The action of the brain factor is independent of that of the hormones released by the endocrine glands. Ecdysterone present in the incubation medium at a concentration of 10^{-6} M does not affect the activation of PSG by the brain factor, nor does 10^{-6} M juvenile hormone II. It is noteworthy that in our system 10^{-6} M juvenile hormone II inhibits the incorporation of [^{3}H]-uridine into RNA of PSG by at least 30% during 3 h incubation in vitro, and this inhibition was abolished by the brain factor. It seems that at least a part of the observed effect of brain implantation reported earlier[3] should be ascribed to a direct, hormone-independent action of brain tissue factor(s). More work is needed on the isolation and structure of brain factor, which may show some similarities to other insect neuropeptides[16–18].

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Development of melatonin rhythm in the pineal gland and eyes of chick embryo

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Abstract. A melatonin rhythm was observed in the pineals of 18-day-old chick embryos incubated under a light-dark regime of 18 : 6 h. A low pineal melatonin content was found during the light phase of the day. Concentrations started to increase 2 h after dark onset and reached maximum levels after 4 h of darkness. The amplitude of the pineal melatonin rhythm increased considerably after 2 days and night-time concentrations in 20-day-old embryos were more than 5 times higher than in 18-day-old ones. Significant day/night differences in melatonin production were found both in pineals and eyes. Exposure of eggs to 1 h of light during the dark period decreased the high melatonin concentrations in the eyes but not in the pineals of the 20-day-old chick embryo. The results suggest that in this precocial bird at least part of the circadian system may already operate during embryonic life.
Key words. Melatonin; circadian rhythm; embryo; ontogeny.

In the mammalian fetus, circadian rhythms are entrained by the rhythmicity of the mother. Maternal melatonin transmits the information on day-length to the fetus[1,2], and this information can influence subsequent body development, especially in photoperiodic species[3,4]. In birds, a direct neuroendocrine signal between mother and developing embryo is naturally excluded. However, through its incubatory behavior the mother could influence the characteristics of endogenously developing rhythms in embryos. As melatonin may be directly involved in the circadian organization of birds[5–7], its possible rhythmic production during embryonic life could be important for the development and entrainment of other behavioral and physiological rhythms.

Melatonin has been found in the pineals of chick embryos[8–10]. Moreover, a circadian rhythm of N-acetyltransferase (NAT), which is responsible for rhythmic melatonin production, was observed in 18-day-old chick embryos[9]. Therefore the aim of the present study was to find out whether pineal melatonin exerts a daily rhythm in chick embryos incubated under a light-dark cycle, and whether melatonin synthesis in the embryonic pineal gland and eye can respond to lighting conditions.

Materials and methods

Broiler breeder hen eggs were incubated in a forced draught incubator (Bios, CSFR) at a temperature of 37.8 + 0.2 °C and a relative humidity of 55–65% under

constant darkness. Eggs were turned automatically every hour. After 7 days of incubation, a lighting regime with 18 h of light and 6 h of darkness per day (LD 18 : 6), with the light off from 14.00 to 20.00 h, was introduced. Lighting was provided by a 28-Watt cool white fluorescent tube (Tesla) that produced illumination in the range of 20–80 lx at the level of the eggs.

During the 18th day of incubation embryos (5/group) were killed at 2-h intervals during the whole of the dark period, and 2 h before and 2 h after the dark period. Pineal glands were rapidly dissected out and placed in closed plastic tubes on dry ice, and stored at −70 °C. Within a week single pineals were homogenized in 0.6 ml of 0.05 M phosphate buffer, pH 7.0. One half of the homogenate was extracted with 1 ml of dichlormethane, and used for melatonin measurement.

In the second experiment, eggs were exposed to LD 16 : 8 (light off from 21.00 to 05.00 h) from the beginning of incubation. All other conditions of incubation were essentially the same as in experiment 1. Pineals were collected during the 20th day of embryonic life. Embryos were in the stage of internal pipping with their beaks in an air chamber of the egg. Pineal glands were collected during the light and dark periods and at the light-to-dark and dark-to-light transitions. For the determination of the melatonin content, pineals were extracted with absolute methanol.

