotonin on the number of rat pineal "synaptic" ribbons and spherules in vitro. Cell Tissue Res 1985; 242: 607–611.
14. Świętosławski J, Karasek M. Day-night changes in the ultrastructure of pinealocytes in the Syrian hamster: a quantitative study. Endokr Pol 1993; 44: 81–87.