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Original article

Regulation of melatonin secretion in the pineal organ of the domestic duck – an *in vitro* study

M. Prusik, B. Lewczuk, N. Ziółkowska, B. Przybylska-Gornowicz

Department of Histology and Embryology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowskiego 13, 10-719 Olsztyn, Poland

Abstract

The aim of study was to determine the mechanisms regulating melatonin secretion in the pineal organs of 1-day-old and 9-month-old domestic ducks. The pineals were cultured in a superfusion system under different light conditions. Additionally, some explants were treated with norepinephrine.

The pineal glands of 1-day-old ducks released melatonin in a well-entrained, regular rhythm during incubation under a 12 hrs light : 12 hrs dark cycle and adjusted their secretory activity to a reversed 12 hrs dark : 12 hrs light cycle within 2 days. In contrast, the diurnal changes in melatonin secretion from the pineals of 9-month-old ducks were largely irregular and the adaptation to a reversed cycle lasted 3 days. The pineal organs of nestling and adult ducks incubated in a continuous light or darkness secreted melatonin in a circadian rhythm. The treatment with norepinephrine during photophases of a light-dark cycle resulted in: 1) a precise adjustment of melatonin secretion rhythm to the presence of this catecholamine in the culture medium, 2) a very high amplitude of the rhythm, 3) a rapid adaptation of the pineal secretory activity to a reversed light-dark cycle. The effects of norepinephrine were similar in the pineal organs of nestlings and adults.

In conclusion, melatonin secretion in the duck pineal organ is controlled by three main mechanisms: the direct photoreception, the endogenous generator and the noradrenergic transmission. The efficiency of intra-pineal, photosensitivity-based regulatory mechanism is markedly lower in adult than in nestling individuals.

Key words: pineal gland, avian pinealocytes, melatonin, diurnal rhythm, circadian rhythm, norepinephrine, duck

Introduction

The pineal organ via its hormone – melatonin (MLT) – is involved in the control of daily and seasonal rhythms of many physiological processes and behavioral activities, and therefore it participates in adaptation to changing environmental conditions. The morphological organization of the pineal organ differs extremely between vertebrate classes and even within the class, especially in reptiles and birds (Vollrath

Correspondence to: M. Prusik, e-mail: mprusik@gmail.com, tel.: +48 89 523 39 49

1981, Przybylska-Gornowicz et al. 2005, 2012, Prusik and Lewczuk 2008a, Lewczuk and Przybylska-Gornowicz 2013). Likewise, the mechanisms regulating MLT biosynthesis and simultaneously the output of this lipophilic hormone from parenchymal cells vary significantly among species (Maronde and Stehle 2007, Prusik and Lewczuk 2008b, Lewczuk et al. 2014b, McStay et al. 2014).

The avian pineal organ shows enormous structural diversity. It represents several morphological types – from the saccular type through the tubulo-follicular form to the solid-follicular one (Boya and Calvo 1978, Vollrath 1981, Ohshima and Hiramatsu 1993, Prusik 2005, Przybylska-Gornowicz et al. 2005, Prusik et al. 2006, Prusik and Lewczuk 2008a). These types differ not only in the architecture and cellular organization, but also in the afferent and efferent innervation. Moreover, the structure and innervation of the avian pineal organ undergo significant changes during postembryonic life as it has been described in the chicken, Japanese quail and turkey (Boya and Calvo 1978, 1979, 1980, Ohshima and Matsuo 1984, Sato and Wake 1984, Ohshima and Hiramatsu 1993, Przybylska-Gornowicz et al. 2005). It is reasonable to expect that such a great variability in the pineal morphology reflects in significant differences in the processes controlling MLT secretion.