In the third experiment, eggs were incubated in a still air incubator (Jäger Kunstglucke, Germany), with a transparent Plexiglass cover which allowed light to fall on the eggs. Eggs were turned manually 5 times a day from the beginning of incubation, but left without turning from the 16th day of embryonic life. The incubator was placed in a light-controlled room with the light off from 21.00 h to 05.00 h (LD 16 : 8). Cool white fluorescent light was used, with a light intensity of approximately 200 lx at the level of the eggs. Samples were collected at 12.00, 24.00 and after 1 h of light exposure starting at midnight. After the embryos had been decapitated, pineal glands were immediately collected. The eyes were dissected, the vitreous humor and lens were discarded and the retina-pigment epithelia stored on dry ice. Single pineal glands and both eyes were homogenized in 0.3 ml and 3.0 ml of absolute methanol, respectively. The homogenate was centrifuged at 5000 × g for 10 min. The supernatant was collected and dried under nitrogen, and the dry residue redissolved in the assay buffer to the original volume (0.3 or 3.0 ml). Recovery of added ^{3}H melatonin (2000 cpm) was over 92% for both pineals and eyes.

Melatonin was measured by radioimmunoassay[11], which was previously validated for the chicken pineal tissue[9] in 50-µl aliquots of samples. Sheep anti-melatonin serum (G/S/704-6483 Stockground Ltd. Guildford, U.K.) and ^{3}H-labeled melatonin with a higher specific activity of 3.056 TBq/mmol (NEN Du Pont, Bad Homburg, Germany), were also used in 50-µl aliquots. Dextran (0.02%) coated charcoal (0.4%) suspension was applied for separation of bound and free hormone. The limit of sensitivity was 1.5–2.0 pg/tube. The intra- and interassay coefficient of variation for pooled night-time plasma containing 380 pg melatonin per ml were 6.0% and 11.6%, respectively.

Data were analyzed by a one-way analysis of variance, followed by Duncan's multiple range test. The difference between light and dark time values was compared by t-test.

Results

A low amplitude melatonin rhythm was observed in pineals of 18-day-old chick embryos incubated from the 7th day in LD 18 : 6 (fig. 1). Concentrations increased 2 h after the onset of darkness and reached maximum levels after 4 h of darkness. High levels persisted to the end of darkness but decreased to basal level after 2 h of light-

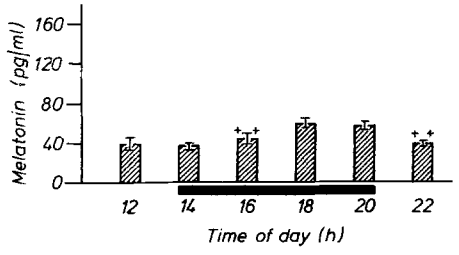

Figure 1. Pineal melatonin content in 18-day-old chick embryos incubated during the first week in constant darkness and then in LD 18 : 6. Columns represent means ± SEM of 5 glands. The dark bar below the abscissa denotes the dark period of the LD cycle. + + : $p < 0.01$ significance of the melatonin increase in comparison to its content at 14 h and the decrease in comparison to its level at 22 h.

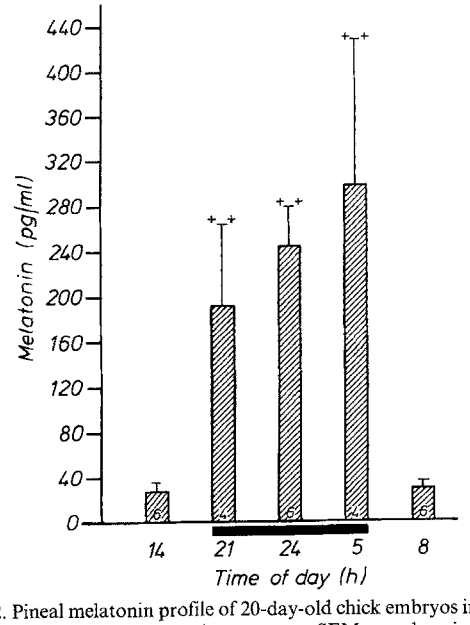

Figure 2. Pineal melatonin profile of 20-day-old chick embryos incubated in LD 16 : 8. Data are presented as means ± SEM; numbers in columns represent sample sizes. The dark bar below the abscissa denotes the dark period of the LD cycle. + + : $p < 0.01$ significant increase of pineal melatonin content in comparison to daytime levels.

