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ORIGINAL ARTICLE

The effect of continuous darkness and illumination on the function and the morphology of the pineal gland in the domestic pig

II. The effect on pinealocyte ultrastructure

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Abstract

OBJECTIVES: The characteristic feature of the pig pinealocytes is the presence of numerous membrane bounded bodies (MBB), which according to our previous results may be involved in the secretory activity. The present study was undertaken to analyze the effect of continuous darkness and illumination on the ultrastructure of the pig pinealocytes.
MATERIAL AND METHODS: The study was performed on three groups of gilts. The first group (control) was kept under a cycle of 14 hrs light (500 lx) and 10 hrs dark per day. The second group was exposed to continuous illumination (500 lx). The third group was kept under red light with intensity less than 1 lx, which was considered as darkness. The pigs were kept for 14 days under above reported conditions and then slaughtered at 08:00. The point count analysis was used in quantitative studies of pinealocyte substructures.
RESULTS: The exposition of pigs to continuous illumination resulted in the decrease in the relative volume of mitochondria and in the numerical density of multivesicular bodies as well as in the increase in the relative volume of MBB in pinealocyte cell bodies. The exposition to continuous darkness led to the increase in the relative volume of mitochondria and the numerical density of dense core vesicles as well as induced some changes in smooth endoplasmic reticulum in pinealocyte cell bodies.
CONCLUSIONS: The obtained results point to mitochondria, MBB, multivesicular bodies, dense core vesicles and smooth endoplasmic reticulum as the structures of the pig pinealocyte, which are controlled by environmental light conditions.

Introduction

Despite a relatively long history of morphological studies of the pineal gland, cytological aspects of the secretory processes occurring in the mammalian pinealocytes are still poorly known [1, 2, 3, 4, 5, 6]. Up until now, the processes of melatonin synthesis and secretion were not arbitrarily localized in subcellular structures of the pinealocyte. However, it is generally believed that melatonin secretion is not related to any form of membrane bounded secretory granules. On the basis of ultrastructural observations two processes, considered as secretory, were found in the mammalian pinealocytes [1, 3, 4, 6]. The first one, called neurosecretory-like, is characterized by formation of dense core vesicles by a Golgi apparatus. The second process is ependymal-like and involves the formation of vacuoles containing flocculent material by granular endoplasmic reticulum. Both processes are probably involved in the synthesis and release of proteinaceous compounds. Apart from the above mentioned two morphologically defined secretory processes, other cytoplasmic structures of pinealocytes, such as various forms of dense bodies and lipid droplets, were also considered as being involved in the secretory processes [1, 3, 6].

The pinealocytes of the domestic pig are distinguished by the presence of numerous electron dense bodies, called membrane bounded bodies—MBB [7, 8]. The relative volume of MBB in the pinealocyte cell bodies ranges from 8% to 16% of the whole cytoplasm. MBB are also numerous in the wild boar and in the miniature pig pineal glands [9]. On the basis of their morphology, membrane bounded bodies were divided into two main types: MBB-1 and MBB-2. Dense bodies of MBB-1 type have variable inner structure and are present both in perikaryon and processes of the pinealocyte. Dense bodies of MBB-2 type are characterized by regular, multilammellar structure and their presence is restricted to pinealocyte perikaryon. The structure and the relative volume of MBB change in various physiological conditions and are also affected by experimental procedures [8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19]. Although the processes of formation and transformation of MBB step by step as well as the detailed chemical composition of MBB remain unknown, the current state of knowledge suggests that MBB may play an important role in the biology of the pig pinealocyte.

Since light is the most important environmental factor regulating the activity of the pineal gland, the present study was undertaken to analyze the effect of continuous darkness and continuous illumination on the ultrastructure of the pig pinealocytes.