The mechanisms regulating MLT synthesis in the avian pineal gland have been studied in a few species, mainly in the chicken, Japanese quail and turkey (Murakami et al. 1994, Prusik 2005, Prusik and Lewczuk 2008b, Piesiewicz et al. 2012, 2015). The pineal glands or isolated pinealocytes of these species cultured under a light-dark cycle secrete MLT in a diurnal rhythm, more or less adjusted to the changes in photoperiod, and incubated in a continuous darkness or a continuous light generate a circadian rhythm of MLT secretion (Okano and Fukada 2003, Csernus et al. 2005, Zeman and Herichová 2011). The interspecies differences concern both the ability of pineal cells *in vitro* to entrain their secretory activity to rapid changes in light conditions and the persistence of the endogenous oscillator activity in a continuous darkness. It has been demonstrated that also norepinephrine (NE) plays an important role in the regulation of MLT secretion in chicken and turkey pinealocytes (Cassone et al. 1990, Prusik 2005, Zawilska et al. 2005, Prusik and Lewczuk 2008b). It is generally accepted that NE in birds, like in mammals, is the final neurotransmitter in the multisynaptic pathway connecting the retina and the hypothalamus with the pineal organ. NE inhibits MLT secretion in avian pinealocytes acting via α_2-adrenoceptors (Cassone et al. 1990, Prusik and Lewczuk 2008b). It is worth to note that the regulation of MLT secretion was studied almost exclusively on young, several-week-old specimens and there are no comparative studies dealing with this subject performed at different stages of post-embryonic life.

In view of the age- and species-dependent morphological variability of the avian pineal organs and the analysis of above-presented investigations on regulation of MLT biosynthesis in birds, we decided to study the mechanisms controlling pineal hormone secretion in the domestic duck. The presence of the diurnal rhythm in content of MLT and 5-methoxytryptophol in the pineal organ and plasma of 2-week-old ducks was initially reported by Zawilska and co-workers (2002). More recently, the daily profiles of ten MLT-synthesis related indoles were characterized in the pineal organs of 14-week-old ducks (Lewczuk et al. 2014a). The aim of the present study was to determine the role of direct photoreception, endogenous oscillator and adrenergic stimulation in the regulation of MLT secretion in pinealocytes of 1-day-old and 9-month-old individuals of the domestic duck. Our study is the first one analyzing the differences in mechanisms controlling MLT secretion between nestlings and adult birds.

Materials and Methods

Chemicals

Medium 199 containing Earle's salt and HEPES (Sigma, USA) was prepared from a powdered form (2.2 g/L $NaHCO_3$, pH=7.3). Antibiotic-Antimycotic Solution (Sigma, USA) was added to the medium immediately before use to provide the final concentrations of antibiotics as follows: penicillin – 100 IU/ml, streptomycin – 100 µg/ml and amphotericin B – 0.25 µg/ml.

Anti-melatonin antibody Prospect 6C was kindly provided by Dr Andrew Foldes, Agriculture Research Western Australia, Australia. ^{3}H-melatonin was purchased from PerkinElmer (USA), gelatin – from Merck (Germany) and all other reagents used in melatonin radioimmunoassay – from Sigma (USA).

Animals and the pineal glands

All experimental procedures on animals were performed in accordance with Polish and EU law. They were approved by Local Ethics Committee for Experiments on Animals in Olsztyn.

The pineal organs were obtained from 1-day- and 9-month-old individuals of the domestic duck (*Anas platyrhynchos f. domestica)*. The nestlings were used in

Table 1. Protocols of experiments performed in superfusion culture of duck pineals.

Experiments		Days					
I	II	1	2	3	4	5	6
group I	group I	12L:12D					0L:24D
group II	group II	12D:12L					0L:24D
group III	group III	12L+ NE:12D					0L:24D
group IV	group IV	12D:12L+ NE					0L:24D
group V	–	12L:12D			0L:24D		
group VI	–	12L:12D			24L:0D		
–	group V	0L:24D					
–	group VI	24L:0D					

the experiments immediately after delivery from a hatchery. The adult ducks (females) were kept under a cycle of 12-hour-long photophase and 12-hour-long scotophase, starting from the 2nd week of the postembryonic life. During the photophase (from 07.00 to 19.00) full-spectrum fluorescent lamps provided light with an intensity of 300 lx at the floor level. During the scotophase the ducks were kept in a complete darkness. The animals had free access to standard food and water.

The nestlings and 9-month-old ducks were killed (by decapitation under isoflurane anesthesia) between 11.00 and 12.00, their pineal glands were immediately removed and placed in separate superfusion chambers.

