FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 42, No. 3, 2004
pp. 155-164

Diurnal rhythms of pinealocyte ultrastructure, pineal serotonin content and plasma melatonin level in the domestic pig

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Abstract: The study was conducted to investigate diurnal changes in pinealocyte ultrastructure, pineal serotonin content and plasma melatonin concentration in the domestic pig. The immature pigs (n=24) were kept under a cycle of 12 h light : 12 h dark, with a photophase between 0800 and 2000. During the photophase the animals were exposed to direct sunlight. After four weeks the gilts were slaughtered at 0900, 1400, 2100 and 0200. The pineals were removed and divided into two parts - one for quantitative ultrastructural study (by a point count method) and one for serotonin assay. Simultaneously, blood samples were taken for melatonin assay. The relative volume of mitochondria in pinealocyte perikarya was significantly higher at 1400 than at 0200 and 0900 as well as at 2100 than at 0200. The relative volume of Golgi apparatus was higher at 0900 and 1400 than at 0200. The relative volume of dense bodies of the MBB-1 type in pinealocyte perikarya was significantly lower at 1400 and 2100 than at 0900. In contrast, the relative volume of MBB-2 was higher at 1400 than at 0900 and 0200. The numerical density of DCV in perikarya was significantly higher at 0200 than at 1400. No significant differences were found in rough endoplasmic reticulum, lysosomes and multivesicular bodies. The pineal serotonin content showed a prominent rhythm with the maximum at 1400. The plasma melatonin concentration was significantly higher at 0200 than at 0900, 1400 and 2100. The obtained results demonstrate that both pinealocyte ultrastructure and pineal biochemistry in the pig undergo significant changes in the course of the diurnal rhythm.

Key words: Pineal gland - Pinealocyte - Melatonin - Serotonin - Ultrastructure - Diurnal cycle - Pig

Introduction

Melatonin, the best known output of the pineal gland, shows a prominent diurnal rhythm of its synthesis and release with the peak at night. In mammals, this rhythm is endogenously driven by the suprachiasmatic nucleus of the hypothalamus and controlled by light acting *via* the retina and the retinohypothalamic tract [10]. The suprachiasmatic nucleus influences the endocrine function of mammalian pinealocytes by the multisynaptic pathway, which reaches the pineal gland as the sympathetic nerve fibers. Norepinephrine released from these fibers is the main neurotransmitter responsible for the nocturnal increase in the activity of serotonin N-acetyltransferase, the enzyme that controls melatonin secretion at a step of conversion of serotonin into N-acetylserotonin [10]. The mechanisms regulating the activity of serotonin N-acetyltransferase in mammalian pinealocytes differ significantly between species investigated up till now: the rat, the Syrian hamster, the sheep and the pig [10, 18]. These differences are probably responsible for the variability in shape and amplitude of the diurnal melatonin profiles observed in mammals. Beside the rhythmic melatonin secretion, another consequence of the diurnal changes in serotonin N-acetyltransferase activity is a prominent rhythm of the pineal serotonin content with high level during the day and low at night [24, 33, 37, 38]. In contrast to the melatonin rhythm, the diurnal changes in pineal serotonin level were rather infrequently investigated.

The studies performed on rodents and lagomorphs - the rat [15, 16, 17, 27], the mouse [2], the Syrian hamster [6, 39], the Chinese hamster [1], the golden hamster [9], the gerbil [45], the chipmunk [13, 14], the ground squirrel [14], the guinea-pig [42] and the rabbit [35] - demonstrated some relationships between morphology of pinealocytes and time of day. Diurnal changes were

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reported in dimensions and structure of nuclei as well as in cross-section area or a number of several cytoplasmic substructures in pinealocytes. It should be noted that the obtained results revealed several considerable disagreements and interspecies differences in morphological aspects of the pineal rhythmicity. Moreover, ultrastructural studies dealing with the pineal rhythm are several times less frequent than those concerning the melatonin level and the serotonin N-acetyltransferase activity. Up till now, the ultrastructure of pinealocytes in the domestic animals (except for the rabbit) during the photophase and scotophase has never been compared.

The morphology and physiology of the pig pineal gland show several species-specific features concerning: (1) diurnal patterns of plasma melatonin level, (2) mechanisms of adrenergic regulation of melatonin secretion, and (3) ultrastructure of pinealocytes.

