Journal of Cellular Biochemistry 60:317–321 (1996)

ARTICLES

Iron Decreases the Nuclear But Not the Cytosolic Content of the Neurohormone Melatonin in Several Tissues in Chicks

Marta I. Pablos, M. Teresa Agapito, Armando Menéndez-Pelaez, Darío Acuña-Castroviejo, Russel J. Reiter, and José M. Recio

Departamento Bioquímica y Biología Molecular y Fisiología, Facultad de Ciencias, Universidad de Valladolid, Spain (M.I.P., M.T.A., J.M.R.); Departamento Morfología y Biología Celular, Facultad de Medicina, Universidad de Oviedo, Spain (A.M.-P.); Departamento Fisiología, Facultad de Medicina, Universidad de Granada, Spain (D.A.-C.); Department of Cellular and Structural Biology, University of Texas, Health Science Center at San Antonio, Texas (R.J.R.)

Abstract This paper describes the influence of iron on both nuclear and cytosolic melatonin contents in several tissues of chicks. The neurohormone melatonin was estimated by means of radioimmunoassay. Iron, administered as $FeCl_3$, decreased the nuclear melatonin level in a variety of tissues, including brain, heart, lung, kidney, and erythrocytes (nucleated cells in chicks) but was not seen in either the liver or gut. All variations related with iron were seen in the nuclear fraction, while only in the pineal gland did the melatonin content of the cytosol change as a result of iron treatment. We also observed a day–night rhythm in the nuclear melatonin: high nuclear levels of melatonin at night and low levels during the light period. This is the first report of nuclear localization of melatonin in any avian cell.

Key words: melatonin, iron, pineal gland, tissues, nucleus, cytosol, chicks, erythrocytes

The neurohormone melatonin is a lipophilic molecule that is primarily synthetized in the pineal gland. Melatonin synthesis exhibits a circadian rhythm (20- to 28-h period) with high levels at night and low levels during the day in all vertebrates investigated [Reiter, 1991: Yu et al., 1993]. Melatonin production begins with tryptophan, and the rate-limiting enzyme in this synthesis is N-acetyl transferase (NAT, EC 2.3.1.87). In chicks, melatonin synthesis is rhytmic and photosensitive and is regulated both by a circadian oscillator in the chicken gland itself and by adrenergic input from the sympathetic nervous system. The latter is governed by norepinephrine, which interacts with α_2-adrenergic receptors and induce a suppression of melatonin synthesis during the light phase [Pratt et al., 1987].

Received June 20, 1995; accepted June 24, 1995.

Address reprint requests to Dr. J.M. Recio Pascual, Departamento Bioquímica y Biología Molecular y Fisiología, Facultad de Ciencias, Prado de la Magdalena s/n. 47005-Valladolid, Spain.

Chaudhuris and Maiti [1989] reported that the diameter of nuclei increased in pineal parenchymal cells during gonadal maturation in both sexes, *Dendrocitta vagabunda*, more recently, it was observed that melatonin preferentially accumulates in the nuclear fraction of the mammalian tissues [Menendez-Pelaez et al., 1993a,b]. These results were obtained using both radioimmunoassay and immunohistochemical techniques. Nuclear melatonin accumulation suggests that melatonin may exert a genomic effect [Menendez-Pelaez et al., 1991] throughout a high-affinity nuclear receptor described previously [Acuña-Castroviejo et al., 1993, 1994].

In previous studies we found that endogenous increases of estrogen-dependent iron produced a significant inhibition of melatonin synthesis [Pablos et al., 1993]. The iron in this case derived from iron storage sites in the organism and melatonin synthesis inhibition was related to the iron concentration in the blood. The present experiments are an extension of these studies in which we investigated the effect of iron on

the nuclear melatonin content of a variety of organs in the chick.