Superfusion culture

The pineal glands were covered with a nylon mesh and placed into the culture chambers (volume 0.5 ml). The lower pool of each chamber was connected via a system of tubes and valves to the containers with culture media. The upper pool of the culture chamber was attached to a multichannel peristaltic pump (Cole Parmer, USA) and a manual fraction collector. The total volume of the superfusion set consisting of culture chamber, tubes and valves was 1.3-1.4 ml. The superfusion was performed at a flow rate of 0.1 ml/min. The medium was continuously gassed with a mixture of 95% O_2 and 5% CO_2. The incubation was performed at 38.5°C in a water bath (Julabo, Germany). The culture chambers were covered with translucent plastic sheets during the incubation in light and with similar light-proof sheets during the incubation in darkness. The chambers were illuminated with a full-spectrum fluorescent lamp providing light with intensity of 100 lx at the surface of sheets covering the perfusion chambers. The medium fractions were collected every 30 minutes during consecutive days designated as the day 1, 2, 3, 4, 5 and 6, and frozen at -20°C until melatonin assay. The time-point 07.00 was taken as the beginning of day 2 and following ones.

Experimental procedures

Experiment I

The experiment was performed on six groups of the pineal organs of 1-day-old ducks (4 glands per each group) cultured under different light conditions for a period of six consecutive days (Table 1).

The pineal organs of the group I were incubated under a light-dark cycle with a photophase from 07.00 to 19.00 during the first five days (12L:12D). At the same time, the explants of the group II were incubated under a reversed dark-light cycle with a photophase from 19.00 to 07.00 (12D:12L). On the last day of the experiment both groups of the pineals were incubated in a continuous darkness (0L:24D).

The explants of the group III were incubated under a 12L:12D cycle and treated with 10 μM of NE during a photophase (from 07.00 to 19.00). The pineals of the group IV were incubated under a 12D:12L cycle and treated with 10 μM of NE between 19.00 and 07.00. Both groups were incubated in the above mentioned conditions during the first five days and in a continuous darkness on the last day of the experiment.

The pineal organs of the group V and VI were incubated under a 12L:12D cycle with photophase from 07.00 to 19.00 during the first three days, and then under a continuous darkness (group V) or a continuous illumination (24L:0D, group VI).