The results of studies concerning melatonin secretion in the domestic pig are controversial. Several investigators reported the presence of nocturnal rise in circulating melatonin level only under certain lighting conditions, like the equatorial lengths of photophase and scotophase [26] or the exposition to high intensity illumination during photophase [11]. In other studies, no nocturnal rise in plasma or serum melatonin was found irrespectively of light conditions [4, 5, 7, 8] or the diurnal rhythm of the pineal hormone level in the blood was observed under a wide range of day-lengths: from 8 to 16 hours [21, 30, 40, 41]. The diurnal profiles of circulating melatonin level in the pig are characterised by low amplitude of day-night changes and resemble type "C" of the Reiter's classification [21, 30, 40, 41]. A long-term blood sampling in gilts showed the lack of repeatability of the diurnal melatonin patterns on consecutive days [20].

Mechanisms of adrenergic activation of melatonin secretion in the pig pineal are completely different from those in the investigated rodent species [18]. Norepinephrine stimulates melatonin synthesis in pig pinealocytes without an induction of transcription and translation of serotonin N-acetyltransferase, most likely by an increase in the affinity of this enzyme to serotonin [18]. In contrast, the synthesis of new molecules of serotonin N-acetyltransferase is necessary for the activation of melatonin secretion in the rat and the Syrian hamster [10]. The specific pathway of adrenergic regulation of the pineal secretory activity in the domestic pig is probably responsible for the characteristic pattern of diurnal changes in plasma melatonin level [18, 20, 21, 40, 41].

The pig pinealocytes possess a unique structural feature: the abundance of cytoplasmic dense bodies called membrane bound bodies - MBB [19, 22, 47]. Based on morphological criteria, MBB were divided into two types: MBB-1 with variable, irregular structure and MBB-2 with regular, multilamellar structure. Dense bodies of the MBB-1 type are present both in cell bodies and cell processes including their terminals located in the perivascular spaces. In contrast, dense bodies of the MBB-2 type occur only in pinealocyte perikarya. The changes in morphology and relative volume of MBB were observed during postnatal development as well as under various physiological and experimental conditions [for review see 47]. Several experimental data suggest that these structures are involved in secretory activity of pig pinealocytes [19, 22, 47].

The present study was undertaken to compare the ultrastructure of pig pinealocytes as well as the pineal serotonin content and the plasma melatonin concentration at four different time-points of the 24-hour cycle. The pigs were kept for one month under regime of 12 hours light : 12 hours dark and exposed to direct sunlight during the photophase. These light conditions combine two environmental factors, which have been reported as promoting diurnal changes in melatonin secretion in the pig: the equatorial lengths of photophase and scotophase [26] with the high intensity of light during photophase [11].

Materials and methods

Animals and experimental protocol. The experiment was performed in Poland from May 11 to June 11. The female crossbred pigs (n=24) aged 95 ± 3 days were purchased from a commercial farm with natural length of photoperiod. After arrival to the animal laboratory the gilts were kept under a cycle of 12 h light : 12 h dark (a photophase from 0800 to 2000). During the photophase the animals were kept in a pig run with a small roof enabling to take the refuge from too intensive sun-light or rain. At 2000 the animals were driven from the pig-run to the adjacent light-proof animal-laboratory room, where they were kept in darkness up to 0800. The gilts had free access to water and were fed twice a day (at 0800 and at 1500).

After four weeks under this light regime, the pigs were slaughtered as follows: 6 gilts between 0845 and 0915 (time-point 0900 - the second hour of the photophase), 6 gilts between 1345 and 1415 (time-point 1400 - the middle of the photophase), 6 gilts between 2045 and 2115 (time-point 2100 - the second hour of the scotophase) and 6 gilts between 0145 and 0215 (time-point 0200 - the middle of the scotophase). During the night the pigs were slaughtered under dim red light with intensity below 2 Lx.

Handling of animals and slaughtering procedure were performed in agreement with "Principles of laboratory animal care" (NIH publication No 86-23, revised 1985) and Polish law on the protection of animals.

Collection of blood and pineal samples. The blood samples were collected during the slaughter by a puncture of the cranial caval vein. The blood plasma was prepared with the use of heparin (Polfa - Poland) as an anticoagulant and stored at -20˚C until melatonin assay.

The pineal glands were removed no later than 3 min after the heart stopped beating and divided in sagittal plane into two parts - the right and the left ones. The right parts were immediately fixed for the ultrastructural investigations whereas the left ones were weighted and stored in liquid nitrogen until serotonin assay.

Ultrastructural investigations. The tissues were fragmented, immersion fixed (2 h, 4˚C) in a mixture of 1% paraformaldehyde and 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4), washed, postfixed in 2% osmium tetroxide (2 h, room temperature) and

embedded in Epon 812. Three blocks from each animal were selected at random for sectioning. Ultrathin sections, stained with uranyl acetate and lead citrate were examined in a Tesla BS 500 transmission electron microscope. The chemicals used for tissue preparation were electron microscope- or analytical-purity grade.