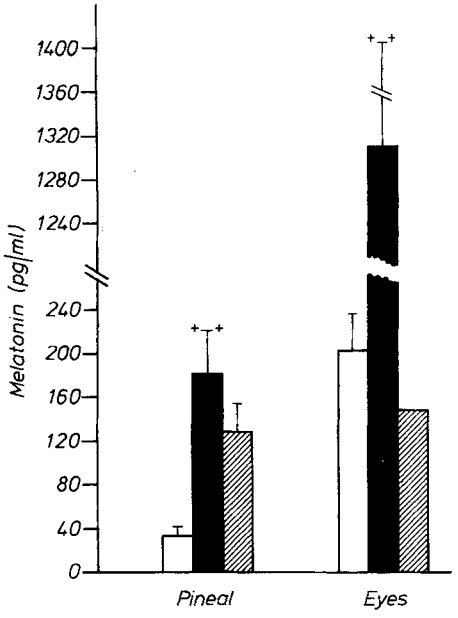

Figure 3. Melatonin content in pineals and eyes of 20-day-old chick embryos (5/group) incubated in LD 16 : 8 and sampled at midday (open), midnight (black) and after 1 h of light exposure at midnight (shaded). Data represent means ± SEM. + + : $p < 0.01$ significance of differences between day and night melatonin content in pineal and dark-time versus both other groups in eyes.

exposure. A comparison of the pooled dark values (56.6 ± 3.6 pg/ml) with the pooled light values (39.9 ± 2.8 pg/ml) showed a significantly higher melatonin concentration ($p < 0.05$) in pineals from dark-time embryos. Pineal melatonin concentrations, as well as the amplitude of the pineal melatonin rhythm, increased considerably during the final stage of embryonic development (fig. 2). Daytime values in 20-day-old embryos remained nearly at the level found in 18-day-old ones, but night-time concentrations increased more than five times in comparison with 18-day-old embryos. As with younger embryos, higher melatonin levels tended to occur late in the dark period.

A significant light-dark difference ($p < 0.01$) in melatonin content was found both in pineals and eyes of 20-day-old chick embryos (fig. 3). Exposure of eggs during the dark period to unexpected light decreased the high melatonin concentrations in eyes to the level found during the light period, but no significant decline was found in the pineals.

Discussion

A melatonin rhythm was observed in pineals of both 18- and 20-day-old chick embryos incubated in a light-dark regime. Melatonin concentrations in 18-day-old embryos were found to be in the same range as in our previous study where melatonin was measured in embryos incubated in the dark[9]. Levels increased considerably during the final stages of embryonic development, which accords with previously published results[8]. This rise reflects processes of maturation which occur in pineal gland biochemistry at the end of embryonic life. A marked increase of pineal NAT activity was observed in chick embryos between the 18th and 20th days of embryonic life[9]. Furthermore, a nearly exponential increase of 24-h melatonin synthesis was found in cultivated chick embryo pineals from the final stages of embryonic development[10].

The development of the rhythm in melatonin production coincides with the development of the visual system in the chick. Chick embryos open and close their eyelids in response to light between the 18th and 20th days of incubation[12], and an increased response of the visual system to a light stimulus was found after embryonic day 19[13]. The finding that a melatonin rhythm is already present in the pineals of 18-day-old chick embryos is in accordance with the presence of enzymes of the melatonin-forming pathway in chick embryo pineals[14,15]. As NAT is responsible for the melatonin rhythm[16], and the NAT activity rhythm was shown to be circadian in 18-day-old embryos[9,17], we can suppose that the melatonin rhythm found in the present experiment is also circadian. The marked increase of both the rhythm amplitude and high dark-time levels between embryonic days 18 and 20 suggests that melatonin could play some role in the development and entrainment of other behavioural and physiological rhythms during the final stages of embryonic life and/or in the timing of hatching in birds. It was suggested that the mechanism of hatching is set after the development of visual function[18], and light could act to trigger the hatching process. Furthermore, exogenous melatonin applied in pharmacological doses was shown to interrupt hatching behavior in chicks[19], but clearly more information is needed to evaluate the situation.