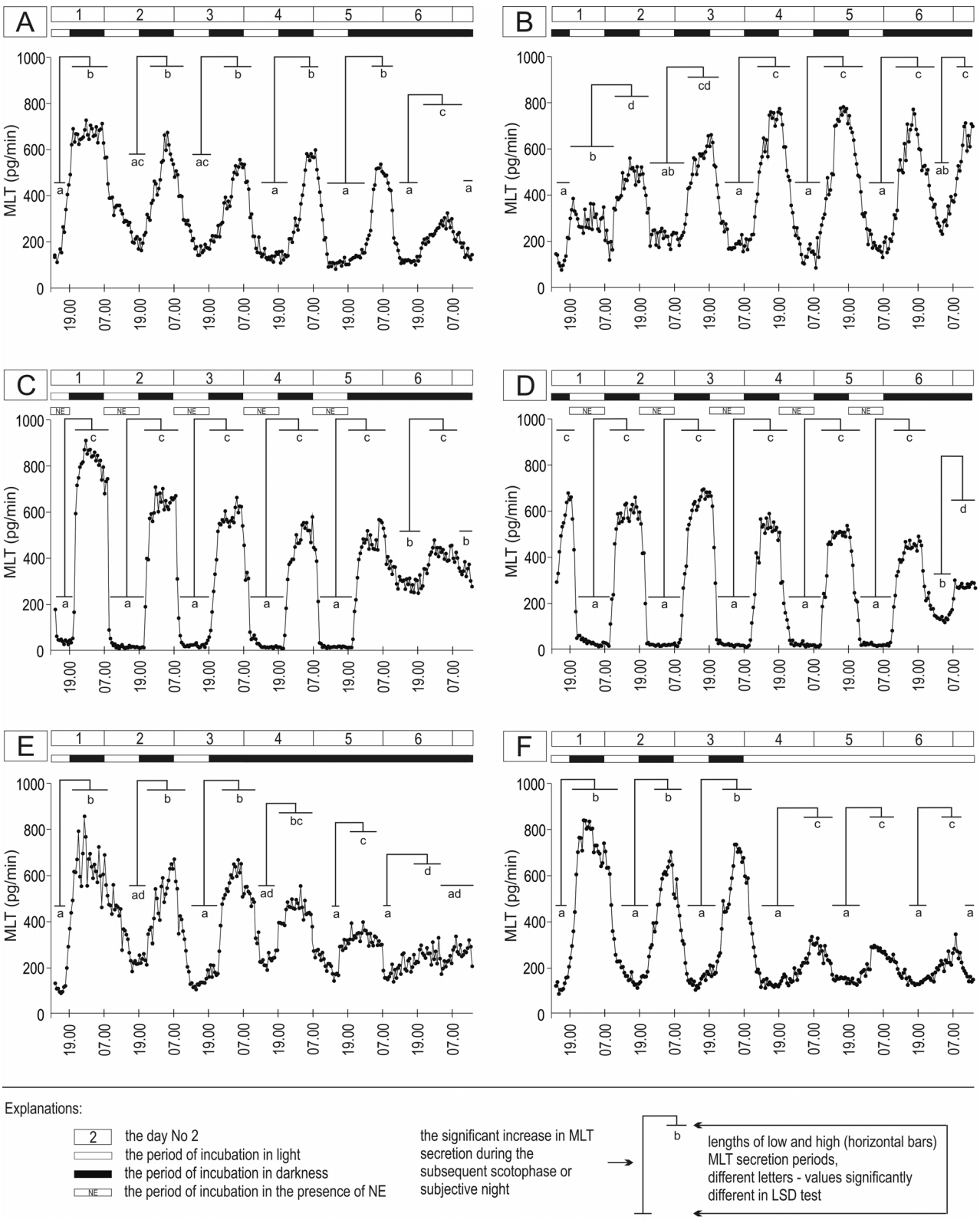

Fig. 1. Experiment I. Secretion of MLT from the pineal organs of nestling ducks (means, n=4). A – Group I, B – Group II, C – Group III, D – Group IV, E – Group V and F – Group VI. The experimental procedure is presented in Table 1.

Experiment II

The chambers with the pineal organs of 9-month-old ducks were randomly assigned to one of six groups (4 glands per group). The pineals of the first four groups (I – IV) were incubated in the same culture conditions like the pineals of nestlings described above (Table 1). The explants of the group V were incubated under a continuous darkness and the pineal of the group VI – under a continuous illumination during the whole experiment.

Melatonin radioimmunoassay

Melatonin concentration in the medium samples was measured by a direct RIA with the use of Prospect 6C antiserum (Paterson et al. 1992) and ^{3}H-melatonin according to the previously described and validated procedure (Fraser et al. 1983, Prusik 2005).

Statistical analysis

The data were analyzed using a repeated measures analysis of variance followed by LSD test using Statistica 10.0 (StatSoft, USA) and SPSS 21.0 (IBM, USA) software. A value of $p \leq 0.05$ was considered as significant.

Results

Experiment 1

The pineal organs of the groups I, V and VI secreted MLT in a diurnal rhythm when they were incubated under a 12L:12D cycle (Fig. 1A, E, F). The time-courses of daily changes in MLT release differed between the first and following days of the culture. During the first day of experiment, MLT secretion increased quickly after the onset of dark phase and reached the maximum within 2-3 hours. Then, it remained at a stable, elevated level through the period of incubation in a darkness. On the second and following days of the incubation under a 12L:12D cycle, MLT secretion increased gradually during the scotophase to reach a peak late in the night between 04.00 and 07.00. The step-wise decrease in MLT secretion started just before or immediately after the onset of photophase. The minimal level of secretion was observed between 14.00 and 17.00 and it was about 2.5-fold lower than the maximum.

The rhythmical secretion of MLT was maintained after the start of incubation in a continuous darkness (groups I and V) or in a continuous light (group VI). The level of MLT release from the pineal organs of the group V showed statistically significant, circadian changes during three days of the culture in a continuous darkness, despite the decrease in the peak high noted on the consecutive days (Fig. 1E). The time-advance characterized the occurrence of successive peaks. The significant changes in MLT secretion, with rises at natural nights, were also noted during three days of the incubation in a continuous light (Fig. 1F). In contrast to the group V, the maximum level of secretion and the time of peak occurrence did not differ between day 4, 5 and 6 of the experiment in the group VI.

The explants of the group II, incubated during the first five days under a 12D:12L cycle, released MLT in a reversed rhythm, starting from the second day of the experiment (Fig. 1B). The highest levels of secretion were noted in the second half of natural day. The reversed rhythm of MLT release was also observed during the culture in a continuous darkness on the last day of the experiment (Fig. 1B).