Fifteen micrographs at × 8 000 magnification were taken from each block (*i.e.* 45 micrographs per animal) using modified systematic random sampling [43]. The upper left corner of each gird aperture showing a pinealocyte perikaryon was photographed until an appropriate sample set was obtained. Altogether 1080 prints photographically enlarged to × 20 000 were used to estimate the relative volume (expressed as the percent of cytoplasm of pinealocyte perikaryon) of the following cell components: mitochondria, Golgi apparatus, rough endoplasmic reticulum, lysosomes, membrane bound bodies type one (MBB-1) and type two (MBB-2) using a point-count method [43]. The numerical density (expressed as the number per 500 μm^2 of cytoplasm area of pinealocyte perikaryon) of dense core vesicles (DCV) and multivesicular bodies was also estimated.

Serotonin assay. The frozen left half-parts of the pineal glands were homogenized (on ice-bath) in glass homogenizers containing 24 mg of PCMPS (p-chloromercuriphenylsulphonic acid in form of monosodium salt) and 20 mg of EDTA (ethylenediaminetetraacetate disodium salt) in 2 mL of phosphate buffer (0.2 M, pH 7.8). Two aliquots (0.9 mL) were taken from each homogenate for duplicate assay. Serotonin was extracted from the homogenates as described by Welch and Welch [44]. The serotonin content was determined based on its native fluorescence measured at excitation wavelength 295 nm and emission wavelength 330 nm in a Baird Nova Spectrofluorimeter (England). Blank samples (prepared using 50 µl distilled water instead of pineal gland) and standard solutions of serotonin were run through all steps of the extraction. Intra-assay coefficient of variation was below 6%. The chemicals used in serotonin assay were analytical purity grade. The organic solvents were purchased from Merck (Germany), other chemicals from Sigma (USA).

Melatonin assay. Concentration of melatonin in blood plasma was measured by a direct RIA using R/R/19540-16876 antibody (a gift from JP Ravault, INRA Nouzilly, France) and 2-^{125}I-iodomelatonin (2200Ci/mM, Du Pont NEN, USA) according to the validated procedure [25, 34]. All other chemicals used in the melatonin assay were purchased from Sigma (USA), except for the precipitation reagent B-60 which was from INRA Nouzilly (France). The samples were run altogether in triplicates. Intra-assay coefficient of variation was below 6%.

Statistical analysis. The data were analyzed using a one-way analysis of variance followed by Duncan test. A value of $p \leq 0.05$ was considered as significant.

Results

Pinealocyte ultrastructure

From the qualitative point of view, the ultrastructure of pinealocytes in the pigs slaughtered at four different time-points of the diurnal cycle was similar (Fig. 1) and closely resembled that previously described in immature gilts [19, 22]. However, the quantitative analysis revealed several differences between pinealocytes of the investigated groups of animals (Fig. 2).

The relative volume of mitochondria in pinealocyte cell bodies was significantly higher in the group of pigs slaughtered at 1400 than at 0200 and 0900. The significant difference in the relative volume of mitochondria was also found between the groups of pigs slaughtered at 2100 and 0200. The relative volume of Golgi apparatus was significantly higher at 0900 and 1400 than at 0200. No significant differences were found in the relative volumes of rough endoplasmic reticulum and lysosomes in pinealocyte cell bodies between the investigated groups of gilts.

The total relative volume of MBB in pinealocyte cell bodies did not show significant diurnal changes. However, the relative volume of MBB-1 in perikarya was significantly lower at 1400 and 2100 than at 0900 and the relative volume of MBB-2 was significantly higher at 1400 than at 0900 and 0200.

The numerical density of DCV in pinealocyte perikarya was significantly higher at 0200 than at 1400. The number of DCV did not differ significantly between the groups of pigs slaughtered at other time-points of the diurnal cycle.

The mean numerical density of multivesicular bodies in pinealocyte perikarya was approx. four times lower in the group of the pigs slaughtered at 0200 than in three other groups of animals, but these differences were not significant.

Pineal serotonin content

The mean serotonin content in the pineal gland ranged from 36.43 ng/mg of wet tissue in the pigs slaughtered at 0200 through 39.98 and 66.60 ng/mg of wet tissue in animals killed respectively at 0900 and at 2100 to 93.42 ng/mg of wet tissue in the gilts slaughtered at 1400 (Fig. 3). The significant differences were found between the groups of pigs slaughtered at 1400 vs. 2100, 0200 and 0900 as well as at 2100 vs. 0200 and 0900. There were no significant differences in the pineal serotonin concentration between the groups of pigs killed at 0200 and 0900.