Material and methods

The female crossbred pigs, aged 95±4 days, were purchased in April (length of day ca 14.5 hrs) from a commercial farm, where they were kept under natural photoperiod. Animals were divided into three groups, which were kept in separated rooms. After seven days of the adaptation period the left jugular veins were cannulated for blood sampling (for details see part I) and then the gilts were kept under conditions of controlled artificial illumination. The first group (control) was exposed to a cycle of 14 hrs of light (500 lx on the level of animal eyes, provided by fluorescent tubes) and 10 hrs of darkness per day. The second group was kept under red light with intensity less than 1 lx and these conditions were considered as darkness. The third group was exposed to continuous illumination (500 lx of fluorescent light on the level of animal eyes). After two weeks the pigs were slaughtered at 08:00 (the group kept in continuous darkness under dim red light with intensity below 1 lx) and the pineal glands were removed no later than 3 min after the heart stopped beating. Handling of animals was performed in agreement with "Principles of laboratory animal care" (NIH publication No. 86–23, revised 1985) and Polish law on the protection of animals.

Pineals were cut into several parts, immersion fixed (2hrs, 4°C) in a mixture of 1% paraformaldehyde and 2.5% glutaraldehyde in 0.2M phosphate buffer (pH 7.4), washed, postfixed in 2% osmium tetroxide and embedded in Epon 812. Four blocks from each animal were selected at random for sectioning. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined by transmission electron microscope. Ten micrographs at a magnification of x 8,000, were taken from each block (i.e. 40 micrographs per animal) using systematic random sampling, photographically enlarged to x 20,000 and used for quantitative study. Point count analysis [20] was employed to estimate the relative volume (expressed as the percent of the cytoplasm of the pinealocyte cell body) of the following cell components: mitochondria (MIT), Golgi apparatus (GA), granular endoplasmic reticulum (GER), lysosomes (LYS), dense bodies type MBB-1 and MBB-2. The numerical density (expressed as the number per $500\mu m^2$ of the cytoplasm surface of the pinealocyte cell body) of dense core vesicles (DCV) and multivesicular bodies (MB) were also estimated.

The results were analyzed using one-way analysis of variance followed by a Student-Newman-Keuls test. Value $p \leq 0.05$ was considered as significant.

Results

Qualitative study

Ultrastructure of the pineal gland in the pigs kept under 14 hrs light:10 hrs dark cycle was similar to the previously described in gilts of the same age [8, 11, 21]. The pinealocyte cell bodies contained various forms of dense bodies (Fig. 1). The endings of pinealocyte processes were filled with dense bodies with granular electron dense content (Fig. 2). Diameter of dense bodies in pinealocyte perikarya and processes varied from 0.5μm to 1.5μm.

Qualitative studies did not reveal prominent differences in the ultrastructure of the pineal gland between the control pigs and the pigs exposed to continuous darkness and to continuous illumination (Figs. 1, 3, 4). The pinealocytes of the gilts kept under continuous darkness were distinguished by the presence of numerous wide cisterns of endoplasmic reticulum, especially their smooth form (Fig. 3). A characteristic feature of the pinealocytes of pigs exposed to continuous illumination was the presence of very numerous dense bodies (Fig. 4).

Quantitative study

The relative volume of MIT in the cytoplasm of the pinealocyte cell bodies was significantly higher in the group of pigs kept under continuous darkness than in the control animals and the gilts exposed to continuous illumination (Fig. 5). The relative volume of MIT was also significantly higher in the control animals than in the pigs exposed to continuous illumination. The differences in the relative volume of GER, GA and LYS in the cytoplasm of pinealocyte cell bodies were not significant (Fig. 5).

The total relative volume of MBB in the cytoplasm of the pinealocyte cell bodies was higher in the pigs kept under continuous illumination than in the control pigs and the pigs exposed to continuous darkness (Fig. 5). The relative volume of dense bodies type MBB-1 in the cytoplasm of the pinealocyte cell bodies was also significantly higher in the gilts exposed to continuous illumination than in two other investigated groups of pigs (Fig. 5). No significant differences were noted in the relative volume of MBB-2.

Numerical density of MB in the pinealocyte cell bodies was significantly lower in the pigs exposed to continuous illumination than in the animals kept under light-dark cycle and in continuous darkness (Fig. 5). The numerical density of DCV in the pinealocyte cell bodies was significantly higher in the pigs kept under continuous darkness than in two other investigated groups of animals (Fig. 5).