The pineals of the groups III and IV, treated with NE during photophase, secreted MLT in the rhythm that was tightly adjusted to the changes in illumination and the presence of NE in medium (Fig. 1C, D). In the case of a 12L:12D cycle (the group III) MLT secretion increased rapidly between 19.00 and 21.00, remained at the constant, high level during the night and then quickly decreased between 07.00 and 08.30 (Fig. 1C). The level of secretion was 15-20-folds higher at night than during daytime. The secretory activity of the pineals of the group IV was adapted to the reversed light-cycle already on the first day of incubation (Fig. 1D). In both groups of explants, the spontaneous decrease in MLT secretion during the subjective day and the spontaneous increase in MLT secretion during the subjective night were observed on the last day of the experiment, when the explants were incubated in a continuous darkness and without exposition to NE (Fig. 1C, D).

Experiment 2

The secretion of MLT showed significant day-night differences in the group I during the whole period of incubation under a 12L:12D cycle, however these variations were largely irregular as concerning their repeatability on the consecutive days (Fig. 2A). On the first day of the experiment, the mean secretion level raised about 1.5-fold during the scotophase and then largely and rapidly decreased at the beginning of the subsequent photophase. During the following four

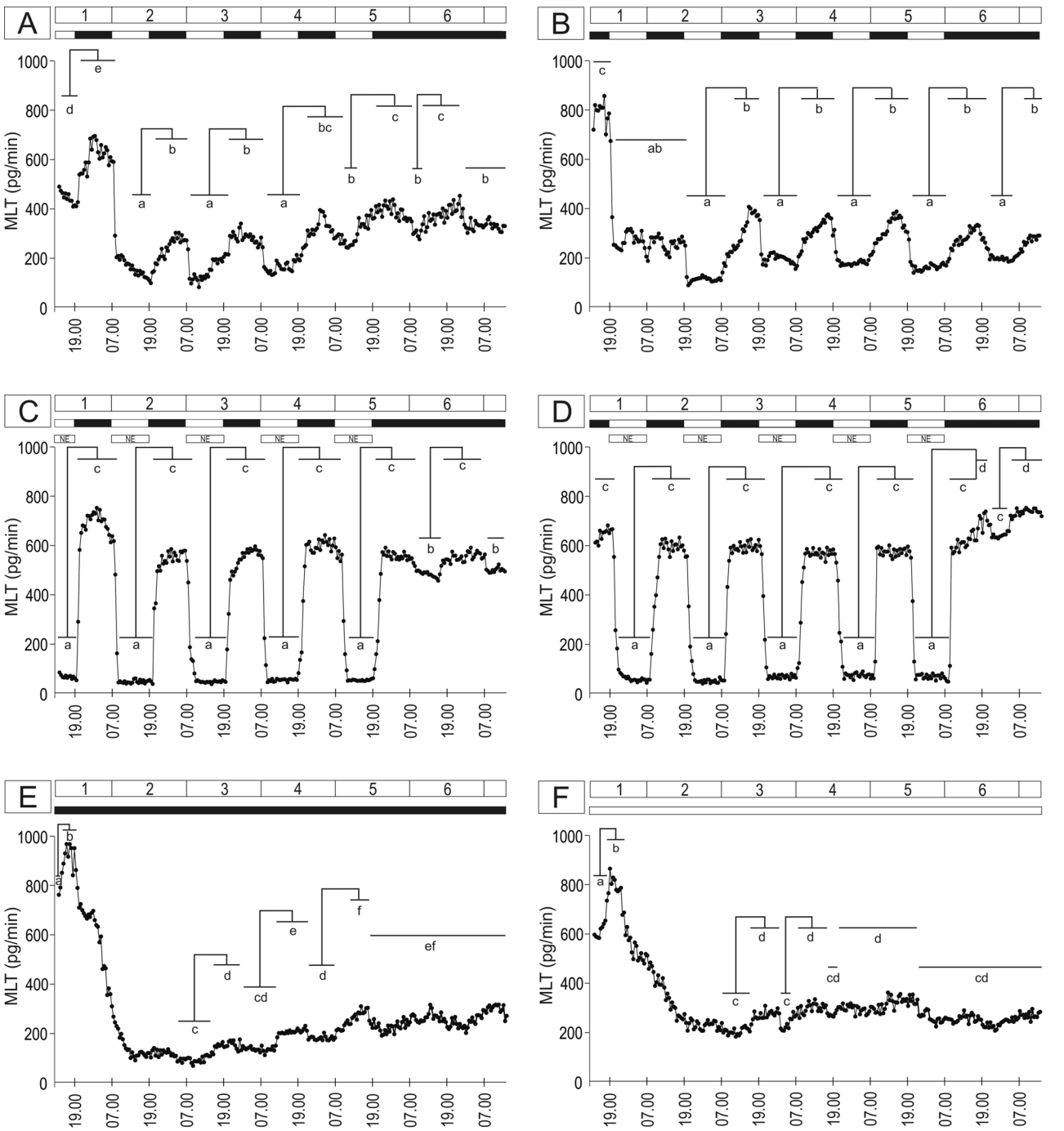

Fig. 2. Experiment II. Secretion of MLT from the pineal organs of adult ducks (means, n=4). A – Group I, B – Group II, C – Group III, D – Group IV, E – Group V and F – Group VI. The experimental procedure is presented in Tab. 1. Explanations – see Fig. 1

days of incubation, the maximum MLT release, noted at night, was about 2-3-fold higher than the minimum one. The peak of secretion occurred earlier on the day 5 than on the days 2, 3 and 4. Simultaneously, the minimum level of MLT release was higher on the day 5 than on the days 2, 3 and 4. The spontaneous increase in MLT secretion was observed during the last day of the experiment, when explants were incubated in a continuous darkness.

In the group II, the secretion of MLT prominently decreased at the ends of scotophases on the first and second days of the experiment (Fig. 2B). No differences in the level of MLT release were noted between the photophase of day 1 and the subsequent scotophase of day 2. The reversed rhythm of MLT secretion, with the peaks at the ends of scotophases, was noted starting from the third day of the experiment. On the last day of the experiment, the release

of MLT increased significantly during the natural day.

The pineal organs of the group III, treated with NE during daytime, released MLT in a cyclic manner with an amplitude extending 1000% (Fig. 2C). The shifts between the periods of low and high secretion of MLT were very fast and well-entrained to changes in the presence of NE in the culture medium. After the start of incubation in a continuous darkness, MLT secretion remained at a high level, however a circadian oscillation with a small amplitude and a peak occurring during the natural night was observed.