Plasma melatonin concentration

The mean plasma melatonin concentration was significantly higher at 0200 than at 0900, 1400, and 2100 (Fig. 4). No significant differences in plasma melatonin level were found between the groups of pigs slaughtered at 0900, 1400 and 2100.

Discussion

The obtained results demonstrated the presence of robust diurnal rhythms of the plasma melatonin concentration as well as the pineal serotonin content in gilts kept under a cycle of 12 h light : 12 h dark and exposed to direct sunlight during photophase.

The plasma melatonin concentration was approx. five times higher in the middle of the night than in the

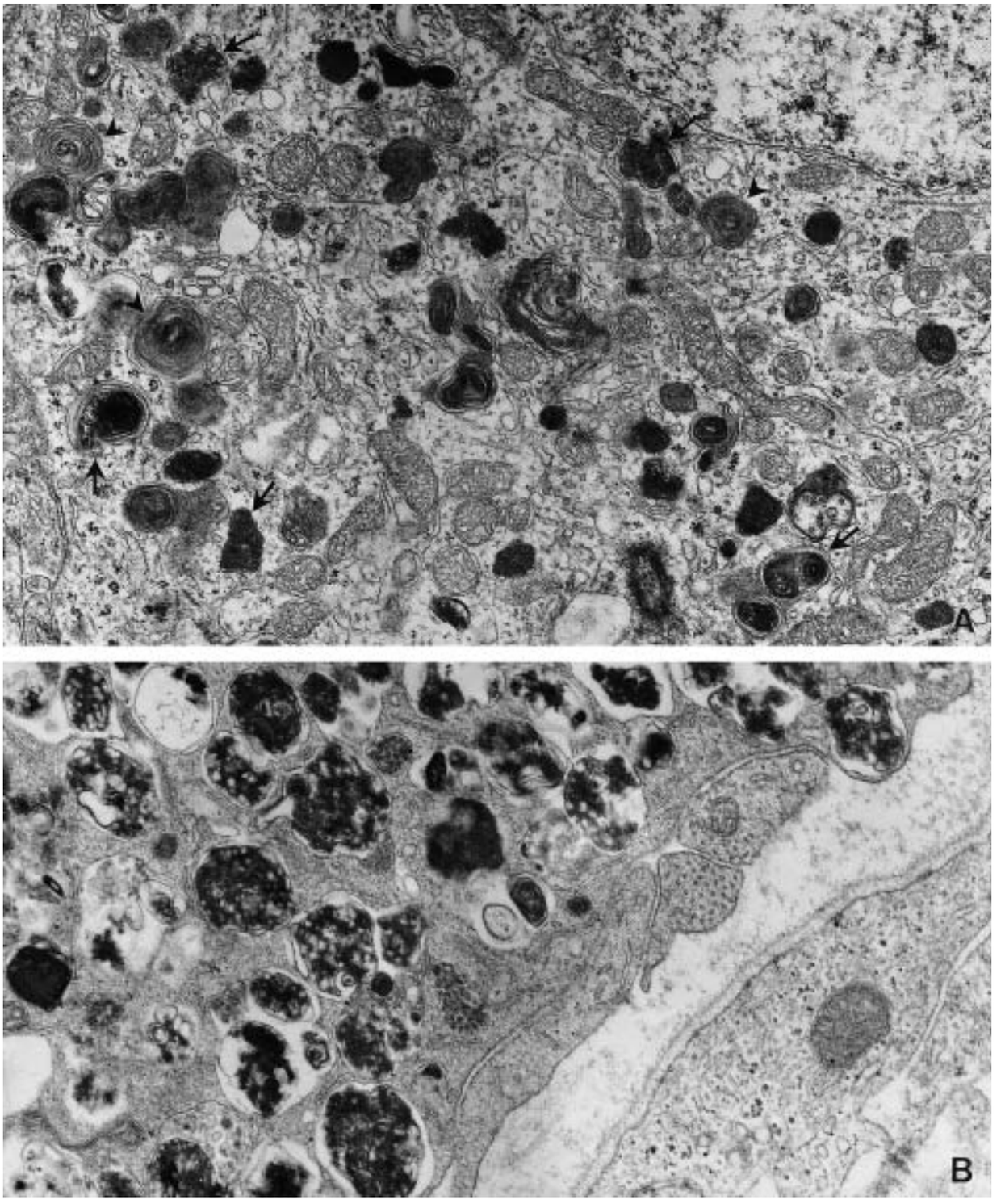

Fig. 1. A. Part of pinealocyte perikaryon with dense bodies, type MBB-1 (arrows) and MBB-2 (arrowheads). The pineal gland of the pig slaughtered at 0200. × 20 000. **B.** Part of ending of pinealocyte process showing numerous dense bodies, type MBB-1. The pineal gland of the pig slaughtered at 0200. × 25 000.