Discussion

The study of the plasma melatonin concentration (see part I—this issue) revealed the presence of significant rise in the level of this pineal hormone between 22:00 and 06:00 in the pigs kept under light-dark cycle as well as between 18:00 and 08:00 in the pigs exposed to continuous darkness. In contrast, no significant circadian changes in the plasma melatonin level were observed in the gilts kept under continuous illumination. However, the differences between the three investigated groups in the mean plasma melatonin level were not significant during the entire sampling period. The mean area under the curve of the plasma melatonin profiles in the pigs exposed to continuous darkness was 105.8% and in the pigs exposed to continuous illumination - 85.9% of the value in the control animals kept under light-dark cycle, but these values did not differ significantly.

The investigations performed in several mammalian species have shown that the exposition to continuous darkness and illumination results in numerous modifications of the pinealocyte ultrastructure including the changes in MIT, endoplasmic reticulum, ribosomes, GA and DCV [1, 2, 4, 6, 22, 23, 24, 25]. Generally, the exposure to continuous illumination caused more prominent changes in the pinealocytes than conditions of continuous darkness, probably due to light-induced reduction in the amount of norepinephrine released from sympathetic nerve terminals in the pineal gland.

The present ultrastructural examinations have shown that the exposition of the pigs to continuous illumination for 14 days resulted in the decrease in the relative volume of MIT and the numerical density of MB well as in the increase in the relative volume of MBB in the pinealocyte cell bodies. On the other hand, the expososure to continuous darkness led to the increase in the relative volume of MIT and the numerical density of DCV as well as induced some changes in smooth endoplasmic reticulum in the pinealocyte cell bodies.

The opposite changes in the relative volume of MIT caused by the exposition to continuous darkness and illumination suggest that biochemical processes occurring in MIT of the pig pinealocytes are modified by the environmental light conditions. Our results are in agreement with those obtained in the rat, in which the changes in the number and structure of MIT were observed following the light deprivation as well as the exposure to continuous darkness [22]. In the previous