MATERIALS AND METHODS

Three-week-old chickens (*Gallus domesticus*, breed) were maintained in a room with regulated temperature (22°C) and humidity and acclimated for 2 weeks to a 12L:12D cycle (lights off at 11.00 h). One h before lights off, a group of six chicks were sacrificed, and blood and several tissues (pineal gland, brain, lung, heart, kidney, gut, and liver) were collected. This group of chicks was designated as light controls. The remainder of the animals were injected intraperitoneally with either of two ferric iron concentrations (50 or 100 mg/L) or with isotonic alone. These animals (six chicks per group) were sacrificed 5 h after lights off. Blood and the tissues described above were collected and frozen until the processing. Nuclear and cytosolic fractions were separated as described before [Blum et al., 1989]. Melatonin in both serum and cellular fractions was measured by radioimmunoassay using a Guildhay antibody [Fraser et al., 1983] and ^{3}H-melatonin. Radioimmunoassay for melatonin in each cellular fraction was validated, and serial dilutions were established for both serum and tissues. The recovery of exogenous melatonin was 64–71%. Pineal N-acetyltransferase (NAT) activity was measured by high-performance liquid chromatography (HPLC) as described previously [Thomas et al., 1990]. Proteins were measured by the BioRad method described by Lonnerdal et al. [1987]. Statistic analysis was performed with Student's *t*-test, in which every group was compared to night controls.

RESULTS

Pineal NAT and serum melatonin activities are shown in Figure 1. Both concentrations of iron decreased nighttime NAT activity and serum melatonin levels by roughly 50%.

The melatonin content in both the pineal cell nuclei and cytosol (Fig. 2) exhibited a light:dark rhythm with high melatonin levels being associated with the dark period. The systemic administration of iron, at both concentrations, reduced nuclear melatonin levels while not influencing that of the cytosol.

Several tissues (brain, lung, kidney, heart, and erythrocytes) exhibited the same profile as did the pineal gland (Fig. 2). These tissues exhibited a nuclear melatonin rhythm with levels being higher during dark period than during the day. In each of these tissues the administration of iron reduced the nuclear melatonin content by 60–80%. On the other hand, no changes in the melatonin concentrations in cytosolic fractions were apparent.

Different changes were seen in gut and liver (Fig. 3). Nuclear melatonin content in the gut did not change after iron injection, but a rhythm in nuclear melatonin was observed (Fig. 3B). In the liver, an increase in nuclear melatonin levels was seen after iron administration (Fig. 3A).

DISCUSSION

The inhibition of NAT activity and the decrease in serum melatonin (Fig. 1) were likely

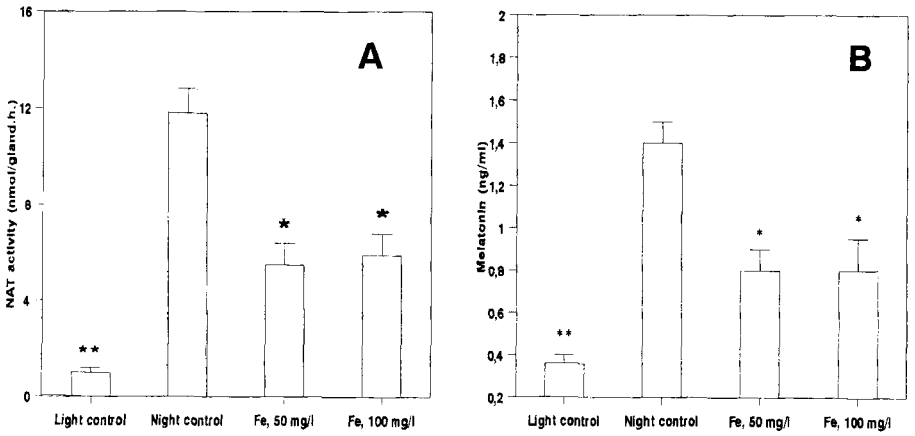

Fig. 1. Pineal NAT activity (A) and serum melatonin levels (B) in light controls, night controls, injected with 50 mg/L of iron, and injected with 100 mg/L of iron. Data mean ±SD. *$P < 0.01$ and **$P < 0.005$ with respect to night controls.