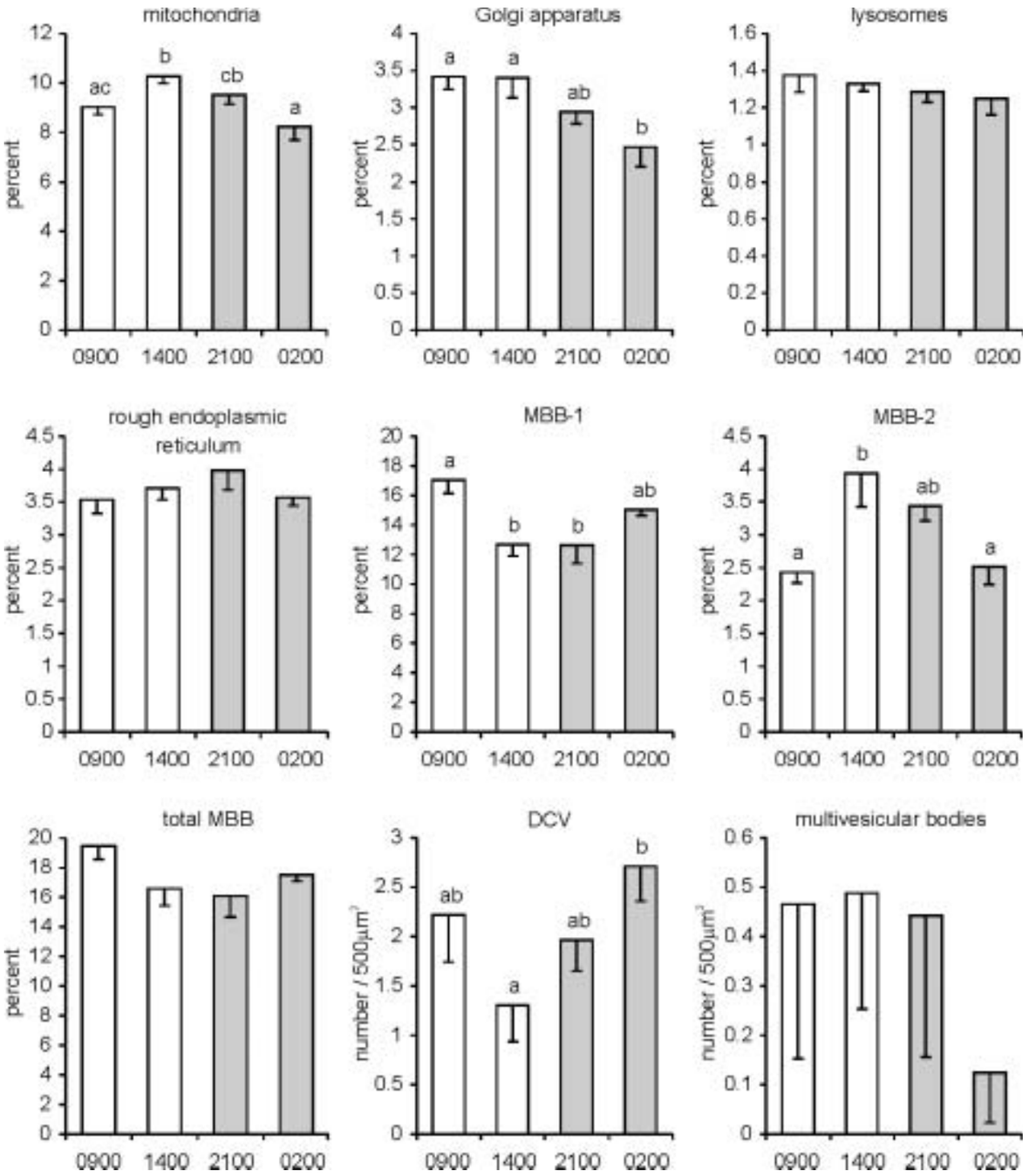

Fig. 2. The mean (±SEM) relative volume of mitochondria, Golgi apparatus, rough endoplasmic reticulum, lysosomes, MBB-1 , MBB-2 and total MBB as well as the mean numerical density of DCV and multivesicular bodies in pinealocyte cell bodies of pigs slaughtered at 0900, 1400, 2100 and 0200. The values signed with different letters are significantly different from each other at $p \leq 0.05$.

