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## SHORT COMMUNICATION

# PROLONGED TREATMENT WITH GLUCOCORTICOID DEXAMETHASONE SUPPRESSES MELATONIN PRODUCTION BY THE CHICK PINEAL GLAND AND RETINA

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*Prolonged treatment with glucocorticoid dexamethasone suppresses melatonin production by the chick pineal gland and retina.* J.B. ZAWILSKA, M. SADOWSKA. Pol. J. Pharmacol., 2002, 54, 61–66.

The chick pineal gland and retina synthesize melatonin in a circadian rhythm with high levels during the night. The rhythmic changes in the hormone production result predominantly from the fluctuation in the activity of serotonin N-acetyltransferase (AA-NAT), a penultimate and key regulatory enzyme in melatonin biosynthesis. The aim of this study was to analyze the effects of an acute and prolonged *in vivo* treatment with a glucocorticoid dexamethasone (4 mg/kg, *ip*) on the nocturnal increase in AA-NAT activity in chick pineal gland and retina. In acute experiments, dexamethasone (single dose)-injected chicks were killed after 2 h, while in prolonged experiments the glucocorticoid was given once daily for 7 days and the animals were killed 26–32 h after the last injection. Acute administration of dexamethasone did not affect AA-NAT activity in the chick pineal gland and retina. In the pineal glands and retinas of chicks that were treated with dexamethasone for one week and then killed at the end of the light phase of the 12:12 h light-dark cycle, AA-NAT activity was significantly higher than the enzyme activity found in tissues isolated from the vehicle-treated (control) animals. In addition to that, the nocturnal increase in pineal and, to a lower extent, retinal AA-NAT activity was significantly lower in dexamethasone-treated birds when compared with the respective control groups. It is suggested that prolonged treatment of animals with dexamethasone reduces the amplitude of the rhythmic melatonin production, a phenomenon which may affect chronobiological processes being under control of this hormone.

***Key words***: *dexamethasone, serotonin N-acetyltransferase, hydroxyindole-O-methyltransferase, melatonin, pineal gland, retina, chick*

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## INTRODUCTION

Pineal gland and, to a lower extent, retina of various vertebrate species produce melatonin, a hormone involved in the regulation of several biological rhythms. The biosynthesis of melatonin occurs in a light-dependent rhythmic fashion controlled by an endogenous circadian clock, with high levels during the dark phase and low levels during the light phase of a natural or imposed light-dark (LD) illumination cycle. The day-night amplitude of the melatonin rhythm provides an important synchronizing information to the biological clock of an organism [1, 14, 18]. Melatonin is synthesized from the amino acid precursor L-tryptophan by a sequential action of four enzymes: tryptophan hydroxylase (EC 1.14.16.4), aromatic L-amino acid decarboxylase (EC 4.1.1.28), serotonin N-acetyltransferase (arylalkylamine N-acetyltransferase; AA-NAT, EC 2.3.1.87), and hydroxyindole-O-methyltransferase (EC 2.1.1.4). Of these enzymes, AA-NAT is considered to play a key role in the regulation of melatonin biosynthesis as changes in its activity are paralleled by alterations in melatonin levels. On the other hand, in pineal glands and retinas of animals kept under standard illumination conditions, the activity of hydroxyindole-O-methyltransferase does not exhibit marked changes throughout the 24 h LD cycle [14, 18].

Glucocorticoids are among variety of endogenous compounds that have been suggested to influence melatonin production in various vertebrate species, including humans, and the existence of the mutual relationship between the pineal gland and the hypothalamo-pituitary-adrenal axis has been postulated by some authors (e.g. [2, 3, 5, 9, 11, 20]). However, both the physiological and pathophysiological significance of this interplay still remains debatable. Furthermore, the question of whether there is a direct link between glucocorticoids and melatonin production and secretion still remains unsolved. The present study was aimed at determining whether an acute and prolonged *in vivo* treatment of chicks with a synthetic glucocorticoid dexamethasone can modulate the nocturnal increase in AA-NAT activity in the pineal gland and retina.

## MATERIALS and METHODS

### Animals

White male leghorn chicks (*Gallus domesticus;* HyLine) were purchased locally on the day of hatching, and kept in temperature-controlled warmed brooders (30 ± 1°C during the first 5 days and 26 ± 1°C afterward) with standard food and tap water available *ad libitum*, for a minimum of three weeks before use. The animals were maintained under a 12 h light : 12 h dark (LD) lighting schedule (lights on between 22.00 and 10.00). The lighting cycle was produced by overhead cool fluorescent lamps providing light intensity at the level of the animals' eyes of approximately 150 lx. The experiments were carried out in strict accordance with the Polish regulations concerning experiments on animals (Dz.U. 97.111.724) and rules followed at the Department of Biogenic Amines.