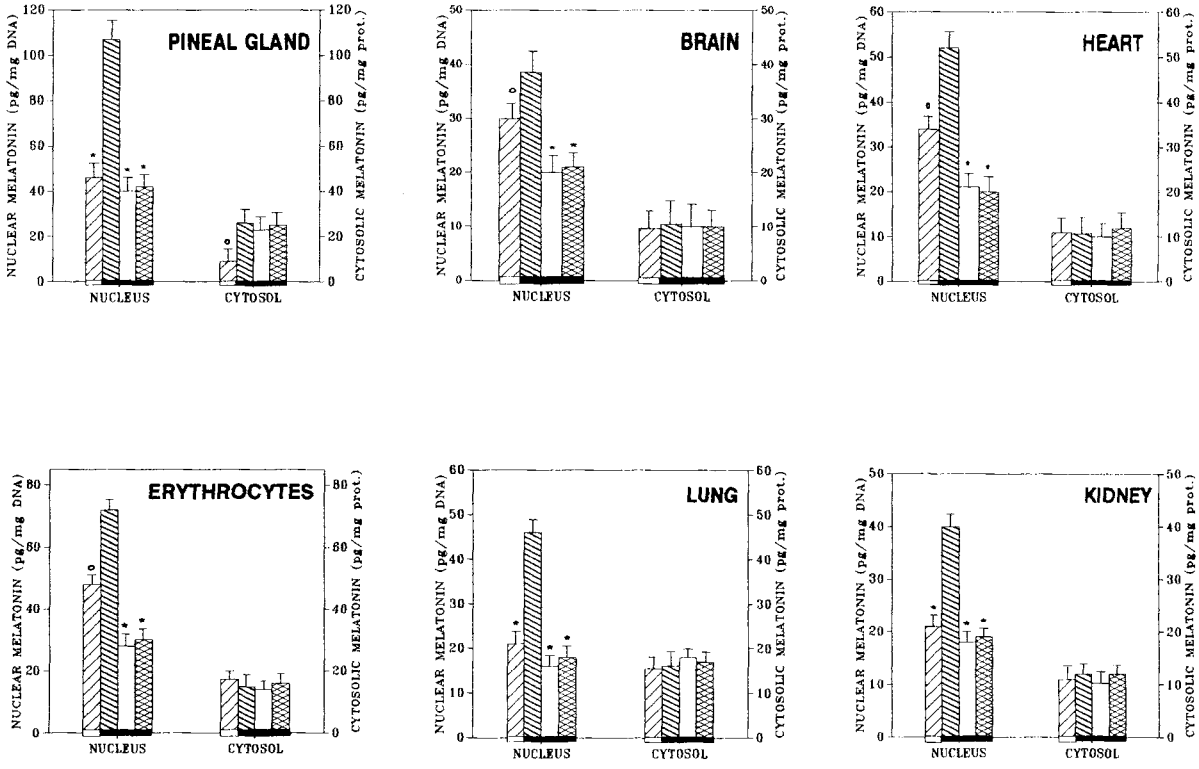

Fig. 2. Nuclear and cytosolic melatonin contents of chick tissues. ▨, light control; ▧, night control; □, injected with 50 mg/L of iron; ▩, injected with 100 mg/L of iron. Solid bars, dark period; empty bars, light phase. Data mean ± SD. *$P < 0.01$, °$P < 0.05$ with respect to night controls.