middle of the day. The obtained results confirmed the presence of diurnal rhythm of the plasma melatonin level in pigs kept under equatorial lengths of photophase and scotophase reported previously by several authors [26, 40] including us [20]. However, it should also be noted that some studies have concluded that the domestic pig does not exhibit diurnal melatonin patterns typical for other mammalian species [4, 5, 7, 8], even when pigs are kept under a cycle of 11.5 h light : 12.5 h dark and exposed to direct sunlight [8]. The sources of this dis-

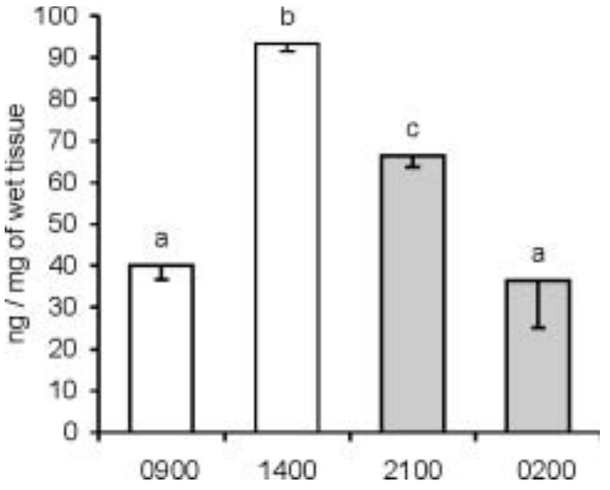

Fig. 3. The mean (±SEM) content of serotonin (ng/mg of wet tissue) in the pineal glands of pigs slaughtered at 0900, 1400, 2100 and 0200. The values signed with different letters are significantly different from each other at $p \leq 0.05$.

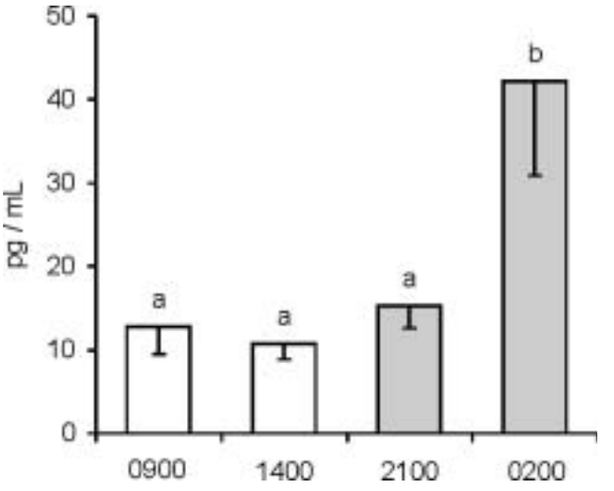

Fig. 4. The mean (±SEM) concentration of melatonin (pg/ml) in blood plasma of pigs slaughtered at 0900, 1400, 2100 and 0200. The values signed with different letters are significantly different from each other at $p \leq 0.05$.

crepancy remain still unknown, although several possibilities have been taken into consideration, experimentally tested and widely discussed [4, 8, 11, 19, 21, 30, 40, 41].

The present results show also a prominent diurnal rhythm of the pineal serotonin content in the domestic pig. The serotonin level was the highest in the middle of light phase. One hour after the onset of darkness, the pineal serotonin content was significantly lower than at 1400. The lowest levels of this amine in the pineal gland were found in the middle of the dark phase and one hour after the onset of light phase. Wyrzykowski *et al.* [46] measured the pineal serotonin content at three time-points during daytime: 0700, 1200 and 1900 in 8-9-month-old gilts kept under two different lighting regiments. The serotonin content in the pineal gland was markedly higher at noon (1200) than in the morning (0700) and in the evening (1900) in pigs kept in natural lighting conditions (June - the length of day about 16 hours). In animals kept in a dark room with limited artificial illumination (light twice a day: from 0600 to 0700 and from 1400 to 1530), the highest amount of serotonin in pineal gland was noted in the natural morning (0700), a bit lower at noon (1200) and nearly twice as low (in comparison with the morning and noon) in the evening (1900).

Up till now, the presence of a 24-hour rhythm of serotonin level in the mammalian pineal gland has been shown only in rodents: the rat [33, 38], the Syrian hamster [37] and the cotton rat [24]. In each of these species, the pineal serotonin level increases gradually from the nocturnal minimum to the midday maximum and then remains at high level until the onset of darkness when it drops. The amplitude between the daytime maximum and the nighttime minimum differs between the examined species.

An interesting feature of the pig pineal gland is an extraordinarily high amount of serotonin comparing to the other investigated mammalian species, reported at first by Sellei and Fruchiger [36] and more recently by Wyrzykowski *et al.* [47]. The latter group examined serotonin content in subcellular fractions and found mainly cytosolic localization of this indoleamine in the pig pineal. The present results also demonstrate a relatively high content of serotonin in the pig pineal gland what confirms the previously reported data.