In acute experiments, chicks received intraperitoneal (*ip*) injections of dexamethasone (4 mg/kg) or vehicle (0.3 ml/animal) at four different time points, i.e. 2 h before the end of the light phase (at 8.00), at the end of the light phase (at 10.00), at the end of the 2nd or 4th h of the dark phase (at 12.00 and 14.00, respectively). The animals were killed 2 h after the injection. In long-term treatment experiments, the animals received *ip* injections of dexamethasone or vehicle once daily for 7 days (always during the late light phase – between 07.45 and 08.15); the chicks were killed 26–32 h after the last injection, at the time points similar to those in the acute experiments. Pineal glands were removed and frozen on dry ice. Eyes were enucleated, hemisected at the equator, vitreous was removed and retina was dissected out and frozen on dry ice. All dissections during the dark phase of the LD cycle were performed under dim red light. The tissues were kept at –70°C until used for biochemical measurements (maximally for 3 days).

### Serotonin N-acetyltransferase activity assay

To measure AA-NAT activity, tissues were sonicated in an ice-cold 0.05 M sodium phosphate buffer (pH 6.8) in a proportion of: pineals – 1 pineal/100 μl, retinas – 1 mg of wet tissue/10 μl. AA-NAT activity was determined in supernatants of pineal and retinal homogenates by the radioisotopic method of Deguchi and Axelrod [4] with some modifications as described by Nowak et al. [13], using tryptamine-HCl (1.5 mM) and acetyl coenzyme A (152 μM) containing 16 nCi of [acetyl-1-$^{14}$C]coenzyme A as substrates.

### Chemicals

The following substances were used: dexamethasone kindly donated by Polfa (Pabianice, Po-

land), acetyl coenzyme A disodium salt supplied by Sigma (St. Louis, MO, USA), tryptamine-HCl supplied by Serva (Heidelberg, Germany), [acetyl-1-$^{14}$C]coenzyme A (sp. activity 60 mCi/mmol) purchased from DuPont New England Nuclear (Boston, MA, USA). Other chemicals were of analytical purity and were obtained mainly from Sigma (St. Louis, MO, USA). Dexamethasone was dissolved in 14% ethanol solution in water.

### Data analysis

Data were expressed as means ± SEM values. To calculate statistical differences between group means, an unpaired Student's *t*-test or a one-way analysis of variance followed by *post-hock* Newman-Keuls test was employed, using GraphPad software (GraphPad, San Diego, CA, USA).

The nocturnal increase in the enzyme activity was calculated as the difference between the nighttime activity (measured in tissues isolated after 2, 4 or 6 h of darkness) and the activity found in tissues isolated at the end of the light phase.

## RESULTS and DISCUSSION

The chick pineal gland and retina are characterized by well-expressed amplitude in daily melatonin production. In line with earlier reports [17, 19], during the first half of dark phase of the LD cycle the activity of AA-NAT (a key regulatory enzyme in the hormone production) exhibited up to 7-fold (retina) or 10-fold (pineal gland) increase compared to the values observed at the end of the light phase (Figs. 1–4). Acute administration of a synthetic glucocorticoid, dexamethasone (4 mg/kg, *ip*), to chicks did not affect AA-NAT activity in the pineal gland and retina (Fig. 1 and Fig. 2).

In the pineal glands and retinas of chicks that were treated with dexamethasone for one week and then killed at the end of the light phase of the LD cycle, when melatonin synthesis is usually still low, AA-NAT activity was significantly higher by 30% (pineal glands) or 53% (retinas) than the enzyme activity found in tissues isolated from the vehicle-treated (control) animals (Tab. 1).