The presence of NE in the culture medium during the photophase enabled the adaptation of secretory activity of the explants of the group IV to a reversed light cycle already on the first day of the experiment (Fig. 2D). Like in the group III, the changes in MLT secretion were highly regular. During the last phase of experiment (day 6), when the pineals were incubated in a continuous darkness, MLT release was very high, but a small spontaneous increase in the secretion level was noted between 04.00 and 14.00.

During the first day of the culture in a continuous darkness (group V) or a continuous light (group VI), the level of MLT secretion initially increased by about 150%, and then largely decreased (Fig. 2E, F). No significant rises in the release of hormone were found on the day 2 in both groups of explants. The MLT secretion showed small, but statistically significant increases during the natural nights on the days 3, 4 and 5 of the incubation in the group V (Fig. 2E) and on the days 3 and 4 in the group VI (Fig. 2F).

Discussion

In our study, the pineal organs of duck nestlings secreted MLT in a diurnal rhythm during the culture under a 12L:12D cycle and in a circadian rhythm, when they were incubated in a continuous darkness. Moreover, the glands efficiently adapted their secretory activity to a reversed light-dark cycle and responded to the adrenergic stimulation. In view of the presented results, the pineal organ of a new-hatched duck has a full suite of enzymes and substrates required for MLT biosynthesis as well as an effective regulatory machinery.

There are no data on the ontogenetic development of the duck pineal gland, either at morphological as well as physiological levels. Up till now, the embryonic development of the avian pineal organ has been investigated exclusively in chick and quail embryos. The release of trace amounts of MLT from the chicken pineal organ *in vitro* was detected as early as on the day 11 of embryonic life, and a large increase in the secretion level was observed during the subsequent days of development (Möller and Möller 1990). The regulation of MLT synthesis by light acting via pineal photoreceptive structures was found for the first time in 13-14-day-old chick embryos (Akasaka et al. 1995, Lamosová et al. 1995, Csernus et al. 2007). It has been demonstrated that the control of MLT secretion by the intrapineal circadian oscillator is switched on slightly later, about day 18 of embryonic life (Akasaka et al. 1995). The daily rhythm of arylalkylamine N-acetyltransferase (AA-NAT) activity was found in the pineal organs of 16-day-old chick embryos (Herichová et al. 2001). As concerning the quail pineal organ, the presence of mature transcripts encoding AA-NAT and MLT receptors: mel-1a, mel-1b and mel-1c has been reported in the pineal glands of 7-11-day-old quail embryos (Obłap and Olszańska 2004). The immunoreactivity of the pineal photopigment, pinopsin, was detected in 8-day-old quail embryos (Yamao et al. 1999).