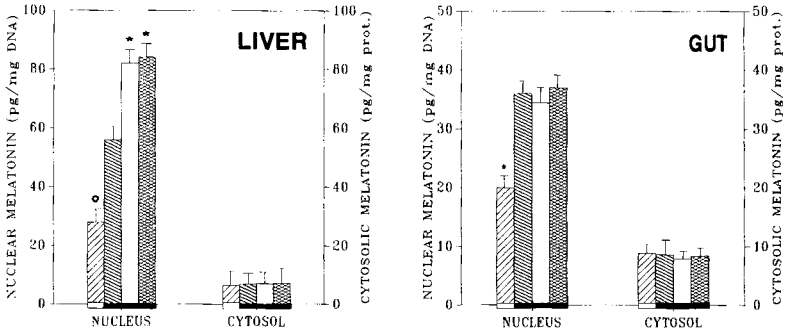

Fig. 3. Nuclear and cytosolic melatonin contents of chick tissues. ▨, light control; ▧, night control; □, injected with 50 mg/L of iron; ▩, injected with 100 mg/L of iron. Solid bars, dark period; empty bars, light phase. Data mean ± SD. *$P < 0.01$; °$P < 0.05$ with respect to night controls.

due to a direct inhibition of NAT by iron. In a previous study we found that iron also suppressed melatonin production in isolated perifused pineal glands [Pablos et al., 1993]. The drop in serum melatonin followed the reduced activity of NAT. Both reductions in NAT activity and melatonin content were highly significant in respect to nighttime controls ($P < 0.01$). The effect of iron on nuclear melatonin content of several tissues (Fig. 2) may be a consequence of the inhibition of pineal NAT activity with a resultant drop in circulating melatonin levels.

The dependence of tissue levels of melatonin on the pineal gland has been studied previously in rats [Menendez-Pelaez et al., 1993b]. Their findings show that perturbations (light, dark-

ness, pinealectomy, and melatonin administration) alter circulating concentrations of melatonin, which also change accordingly intracellular levels of the indole in most organs studied. Thus, pinealectomy, while lowering blood melatonin levels, also diminished nuclear concentrations of the constituent. Conversely, exogenous melatonin administration caused high circulating levels and correspondingly elevated nuclear concentrations of that hormone. Melatonin in the nuclei of tissues may relate to the apparent genomic actions of this indole, e.g., the regulation of δ-aminolevulinate synthase [Menendez-Pelaez et al., 1991]. The organs in which these changes were reported included cerebral cortices, kidney, spleen, and pineal gland.

In contrast to the organs mentioned above, in the gut, intracellular concentrations of melatonin are reportedly less dependent on the pineal gland [Menendez-Pelaez et al., 1993b]. Thus, pinealectomy was without effect on the nuclear melatonin rhythm in this tissue. The persistence of a melatonin rhythm in the gut even in pinealectomized rats probably relates to the fact that the gut synthesizes seemingly large quantities of melatonin [Huether et al., 1992; Yaga et al., 1993]. The apparent inability of iron to reduce gut melatonin (Fig. 3B) could be because this tissue rapidly replenishes melatonin supplies as they are depleted.

Hepatocyte nuclear melatonin content increased significantly after the administration of iron (Fig. 3A). The increase may have been due to iron is sequestered by liver ferritin thereby negating and depressive effect on intrahepatocyte melatonin levels. The continued uptake of melatonin by the hepatocytes could then explain the rise in intranuclear concentrations of the indole.

The higher concentrations of melatonin in the nuclear verses the cytosolic fractions could relate to the binding of melatonin in the nucleus. Nuclear binding sites for melatonin were recently described in rat liver cells [Acuña-Castroviejo et al., 1993; Acuña-Castroviejo et al., 1994], but they may well exist in many tissues. The high-affinity sites in the nucleus are saturable, and binding is rapid and reversible. The site is also specific for melatonin [Acuña-Castroviejo et al., 1994].

In summary, the neurohormone melatonin accumulates in the nuclei of cells in a variety of organs in the chick; this is the first demonstration of the nuclear accumulation of melatonin in an avian species. When iron is administered to chicks, nuclear melatonin levels were decreased in cells of the brain, lung, heart, kidney and in erythrocytes but were increase in hepatic cell nuclei.

ACKNOWLEDGMENTS

This paper is in memory of A. Menendez-Pelaez, a dear friend and a great scientist. Part of this work was supported by La Junta de Castilla y Leon with the "Programa de Apoyo a Proyectos de Investigación" (B.O.C. y L. de 26 de Diciembre 1994) ref Va/06/94.

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