Summing up, the results of studies on plasma melatonin and pineal serotonin levels let us conclude that the secretory activity of the pineal gland shows obvious diurnal changes in the investigated pigs. These data provide us with a good motivation for a detailed study on diurnal changes in pinealocyte ultrastructure.

The changes in pinealocyte ultrastructure occurring in the course of light-dark cycle were not as prominent as in a case of the investigated biochemical parameters. However, the quantitative studies revealed significant differences in the relative volumes of mitochondria, Golgi apparatus, MBB-1 and MBB-2 as well as in the numerical density of DCV between pinealocytes of gilts killed at different time-points of photophase and scotophase. No significant diurnal changes were found in the other investigated structures: lysosomes, rough endoplasmic reticulum and multivesicular bodies.

The relative volume of mitochondria in pinealocytes of the investigated pigs increased significantly between 0900 and 1400, remained at high level up to 2100 and

then decreased during night to the lowest value noted at 0200. The present results are rather surprising, because the opposite diurnal rhythms of the relative volume of mitochondria, with the peaks at night, have been described in the majority of investigated rodents - the rat, the Syrian hamster and the gerbil (see below). The explanation of this discrepancy is difficult. On the other hand, the present results correspond very well with the data obtained in the organ culture [23] that showed an increase in the relative volume of mitochondria in pig pinealocytes cultured in medium without norepinephrine and a decrease in response to the incubation in the presence of norepinephrine (*in vivo* norepinephrine is released in the pineal at night). In contrast to the *in vitro* study, the treatment of gilts with sympathicolytic and sympathicomimetic drugs during four consecutive days did not influence significantly the relative volume of mitochondria in animals slaughtered at midnight [19]. The decrease in the relative volume of mitochondria in pinealocytes was noted in pigs kept in continuous illumination during two weeks and the increase - in animals kept in continuous darkness during two weeks [22].

The relative volume of Golgi apparatus was significantly lower in pinealocytes of pigs killed at 0200 than during the day at 0900 and 1400. Like in the case of mitochondria, the present results are in agreement with our *in vitro* studies, showing a decrease in the relative volume of Golgi apparatus in response to the treatment of pig pinealocytes with norepinephrine [23]. The relative volume of Golgi apparatus in pig pinealocytes was not affected by the administration of sympathicolytic and sympathicomimetic drugs [19] or by the exposition of gilts to continuous darkness and continuous illumination [22]. In the rodents investigated up till now, except for the golden hamster, the relative volume of Golgi apparatus did not show significant diurnal fluctuations [9, 13, 15, 39, 45]. It should be underlined that according to the commonly accepted model, the secretion of melatonin occurs without participation of Golgi apparatus.

It is generally believed that Golgi apparatus is involved in formation of DCV in mammalian pinealocytes [12]. In our study, the numerical density of DCV in perikarya of pig pinealocytes was significantly higher in the middle of the night than in the middle of the day. The diurnal rhythm in the number of DCV in pinealocytes was described in several species [2, 9, 13, 15, 27, 35, 39, 45]. In rabbit pinealocytes, the number of DCV near Golgi apparatus was the highest at 1200 and the lowest at 2400 [35]. In contrast, the number of DCV in endings of pinealocyte processes was the highest at the night-onset [35]. The numerical changes in DCV were found to be particularly pronounced in mouse pinealocytes, where the late-photoperiod maximum was more than four-fold higher than the late-scotophase minimum [2]. In the majority of other studies performed on the rodents, the highest number of DCV was observed during the light phase or at light-dark transition (see below).

It is well established that the number of DCV in pinealocytes of mammals [2, 12] including the domestic pig [19, 22] is controlled by norepinephrine and strongly depends on the environmental light conditions. The increase in the numerical density of DCV in pig pinealocytes was noted after the administration of clorgyline (MAO-inhibitor), the exposition of animals to continuous darkness and the treatment with norepinephrine in the organ culture [19, 22, 23]. The present data confirm the lack of any close relationship between the dimensions of Golgi apparatus and the number of DCV in pig pinealocytes, previously found in the *in vitro* study [23].