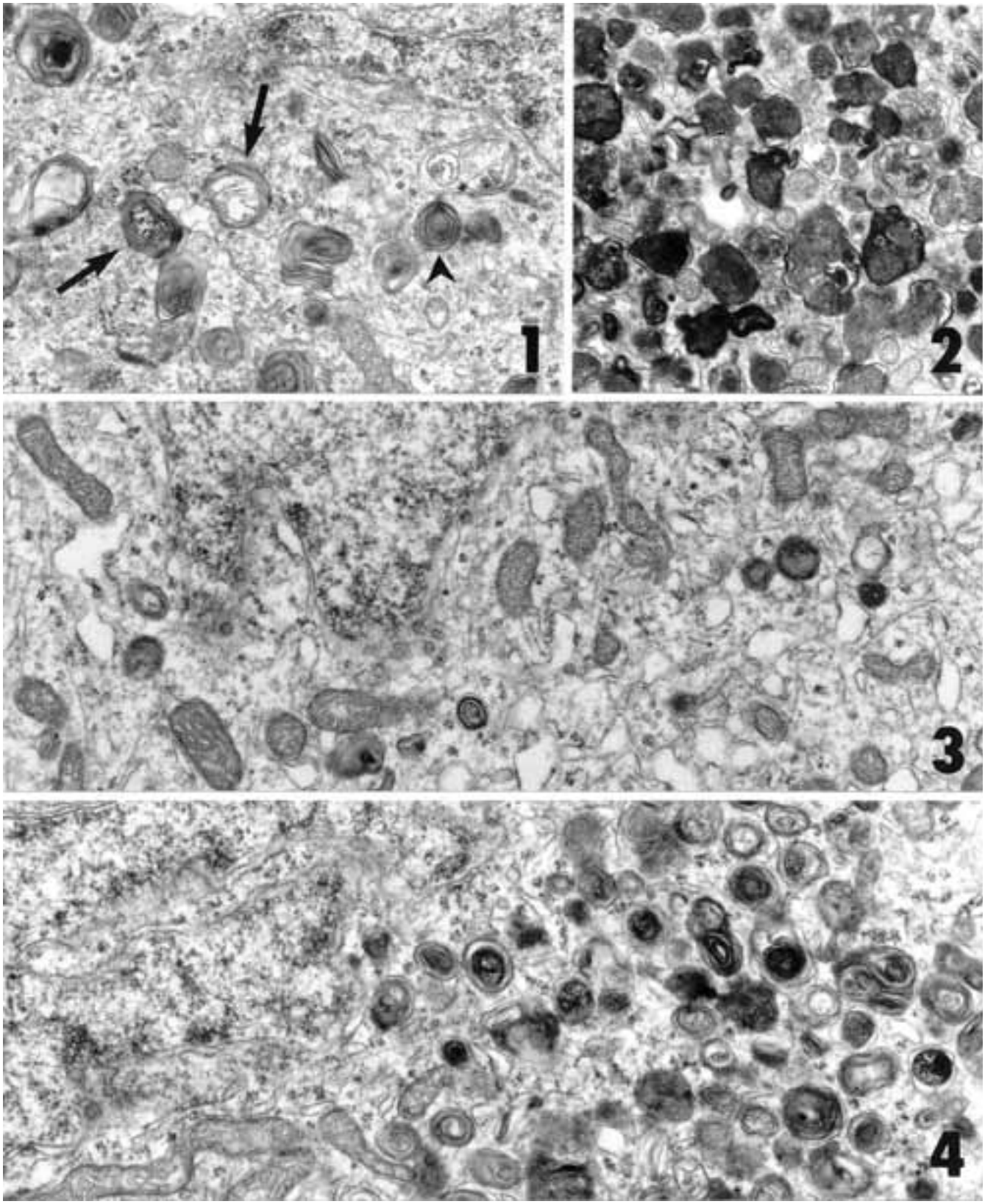


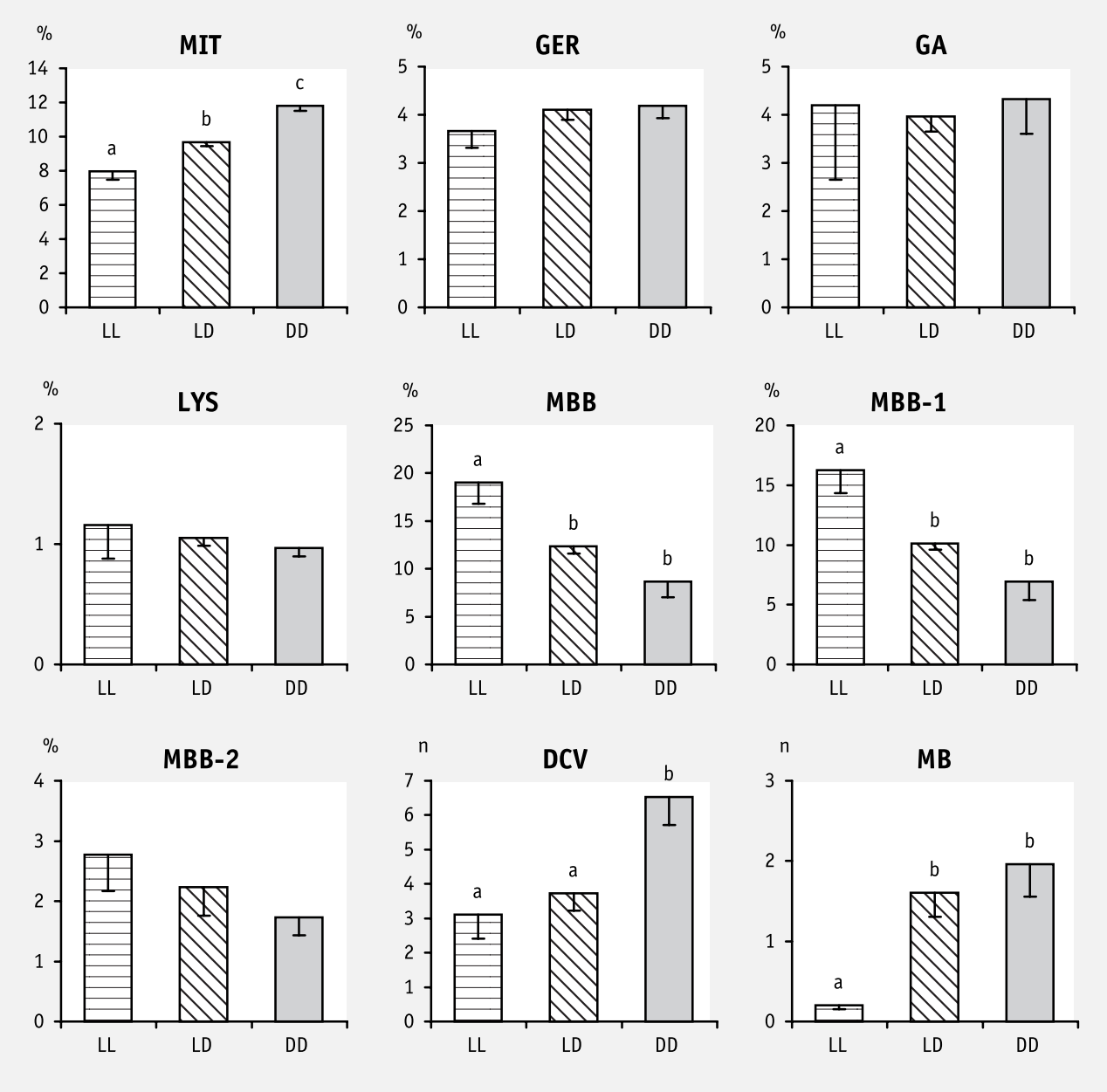
Fig. 1. Part of the pinealocyte cell body in the pineal gland of the pig kept under light-dark cycle. Dense bodies type MBB-1 —arrows, dense bodies type MBB-2—arrow head. x 20,000

Fig. 2. Part of the ending of the pinealocyte process in the pineal gland of the pig kept under light-dark cycle. x 20,000

Fig. 3. Part of the pinealocyte cell body in the pineal gland of the pig kept under continuous darkness. x 20,000

Fig. 4. Part of the pinealocyte cell body in the pineal gland of the pig kept under continuous illumination. x 20,000

Fig. 5. The mean (±SEM) relative volume of MIT, GER,



GA, LYS, MBB (all forms), MBB-1 and MBB-2 as well as numerical density of MB and DCV in the pinealocyte cell bodies of the pigs kept under light-dark cycle (LD), continuous darkness (DD) and continuous illumination (LL). The values signed with different letters are significantly different from each other at $p \leq 0.05$.

study the administration of sympathicolytic and sympathicomimetic drugs to female pigs did not result in significant changes in the relative volume of MIT in the pinealocytes [11]. In gilts kept under 12 hrs light:12 hrs dark cycle, the relative volume of MIT was significantly higher in the pigs slaughtered at 14:00 than at 02:00 [21].

Special attention should be paid to the effect of the deprivation of light-dark cycle on the dense bodies of type MBB, the characteristic structures of the pig pinealocytes. Exposition of the domestic pigs to continuous illumination with intensity of 500 lx during 14 days resulted in the significantly higher relative volumes of MBB and its form—MBB-1 in the cytoplasm of the pinealocyte cell body than in two other investigated groups. Opposite changes, but not significant versus control group, were observed after two-week-long light deprivation. The obtained results provide strong evidence that the conditions of the environmental lighting influence the process of formation and/or transformation of MBB. The effect of light on MBB is probably mediated by the sympathetic innervation, a main route controlling the circadian rhythmicity of the function of the mammalian pineal gland. In our previous study an increase in the relative volume of MBB was noted

after administration of sympathicolytic drugs (propranolol, α-methyl-p-tyrosine) and a decrease after treatment with clorgyline, a monoamine oxidase inhibitor [11]. The dependence on the environmental light conditions and the regulation by sympathetic innervation suggest the existence of some similarities in the mechanisms regulating the melatonin secretion and the system of MBB. However, the relative volume of MBB did not show significant diurnal rhythm in the pigs kept under 12 hrs light:12 hrs dark cycle [21].