The results of our study showed differences in the time-course of nocturnal MLT rise between the first and following days of incubation, occurring in the superfusion culture of the pineal organs of one-day-old ducks, performed under a 12L:12D cycle. These differences were not observed in the culture of the pineal organs of adult birds, and therefore they could be related to the developmental processes taking place shortly after hatching. The changes in the expression and activity of the enzymes involved in MLT synthesis pathway, going on during the first two weeks of posthatching life were reported in the chicken pineal gland (Piesiewicz et al. 2012, 2015).

The results of experiments performed in the present study clearly show that MLT synthesis in the domestic duck, like in other avian species investigated up till now, is controlled by three main components: a light received directly by pinealocytes, an endogenous generator and NE.

The direct photosensitivity of avian pinealocytes is possible due to the presence of two photopigments – pinopsin and melanopsin (Yamao et al. 1999, Okano and Fukada 2003, Holthues et al. 2005, Prusik and Lewczuk 2008b). Pinopsin is responsible for the inhibitory action of light on MLT synthesis, while melanopsin is considered to be involved in the entrainment of the endogenous circadian oscillator activity to the environmental light conditions (Holthues et al. 2005). Although, the data concerning photosensitivity of pinealocytes, necessary for interspecies comparative analyses, are largely limited, it could be stated that the role of light directly received by the pineal organs in the regulation of MLT secretion differs significantly between birds. The secretory activity of the turkey pineal organ *in vitro* adjusts extremely

precisely and quickly to the light-dark shifts (Prusik 2005). The regulatory action of light on MLT secretion is much less effective in the chicken pineal organ (Csernus et al. 1998) and – as demonstrated in the present study – in the duck pineal organ.

The obtained results showed prominent differences in a course of the diurnal MLT secretion rhythm between the pineal organs of nestlings and adult individuals of the domestic duck. The pineal glands of 1-day-old ducks secreted MLT in a well-entrained, regular rhythm, while the diurnal changes in MLT secretion from the pineals of 9-month-old ducks were largely irregular. Moreover, the adaptation of secretory activity to a reversed light-dark cycle occurred more slowly in the pineals of adults than in the glands of nestlings. Our data point to important changes in the efficiency of intra-pineal, photosensitivity-based regulatory mechanism occurring during the post-hatching development of the domestic duck.

Up till now, the effect of age on the ability of avian pinealocytes to entrain to a new light-dark-cycle *in vitro* was studied exclusively in the chicken model (Csernus et al. 1998). In superfusion culture of the pineals of 15-week-old chickens, performed under a reversed 12D:12L cycle, the first peak of MLT secretion was noted on the third day of incubation. The glands of 3-week-old birds demonstrated a faster accommodation to the reversed light regime, as MLT secretion peaked on the second day of the experiment. Pineal cells of 13- and 14-day-old chick embryos secreted MLT in a reversed pattern already on the first day of the culture under a 12D:12L cycle (Akasaka et al. 1995). However, it should be stressed that an endogenous oscillator starts to control MLT secretion in the chicken pineal on the day 18 of embryonic development and the lack of a circadian regulation in pinealocytes of 13- and 14-day-old embryos probably creates favorable conditions for quick adaptation of the secretory activity to changes in illumination.