The obtained results demonstrated significant diurnal changes in the relative volumes of MBB-1 and MBB-2 in pinealocyte cell bodies. The abundance of these bodies is a unique structural feature of the pig pineal [47]. The relative volume of MBB-1 in perikarya decreased during the first part of the photophase and it was significantly lower at 1400 and 2100 than at 0900. In contrast, the relative volume of MBB-2 increased significantly between 0900 and 1400. As a consequence of the opposite changes in MBB-1 and MBB-2, the total relative volume of dense bodies in pinealocyte perikarya did not vary significantly in the course of the diurnal rhythm. Our previous studies have undoubtedly demonstrated that the number of MBB in pinealocyte cell bodies is controlled by light [22] and the adrenergic innervation [19]. The opposite changes in the total relative volume of MBB in pig pinealocytes were found after the exposition to continuous light and continuous darkness for two weeks [22] as well as after the repeated treatment with sympathicolytic and sympathicomimetic drugs [19]. The exposition to continuous illumination and the administration of α-methyl-p-tyrosine (an inhibitor of norepinephrine synthesis) or propranolol (a β-antagonist) resulted in the increase in the total relative volume of MBB [19, 22]. Moreover, the recently performed *in vitro* studies demonstrated that the treatment with β-agonists during five consecutive days resulted in decreased relative volume of MBB in pinealocyte cell bodies [32].

The current state of our knowledge about the ultrastructure of mammalian pinealocytes over a 24 h period comes almost exclusively from the studies performed on the rodents: the rat [15, 16, 17, 27], the golden hamster [9], the Syrian hamster [39], the chipmunk [13] and the gerbil [45].

In the most frequently investigated rat pinealocytes, Lew *et al.* [17] reported the peak in dimensions of nucleus in the middle of the light period. At the same time, the presence of deep nuclear indentations and close association of mitochondria with nuclear folds were observed [17]. According to the same authors, the volume of nucleolus was the highest one hour after light

onset [16]. In contrast, Karasek *et al.* [15] found that the sizes of nuclei and nucleoli in rat pinealocytes were higher during scotophase than during photophase. Among the cytoplasmic substructures of rat pinealocytes, the cross-sectional areas of mitochondria and rough endoplasmic reticulum were higher at night than during daytime, whereas the areas of lysosomes, Golgi apparatus and vacuoles containing flocculent material show no diurnal changes [15]. Inconsistent data were obtained in the case of DCV [15, 27]. McNulty *et al.* [27] observed a peak in the number of DCV at night, while Karasek *et al.* [15] found higher number of DCV during the day than during the night. The discrepancies reported in diurnal changes of the nuclear dimensions and the number of DCV in rat pinealocytes may have their sources in differences in light regimes and measurement techniques used in the studies.

The analysis of morphological changes occurring during the diurnal rhythm in pinealocytes of the golden hamster showed the peak values for nucleus, nucleolus, rough and smooth endoplasmic reticulum, Golgi apparatus, dense bodies and DCV at the light:dark interface [9]. In Syrian hamsters, the relative volumes of mitochondria, lysosomes, rough endoplasmic reticulum, vacuoles containing flocculent material and lipid droplets in pinealocyte perikarya were higher at night than during the day [39]. In contrast, the number of DCV in the pinealocyte cell bodies and in the endings of processes was higher during daytime than at night [39]. In gerbil pinealocytes, the diurnal changes were reported in mitochondria, smooth and rough endoplasmic reticulum, DCV and microtubules [45]. Microtubules and DCV reached the peak one hour before light-off, while mitochondria and both forms of endoplasmic reticulum - one hour after light-off.

A prominent number of morphological studies on diurnal variations in mammalian pinealocytes concerned synaptic ribbons which generally showed marked changes in the course of day-night rhythm and in response to modifications of illumination conditions [1, 6, 14, 27, 42]. However, these structures are very rarely observed in pig pinealocytes and for this reason they were not taken into consideration in the present study.

The comparison of the results obtained in the domestic pig and in the other investigated mammals demonstrates large interspecies differences in the diurnal changes occurring in the pinealocyte ultrastructure. They include both the variability of subcellular components showing rhythmic changes in the course of light-dark cycle as well as in the time-points when the maximum and minimum values occur. These interspecies differences probably reflect a considerable heterogeneity in the pinealocyte ultrastructure [3] and in the innervation of the pineal gland [28, 29, 31] as well as in the mechanisms governing melatonin secretion in mammals [10, 18]. The functional significance of the variability in the subcellular organization of mammalian pinealocytes and in the morphological manifestation of diurnal changes occurring in these cells remains completely unknown.

Acknowledgements: The authors wish to express their deep thanks to Dr. J.P. Ravault (INRA, Nouzilly, France) for melatonin antibodies and to Dr. Henryk Zieliński (Polish Academy of Sciences, Olsztyn) for his kind help in serotonin assay. Thanks are also due to Krystyna Targońska, Anna Koncicka and Jacek Sztorc for their skillful technical assistance.

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Accepted March 4, 2004