Our previous study has shown that the system of MBB undergoes significant qualitative and quantitative changes during development [8] as well as in various stages of reproductive activity [10]. The influence of the reproductive system on MBB was confirmed by several experiments, which show the changes in MBB after ovariectomy [16], administration of ovarian steroids to normal [15, 17] and ovariectomized pigs [14] as well as after treatment with prolactin [12, 13]. It was also demonstrated that the administration of melatonin resulted in the significant changes in MBB [18, 19]. The effect of melatonin on MBB was dependent on the time of administration and duration of treatment [19, 26].

The changes in the structure and the relative volume of MBB in response to the experimental procedures as well as their numerous presence in the pinealocytes, the differences in the structure of MBB located in cell body and processes, and the exocytosis of MBB from endings of cell processes [27] suggest that MBB may be involved in secretory processes of the pig pinealocytes. The present results showing the effect of continuous illumination and darkness strongly support this hypothesis, but further studies on biogenesis and transformations of MBB as well as on chemical composition of MBB are necessary for the definitive explanation of the role of MBB.

In the present study the exposition to continuous illumination led to significant decrease in the numerical density of MB in the pinealocyte cell bodies. These results are in agreement with previous observations showing decrease in the numerical density of MB in the pinealocytes of gilts treated with α-methyl-p-tyrosine [11] as well as negative correlation between the relative volume of MBB and numerical density of MB in the pig pinealocytes [unpublished].

The obtained results demonstrated the significant differences in the numerical density of DCV in the pinealocyte cell bodies between the pigs kept under continuous darkness and two other investigated groups. The changes in the number of DCV in cell bodies and/or processes of pinealocytes were described during diurnal light-dark cycle [28, 29, 30, 31] as well as following sympathectomy [23, 30, 32, 33], blinding [33, 34], exposition to continuous darkness and illumination [23, 24, 25] in several laboratory rodents. The sympathetic control of DCV number in rodent pinealocytes was also confirmed by the results of the *in vitro* studies [35, 36, 37]. Our previous experiment has demonstrated the opposite changes in the numerical density of DCV in pinealocyte cell bodies of the pig after treatment with clorgyline (increase) and α-methyl-p-tyrosine (decrease), which suggest adrenergic control of the number of DCV in the pig pinealocytes [11]. However, the studies of the ultrastructure of the pig pinealocytes during scotophase and photophase of diurnal rhythm did not show significant differences in the numerical density of DCV [21].

The pinealocytes of the pigs exposed to continuous darkness were characterized by the presence of wide cisterns of the smooth endoplasmic reticulum. Several changes in the smooth endoplasmic reticulum of the mammalian pinealocytes were observed in response to experimental factors including deprivation of light-dark cycle [1, 2, 3, 6, 23]. However, the interpretation of the changes in the smooth endoplasmic reticulum may be only very speculative due to variability of the functions of this cell component and difficulties in its quantitative, morphometrical analysis.

In summary, the obtained results point to mitochondria, membrane bounded dense bodies, multivesicular bodies, dense core vesicles and smooth endoplasmic reticulum as the structures of the pig pinealocyte, which are controlled by environmental light conditions.