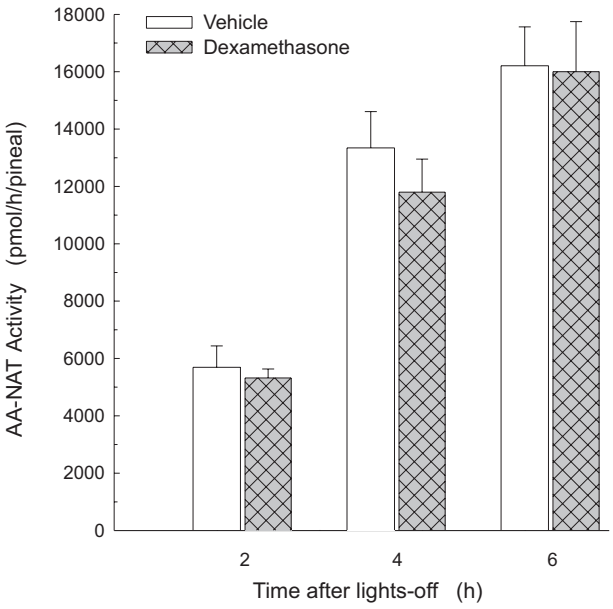


*Fig. 1.* Effect of acute treatment with dexamethasone on the nocturnal increase in AA-NAT activity in the pineal gland of chick. The animals received dexamethasone (4 mg/kg, *ip*) or vehicle. Chicks were killed 2 h after the injection (at the end of the light phase, at the end of the 2nd, 4th, or 6th h of the dark phase of the LD cycle). AA-NAT activity at the end of the light phase was: vehicle, 1885 ± 123 pmol/h/pineal; dexamethasone, 1714 ± 87 pmol/h/pineal. N = 10–13 animals/group. Comparison between groups was performed using the one-way analysis of variance followed by *post-hock* Newman-Keuls test

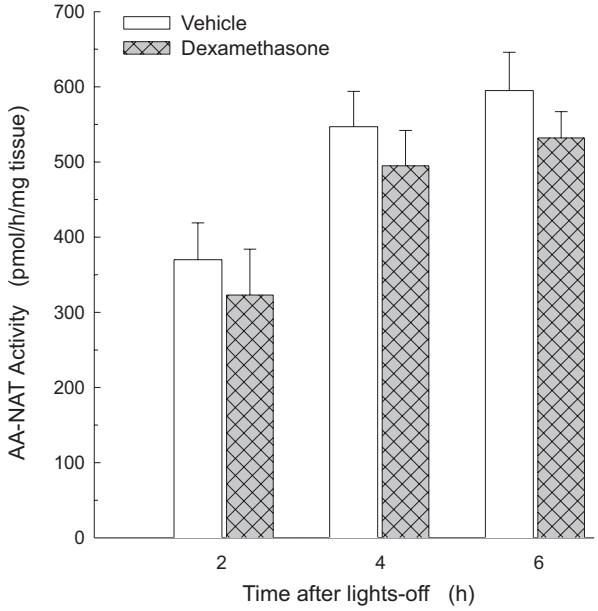


*Fig. 2.* Effect of acute treatment with dexamethasone on the nocturnal increase in AA-NAT activity in the retina of chick. The animals received dexamethasone (4 mg/kg, *ip*) or vehicle. Chicks were killed 2 h after the injection (at the end of the light phase, at the end of the 2nd, 4th, or 6th h of the dark phase of the LD cycle). AA-NAT activity at the end of the light phase was: vehicle, 108 ± 7 pmol/h/mg tissue; dexamethasone, 109 ± 9 pmol/h/pineal. N = 10–13 animals/group. Comparison between groups was performed using the one-way analysis of variance followed by *post-hock* Newman-Keuls test

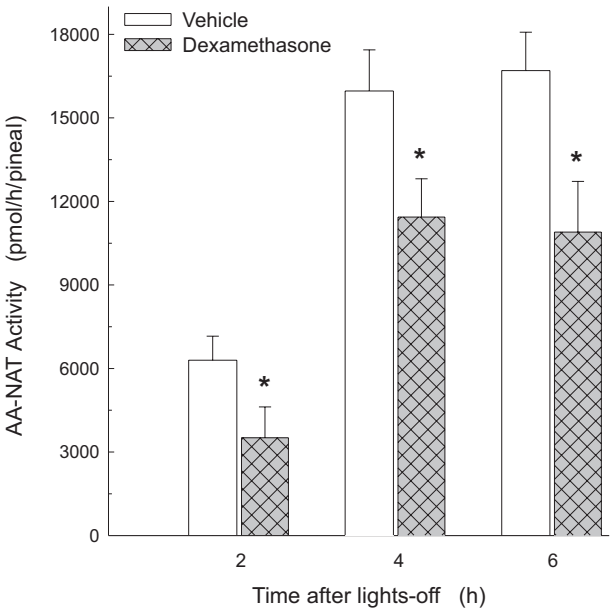


*Fig. 3.* Effect of prolonged treatment with dexamethasone on the nocturnal increase in AA-NAT activity in the pineal gland of chick. The animals received dexamethasone (4 mg/kg, *ip*) or vehicle once daily for 7 days. Chicks were killed 28–32 h after the last injection (at the end of the 2nd, 4th, or 6th h of the dark phase of the LD cycle). N = 9–12 animals/group. * $p < 0.05$ vs. vehicle-treated control. The statistical differences between group means were calculated using the one-way analysis of variance followed by *post-hock* Newman-Keuls test

