ABSTRACT

The current state of knowledge about the secretory activity of the avian pineal organ during embryonic period is the result of studies performed mainly on the chicken. Research on other species has been scarce. The pineal glands of birds differ much in histological structure and ultrastructure, the content and daily profiles of indolic compounds, and the role of particular mechanisms in the regulation of melatonin (MLT) secretion. Significant interspecies differences indicate the necessity to conduct comparative studies, including these concerning the embryonic development of the gland.

The aim of study was to characterize the ontogenesis of 5-hydroxyindoles and 5-methoxyindoles synthesis pathways and the mechanisms regulating MLT secretion in the goose pineal gland during embryonic development.

The studies were performed *in vivo* and *in vitro* on pineal organs of goose embryos collected from day 14 to 28 of egg incubation. The first stage of *in vivo* studies concerned the characterization of ontogenesis of metabolic pathways involved in the synthesis of MLT and determination the occurrence of diurnal variations in the content of 5-hydroxyindoles and 5-methoxyindoles in the pineal organ. Indolic compounds were assayed by high-performance liquid chromatography (HPLC) with gradient separation and fluorescence detection. The second stage of *in vivo* studies was aimed at understanding the ontogeny of the catecholamine synthesis pathway in the pineal organ. The contents of catecholamines and their metabolites in the pineal organs were determined by HPLC with electrochemical detection.

In vitro experiments were performed in superfusion culture to characterize the mechanisms regulating secretory activity of the goose pineal organ during the embryonic period. The cultures were conducted under different light conditions, also with the addition of norepinephrine (NE) to the culture medium. MLT levels were determined by radioimmunoassay in all collected medium samples. To further characterize the changes in indole metabolism associated with embryonic age and occurring during the culture, 5-hydroxyindoles and 5-methoxyindoles were measured in selected medium samples by HPLC with fluorescence detection.

In vivo studies showed that the embryonic ontogeny of 5-hydroxyindole and 5-methoxyindole synthesis pathways in the goose pineal organ is composed of three phases. During the first phase, the pineal organ produces and metabolizes 5-HT, but it lacks the ability to perform 5-HT acetylation and methylation of 5-hydroxyindoles. The process of serotonin (5-HT) acetylation is turned on in the second phase, which begins around day 16 of eggs incubation. During the third phase, starting from day 18 of incubation, the pineal organ produces 5-methoxyindoles, including MLT. The levels of 5-hydroxyindoles and 5-methoxyindoles increase with the age of the embryos and are highest in embryos at 26 to 28 days of age. The pineal organ of goose embryos is characterized by a high content of 5-HT, compared to a 16-day-old chicken; however, this level is much lower than that in the organs of a 14-week-old goose. This finding suggests that a species-specific type of indole metabolism is determined during embryonic life and further matures after hatching. Diurnal rhythmicity develops very early in the embryonic pineal organ of the goose when

the eggs are incubated under a 12 hours light : 12 hours dark cycle. The pineal contents of 5-hydroxytryptophan (5-HTRP), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) show diurnal variations in 14-day-old embryos, being higher during scotophase than during photophase. Significant diurnal fluctuations in the pineal content of N-acetylserotonin (NAS), MLT and 5-methoxyindoleacetic acid (5-MIAA) were noted from day 20 of development. The course and amplitudes of the diurnal rhythms change during development.

The analysis of the results obtained in *in vitro* experiments indicates that the rhythmic release of 5-HTRP and 5-HIAA from the pineal organ occurs several days before the development of diurnal rhythm in the release of NAS, MLT, and 5-MIAA. MLT secretion in the culture increases with embryonic age, most intensely after day 22, resulting in changes in the ratio of indoles released into the medium occurring with embryonic age. Furthermore, isolation of embryonic pineal organs from their natural niche was found to induce the development of MLT biosynthesis and its diurnal rhythmicity. The modifications occurring during culture are similar to age-dependent changes. This finding is important when analyzing the results obtained in long-term cultures of avian embryonic pineal cells, such as monolayer cultures.

Regarding the regulatory mechanisms, this study demonstrated that MLT secretion is dependent on the light-dark diurnal cycle in all age-groups of embryos, although it should be noted that 6-h light exposure during the dark phase of the day does not affect MLT secretion. Endogenous oscillator activity is revealed in the regulation of MLT secretion in the pineal organs of embryos 24 days of age and older, but is unable to generate a rhythm after just one cycle. The present study demonstrates for the first time a strong inhibitory effect of NE on MLT secretion by embryonic pineal cells, both during the light and dark phases. The treatment of explants with NE during consecutive photophases resulted in a high-amplitude, well-entrained rhythm of MLT secretion. For the first time, NE was found to induce a very large increase in 5-HT release from the avian pineal organ. The increase in NE and its metabolite vanillylmandelic acid in the last days of the embryonic period suggests that adrenergic regulation of pineal activity may develop before or shortly after hatching.