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REFERENCES

1 Karasek M. Ultrastructure of the mammalian pineal gland: its comparative and functional aspects. In: Reiter RJ, editor. Pineal Research Reviews, Vol. 1. New York: Liss AR Inc; 1983. p. 1–48.
2 Karasek M. Quantitative aspects of ultrastructure of the mammalian pinealocyte. In: Reiter RJ, Karasek M, editors. Advances in Pineal Research, Vol. 1. London, Paris: John Libbey Ltd; 1986. p. 9–18.
3 Karasek M. Functional ultrastructure of the mammalian pinealocyte. In: Reiter RJ, Fraschini F, editors. Advances in Pineal Research, Vol. 2. London, Paris: John Libbey Ltd; 1987. p. 19–33.
4 Pevet P. Anatomy of the pineal gland of mammals. In: Relkin R,

editor. The pineal gland. Elsevier Biomedical; 1983. p.1–75.
5 Reiter RJ, Vaughan MK, King TS, Karasek M. The mammalin pineal gland: pharmacologic regulation and physiologic consequences. In: Steger RW, Johns A, editors. Handbook of pharmacologie methodologies for the study of the neuroendocrine system. Boca Raton: CRC Press; 1985. p. 331–384.
6 Vollrath L. The pineal gland. In: Handbuch der mikroskopischen Anatomie des Menschen. VI/7. Berlin, Heidelberg: Springer Verlag; 1981.
7 Karasek M, Wyrzykowski Z. The ultrastructure of pinealocytes in the pig. Cell Tissue Res 1980; **211**:151–161.
8 Przybylska-Gornowicz B, Lewczuk B. Cytoplasmic dense bodies in pig pinealocyte during postnatal development. Quantitative, ultrastructural study. Folia Morphol (Warsz) 1997; **56**:13–21.
9 Wyrzykowski Z, Wyrzykowska K, Przybylska B. Pineal gland ultrastructure in wild boar and miniature pig. Folia Morphol (Warsz) 1987; **46**:161–173.
10 Przybylska B, Wyrzykowski Z, Wyrzykowska K, Karasek M. Ultrastructure of pig pinealocytes in various stages of the sexual cycle: a quantitative study. Cytobios 1990; **64**:7–14.
11 Lewczuk B, Przybylska-Gornowicz B. Effects of sympathicolytic and sympathicomimetic drugs on pineal ultrastructure in the domestic pig. J Pineal Res 1997; **23**:198–208.
12 Przybylska B, Dusza L, Lewczuk B, Ciesielska-Myszka L. Effect of exogenous prolactin on ultrastructure of pinealocyte in female pigs during puberty. Arch Vet Pol 1994; **34**:91–98.
13 Przybylska B, Dusza L, Lewczuk B, Wyrzykowski Z. Influence of prolactin on ultrastructure of pinealocyte in sexually immature female pigs: a quantitative study. Cytobios 1992; **71**:75–83.
14 Przybylska B, Wyrzykowski Z, Kaleczyc J. Effect of ovariectomy followed by administration of ovarian steroid hormones on the pig pinealocyte ultrastructure. Steroids 1993; **58**:466–471.
15 Przybylska B, Wyrzykowski Z, Wyrzykowska K. The effect of estradiol on quantitative changes in the ultrastructure of pinealocyte structures in sexually immature female domestic pigs. Z Mikrosk-Anat Forsch 1988; **102**:533–540.
16 Wyrzykowski Z, Przybylska B, Wyrzykowska K, Kaleczyc J. Influence of bilateral ovariectomy on the morphology and ultrastructure of the pineal gland in the pig (Sus scrofa): quantitative and qualitative study. Folia Morphol (Warsz) 1992; **51**:93–108.
17 Wyrzykowski Z, Przybylska B, Wyrzykowska K. The effect of progesterone and progesterone + estradiol on the morphology of the pineal gland in immature female pigs. Z Mikrosk Anat Forsch 1990; **104**:265–272.
18 Przybylska B, Lewczuk B, Wyrzykowski Z, Karasek M. Effects of p-chlorophenylalanine, amiflamine and melatonin treatment on the ultrastructure of pinealocytes in Sus scrofa. Cytobios 1994; **77**:233–246.
19 Lewczuk B, Przybylska-Gornowicz B. The effect of exogenous melatonin on the pinealocyte ultrastructure in the domestic pig (Sus domesticus) depends on the time of its administration. Polish J Vet Sci 2000; **3**:29–38.