The synthesis of MLT in the avian pineal organ is also controlled by the system of clock genes generating molecular oscillations due to the action of autoregulatory, transcriptional-translational feedback loop (Okano and Fukada 2003, Csernus et al. 2005, Prusik and Lewczuk 2008b, Turkowska et al. 2014). To check the activity of this endogenous generator in the duck pineal gland, the explants were incubated under continuous dark and light conditions. The obtained results show that 1) the pineal organs of one-day-old and adult ducks generate a circadian rhythm of MLT secretion during incubation in a continuous darkness and in a continuous illumination, 2) the information about changes in the phase of a light-dark cycle is acquired and stored by the circadian oscillator of duck pinealocytes. The circadian rhythm of MLT secretion from the investigated explants persists in the absence of a light-dark cycle for at least 2-3 days, therefore it could be concluded that the stability of the pineal circadian oscillator activity in the duck is similar to that in the chicken, turkey and house sparrow and higher than in the Japanese quail (Csernus et al. 1998, Murakami et al. 1994, Prusik et al. 2005).

The role of the sympathetic innervation in the regulation of MLT secretion in the avian pineal gland is still a matter of discussion (Cassone et al. 1990, Barret and Underwood 1992). To determine the significance of the adrenergic signaling in the control of secretory activity of duck pinealocytes, the explants were exposed during the light phases of daily cycles (12L:12D and 12D:12L) to NE. The treatment with NE resulted in: 1) a precise adjustment of MLT secretion rhythm to the presence of this catecholamine in the culture medium, 2) a very high amplitude of the MLT secretion rhythm, 3) a rapid adaptation of the pineal secretory activity to a reversed light-dark cycle, 4) a very fast changes in MLT secretion at the beginning and at the end of the period of adrenergic stimulation. The effects of NE were similar in one-day-old and adult ducks, therefore it seems that NE plays an important role in the regulation of MLT secretion starting (at least) from the first day of posthatching life.

Based on morphological studies, it is generally accepted that the postembryonic development of the avian pineal gland comprises a reduction of the photoreceptive structures and a development of the efferent innervation (Calvo and Boya 1979, Sato and Wake 1984). This idea is also supported by the biochemical data showing the increase in NE content in the chicken pineal organ during the first two months of life as well as the presence of rhythmic changes in the pineal NE content in 30- and 57-day-old chickens and their absence in younger individuals (Zawilska et al. 2005). These data suggest that the significance of the adrenergic regulation of MLT secretion in the chicken pineal organ increases markedly with age. However, other studies have shown that NE is able to decrease the nocturnal MLT production in embryonic chick pineal cells (Lamosová et al. 1995, Macková et al. 1998). Moreover, the sympathetic fibres are present in the quail pineal gland at least 3 days before hatching (Zeman and Herichová 2011).

The highly effective inhibitory action of NE on MLT secretion in the investigated pineal organs suggests that the adrenergic innervation plays a key role in the regulation of MLT secretion in the domestic duck. NE seems to be responsible for a precise adaptation of the secretory activity to the continuously

changing length of photoperiod. It should be stressed that the light stimuli alone failed to ensure a precisely entrained, diurnal rhythm of MLT secretion in the pineal organs of adult ducks. The strong argument supporting this concept has also been provided by our *in vivo* study, which shows the well-entrained, prominent diurnal rhythm of the content of vanillylmandelic acid in the duck pineal organ (Lewczuk et al. 2014a). The level of this NE metabolite was markedly higher during the photophase than during the scotophase, that confirms the more intensive release of NE during day than at night and role of the adrenergic innervation as a source of precise information about the length of photoperiod (Lewczuk et al. 2014a).

The differences in MLT secretion between the pineal organs of nestling and adult ducks concerned also the initial period of superfusion culture. The release of MLT from the glands of adult birds notably dropped at the end of the first day of incubations performed under a 12L:12D cycle or in a continuous darkness. The decline was not noted when the pineal organs of 1-day-old ducks were incubated in the same conditions. This phenomenon was not reported in the experiments performed on the pineal organs of other avian species and its mechanism is unknown. It should be emphasized that the described differences in MLT secretion between the first and following days of incubation were not observed when the glands were incubated in the presence of NE during the photophases.

In conclusion, MLT secretion in the duck pineal organ is controlled by three main mechanisms: the direct photoreception, the endogenous generator and the noradrenergic transmission. The efficiency of the mechanism based on light sensitivity of pinealocytes is markedly lower in adult than in nestling individuals. The activity of circadian generator persists in a continuous darkness and in a continuous light. The oscillator is able to acquire and store the information about the phase of light-dark cycle. NE seems to play a key role in the synchronization of the pineal secretory activity with the environmental light condition in both 1-day-old and 9-month-old birds.

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