In the present experiment a significant light-dark difference in melatonin content was observed in the eyes of 20-day-old embryos. A light-dark difference in NAT activity in chick retinae was also first observed on embryonic day 20, but the incubation of retinae with forskolin or dibutyryl cyclic AMP already increased NAT activity in 14-day-old embryos[20]. These results demonstrate that the development of the molecular mechanisms regulating NAT activity precedes the onset of either the light/dark difference or the circadian rhythmicity of enzyme activity. In this way, the development of photoperiodic regulation of NAT activity and melatonin synthesis may be related to the development of photoreception.

Because the emergence of a day/night rhythm in melatonin production is not the only and the best index of when light first influences melatonin biosynthesis[21], the melatonin response to the light pulse occurring during the dark period was also examined. Exposure of eggs to 1 h of light at midnight decreased the high melatonin concentration in the eyes but not in the pineals of 20-day-old chick embryos. The suppression suggests that at this age photosensitive elements, at least in the retina, are mature to the extent that a light signal is able to influence

melatonin biosynthesis. A failure of the light pulse to suppress melatonin production in pineals may simply reflect differences in the accessibility of these two organs to environmental light. The higher intensity, longer duration of pulse or repeated exposure to light might suppress melatonin production in embryonic pineals as well. On the other hand, different sensitivity to light or a delayed development of photosensitive elements in pineals in comparison with eyes are also possible. In 1-day-old Japanese quail, high dark-time melatonin concentrations were suppressed to basal level 5 min after light exposure (Zeman and Gwinner, in preparation), indicating that immediately after hatching the response of the system to unexpected light already resembles the response of the mature system.

Our results suggest that the daily rhythm of melatonin production as part of the avian circadian system is developed before hatching in this precocial bird. The rhythmic melatonin production is synchronized to a light-dark cycle during incubation, and at the end of embryonic life it can respond almost instantaneously to a light pulse applied during the dark period.

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Regulation of rat renal ($Na^+ + K^+$)-adenosine triphosphatase mRNA levels by corticosterone*

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Abstract. We investigated the mechanisms for glucocorticoid regulation of rat renal NaK-ATPase activity. Our findings suggest that the magnitudes of corticosterone-induced increases in α_1 mRNA and β_1 mRNA levels are similar in the kidney of the adult adrenalectomized rats. The results also suggest that corticosterone restores NaK-ATPase activity in adrenalectomized rats prior to any enhanced sodium delivery.

Key words. Corticosterone; adrenalectomy; NaK-ATPase subunits; NaK-ATPase mRNAs; glomerular filtration rate.

($Na^+ + K^+$)-adenosine triphosphatase (NaK-ATPase, EC 3. 6. 1. 3) is an integral plasma membrane protein which maintains the electrochemical Na^+ and K^+ gradients required for many vital functions. These include the regulation of cell volume, the propagation of action potentials in excitable tissues and the active transport of nutrients. The enzyme is believed to exist as a heterodimer consisting of a catalytic α subunit and a β subunit. Both subunits are encoded by multigene families and the biochemically distinct isoforms of each subunit are differentially expressed with regard to tissue type[1-3]. Glucocorticoids increase NaK-ATPase activity in the kidney[4-6]. These hormones also increase renal blood flow and glomerular filtration rate[7]. The elevated NaK-ATPase activity in the kidney could be due to the enhanced glomerular filtration rate and filtered sodium load[8]. In isolated superfused distal tubules, glucocorticoids increase NaK-ATPase activity[9] suggesting a direct action of these hormones at the tubular epithelial cells. One objective of our investigation was to test whether a change in net Na^+ reabsorption could mediate the change in NaK-ATPase activity on treatment with corticosterone. The results indicate that corticosterone restores renal NaK-ATPase activity in adrenalectomized rats prior to any enhanced sodium delivery.

Glucocorticoid-mediated increases in renal NaK-ATPase activity and in quantities of the phosphorylated intermediate[10] suggest that these hormones mediate an enhanced NaK-ATPase activity via an increase in the number of active enzyme units. Recent studies demonstrate