20 Weibel ER. Stereological methods. Vol. 1. London: Acad. Press; 1979.
21 Lewczuk B, Przybylska-Gornowicz B, Wyrzykowski Z. Circadian rhythm in the morphology and function of the domestic pig pineal gland. Proceedings of 7th European Pineal Society Colloquium; Mar 28–31, 1996; Sitges, Spain, p.8.
22 Bostelman W. Das ultrastrukturelle and enzymhistochemische Vehalten der Rattenzirbeldruse nach Funktionsphasenwechsel durch Dauerbeleuchtung und standige Dunkelheit. [(Ultrastructural and enzyme histochemical behavior of the rat pineal body following functional phase change caused by continuous illumination and constant darkness) (in German with English abstract)]. Endokrinologie 1968; **53**:365–384.
23 Romijn HJ. The ultrastructure of the rabbit pineal gland after sympathectomy, parasympathectomy, continuous illumination and continuous darkness. J Neural Transm 1975; **36**:183–194.
24 Upson RH, Benson B, Satterfield V. Quantitation of ultrastructural changes in the mouse pineal in response to continuous illumination. Anat Rec 1976; **184**:311–323.
25 Clabough JW. Ultrastructural features of the pineal gland in normal and light deprived golden hamsters. Z Zellforsch-Mikrosk Anat 1971; **114**:151–164.
26 Przybylska B, Lewczuk B, Dusza L, Wyrzykowski Z. Effect of long-term administration of melatonin on ultrastructure of pinealocytes in gilts. Folia Morphol (Warsz) 1994; **53**:129–136.
27 Przybylska B, Masson-Pevet M, Pevet P. Ultrastructural visualization of exocytosis in the pig pineal gland. Cell Tissue Res 1991; **264**:377–379.
28 Karasek M, King TS, Hansen JT, Reiter RJ. A quantitative ultrastructural study of the pinealocyte of the chipmunk (*Tamias striatus*) during the daytime and at night. J Neurosci Res 1982; **7**:397–401.
29 Karasek M, Stankov B, Lucini V, Scaglione F, Esposti G, Mariani M, et al. Comparison of the rat pinealocyte ultrastructure with melatonin concentrations during daytime and at night. J Pineal Res 1990; **9**:251–257.
30 Benson B, Krasovich M. Circadian rhythm in the number of granulated vesicles in the pinealocytes of mice. Effects of sympathectomy and melatonin treatment. Cell Tissue Res 1977; **184**:499–506
31 Romijn HJ, Mud MT, Wolters PS. Diurnal variations in number of Golgi-dense core vesicles in light pinealocytes of the rabbit. J Neural Transm1976; **38**:231–237.
32 Karasek M, King TS, Hansen JT, Reiter RJ. Quantitative changes in the numbers of dense-core vesicles and 'synaptic' ribbons in pinealocytes of the Djungarian hamster (*Phodopus sungorus*) following sympathectomy. Cytobios 1982; **35**:157–162.
33 Lin HS, Hwang BH, Tseng CY. Fine structural changes in the hamster pineal gland after blinding and superior cervical ganglionectomy. Cell Tissue Res 1975; **158**:285–299.
34 Upson RH, Benson B. Effects of blinding on the ultrastructure of mouse pinealocytes with particular emphasis on the dense-cored vesicles. Cell Tissue Res 1977; **183**:491–498.
35 Haldar-Misra Ch, Pevet P. The influence of noradrenaline on the process of protein/peptide secretion in the mammalian pineal organ. Comparative in vitro studies. Cell Tissue Res 1982; **224**:33–44.
36 Karasek M. Ultrastructure of rat pineal gland in organ culture: influences of norepinephrine, dibutyryl cyclic adenosine 3,5-monophosphate and adenohypophysis. Endokrinologie 1974; **64**:106–114.
37 Romijn HJ, Gelsema AJ. Electron microscopy of the rabbit pineal organ in vitro. Evidence of norepinephrine-stimulated secretory activity of the Golgi apparatus. Cell Tissue Res 1976; **172**:365–377.