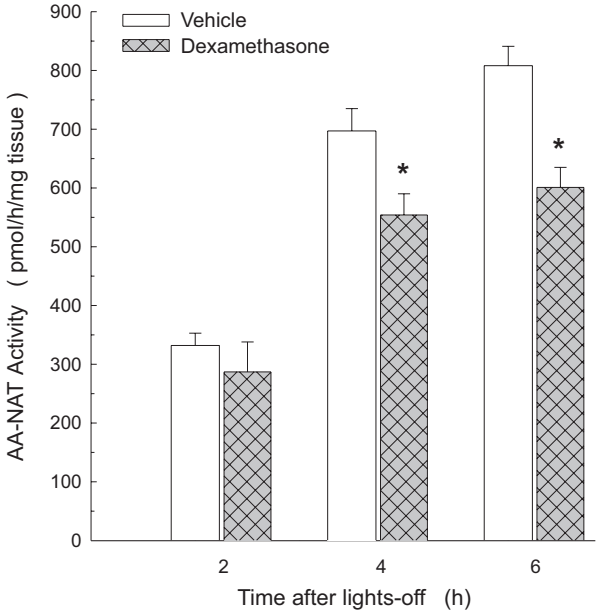


*Fig. 4.* Effect of prolonged treatment with dexamethasone on the nocturnal increase in AA-NAT activity in the retina of chick. The animals received dexamethasone (4 mg/kg, *ip*) or vehicle once daily for 7 days. Chicks were killed 28–32 h after the last injection (at the end of the 2nd, 4th, or 6th h of the dark phase of the LD cycle). N = 9–12 animals/group. * $p < 0.05$ vs. vehicle-treated control. The statistical differences between group means were calculated using the one-way analysis of variance followed by *post-hock* Newman-Keuls test

*Table 1.* Effect of prolonged treatment with dexamethasone on the day-time AA-NAT activity in the pineal gland and retina of chick

| Treatment | Pineal gland AA-NAT activity (pmol/h/pineal) | Retina AA-NAT activity (pmol/h/mg tissue) |
|---|---|---|
| Vehicle | 1793 ± 75 | 95 ± 12 |
| Dexamethasone | 2323 ± 287* | 145 ± 18* |

Chicks received dexamethasone (4 mg/kg, *ip*) or vehicle once daily for 7 days. The animals were killed 26 h after the last injection, at the end of the light phase. Results are means ± SEM values. N = 9–12 animals/group. * $p < 0.05$ vs. vehicle-treated control. The statistical differences between group means were calculated using the unpaired Student's *t*-test

At all studied time points, the nocturnal increase in pineal AA-NAT activity was markedly lower in dexamethasone-treated chicks when compared with the respective control groups (Fig. 3). The nocturnal increase in retinal AA-NAT activity measured after 4 and 6 h of darkness was significantly lower in dexamethasone-treated birds compared with the controls (Fig. 4). In contrast to AA-NAT, in the two studied tissues the activity of hydroxyindole-O-methyltransferase, the last enzyme in melatonin biosynthetic pathway which did not show any marked day-night differences, remained unaffected by the dexamethasone treatment (data not shown).

Despite several experimental data, the interaction between the pineal gland and the hypothalamic-pituitary-adrenal axis still remains a controversial issue. In humans, no evidence for such an interplay has been found by some authors (e.g. [8, 16]), while others have demonstrated an attenuation of the nocturnal melatonin production by dexamethasone [5, 10], an enhancement of daytime cortisol levels by melatonin [3], and a significant inverse relation between diurnal rhythms of plasma melatonin and cortisol levels in old demented patients [6]. In *in vitro* experiments, dexamethasone has been shown to decrease melatonin secretion from superfused rat pineal glands, to inhibit the nocturnal AA-NAT2 activity of trout pineal gland, and to reduce melatonin secretion by cultured trout pineal

cells [2, 20]. The action of dexamethasone appears to be specific for AA-NAT, the key regulatory enzyme in melatonin biosynthesis, as the drug did not modify the activity of hydroxyindole-O-methyltransferase (the last melatonin synthesizing enzyme) of trout pineal [3], chick pineal and retina (J.B. Zawilska and M. Sadowska, unpublished observations). The mechanisms underlying the inhibitory effect of dexamethasone on AA-NAT activity and melatonin production remain to be elucidated. The presence of glucocorticoid receptors in pineal glands of rat, tree shrew and trout, as well as in chicken retina, has recently been demonstrated [2, 7, 12, 15], suggesting that the analyzed action of dexamethasone likely results from the activation of specific nuclear receptors localized in these tissues. However, at present it is unknown whether it is mediated through glucocorticoid responsive elements in the AA-NAT gene promotor or results from glucocorticoid receptor-dependent alterations in sensitivity of membrane-bound receptors involved in the regulation of melatonin synthesis, or both [2].

In summary, the results of our *in vivo* experiments carried out on chicks indicate that prolonged treatment with the synthetic glucocorticoid, dexamethasone, reduces the amplitude of the diurnal rhythm of melatonin production in the retina and pineal gland, a phenomenon which may affect chronobiological processes being under control of this hormone.

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