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Melatonin Reduces Dopamine Content in the Neurointermediate Lobe of Male Syrian Hamsters

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ALEXIUK, N. A. M. AND J. P. VRIEND. *Melatonin reduces dopamine content in the neurointermediate lobe of male Syrian hamsters.* BRAIN RES BULL 32(4) 433–436, 1993.—The effect of daily late afternoon administration of melatonin on the in situ activity of tyrosine hydroxylase (TH) was studied in the posterior pituitary (neurointermediate lobe) of the male Syrian hamster. After 3 weeks of melatonin administration, TH activity was significantly reduced in the posterior pituitary. This was associated with a significant decrease in norepinephrine (NE) content. After 5 weeks, TH activity and NE content were no longer significantly different from controls. Dopamine (DA) content of the posterior pituitary was decreased progressively by melatonin administration, with a reduction of greater than 50% after 5 weeks of treatment. These data provide evidence that melatonin has a potent inhibitory effect on the regulation of the dopaminergic system of the neurointermediate lobe—an effect that appears unrelated to changes in axonal TH.

Melatonin Tyrosine hydroxylase Dopamine Posterior pituitary

THE serotonin derivative, melatonin, is secreted by pinealocytes during darkness in response to sympathetic noradrenergic stimulation. Secretion of this hormone is characterized by a rhythmic release that is synchronized to the daily light–dark cycle. Melatonin has been examined extensively for its dramatic effects on mammalian reproductive cycles (11,13) and circadian rhythm regulation. Although the question of melatonin's site(s) and mechanism of action continues to be in dispute (7), increasing evidence suggests that this indoleamine is producing some of its physiological effects by altering the metabolism of monoaminergic neurotransmitter systems within the hypothalamic-pituitary complex (1,4).

The significant inhibitory effects of melatonin administration (1,4), short photoperiod exposure (12), and light deprivation (6) upon catecholamine synthesis in hypothalamic and extrahypothalamic regions, have been demonstrated by several investigators. Although work has been done on retinal and tuberoinfundibular DA (TIDA) systems, the temporal effects of melatonin on tuberohypophysial DA (THDA) remains unexamined.

The present study was designed to determine the effects of melatonin administration on the in situ activity of tyrosine hydroxylase (TH)—the enzyme catalyzing the formation of L-DOPA and considered to be rate-limiting in catecholamine synthesis (8). This investigation tests the hypothesis that melatonin treatment produces an inhibitory effect on TH activity in the neurointermediate lobe of the pituitary gland. Male Syrian hamsters were treated for 1, 3, or 5 weeks to establish the temporal sequence of melatonin's effect on TH activity and catecholamine synthesis.

A second objective was to relate TH activity to changes in the tissue content of DA and NE in the neurointermediate lobe of the pituitary.

METHOD

Forty-eight 9-week-old male Syrian (golden) hamsters (strain Lak: LVG, Charles River, St. Constance, Quebec) were used in this study. The hamsters were maintained under controlled lighting and temperature conditions (22° ± 2°C). The photoperiod began at 0400 h and ended when lights were turned off at 1800 h. The light intensity at the level of the cage was approximately 200 footcandles. Food (Teklad rodent diet) and water were provided ad lib and the hamsters were housed 4 per cage.

The hamsters were acclimatized to laboratory conditions for approximately 1 week. The 48 hamsters were divided into 3 groups of 16. Each of these 3 groups were divided further into 2 groups. One group of 8 received daily SC injections of 0.1 ml of physiological saline; while the remaining 8 hamsters received daily injections of 25 micrograms of melatonin (N-acetyl-5-methoxytryptamine) in 0.1 ml of saline. The injections were administered between 1600 and 1700 h.

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After daily treatments for 1 week, all hamsters from the first group (both saline and melatonin-treated), received an IP injection of an aromatic-L-amino-acid decarboxylase inhibitor, NSD-1015 (m-hydroxybenzylhydrazine, Sigma)—100 mg/kg—40 min before sacrifice. After 3 weeks of treatment with saline or melatonin, all 16 hamsters from the second group were injected with NSD-1015 and after 40 min were sacrificed via decapitation. This was repeated also for the third group of hamsters following 5 weeks of saline or melatonin treatment. All animals were sacrificed between 1100 and 1300 h. The posterior pituitaries (neurointermediate lobes) were removed and immediately frozen on dry ice. Testicular weights were determined at this time to verify the physiological effectiveness of melatonin (13).

All tissue samples remained frozen until they were processed for high performance liquid chromatography with electrochemical detection (HPLC-EC). At this time, the tissues were homogenized in the running buffer containing the internal standard dihydroxybenzylamine (DHBA 10 ng/ml) and centrifuged at 12,000 g for 7 min. The precursor 3, 4-dihydroxyphenylalamine (L-DOPA) and the monoamines dopamine (DA) and norepinephrine (NE) were separated and assayed via HPLC-EC.

TH is considered to be the rate-limiting enzyme in catecholamine synthesis (8) under normal conditions. Therefore, the accumulation of L-DOPA following the administration of NSD-1015 is regarded as a measure of TH activity and catecholamine synthesis.

The posterior pituitary extracts were separated using an HPLC system that consisted of a Beckman solvent delivery system (Model 114M), and Altex injector (Model 210A), and a 10 cm C-18 reverse-phase column (Chromatography Sciences Co., Canada). The electrochemical detector (ESA Model 5100A) was equipped with a high sensitivity cell (ESA Model 5011). The detector was set at a reduction potential of D1 = +0.04 volts; guard cell = +0.40; D2 = -0.34. A Shimadzu integrator (Model C-R3A) was used to record and integrate peak areas and calculate the content of monoamines, precursors, and metabolites. The mobile phase (running buffer) consisted of 0.05 M sodium phosphate monobasic, 1.2 mM heptanesulphonic acid, 0.2 mM EDTA, and 3% methanol. The mobile phase was brought to a pH of 3.0 with phosphoric acid.

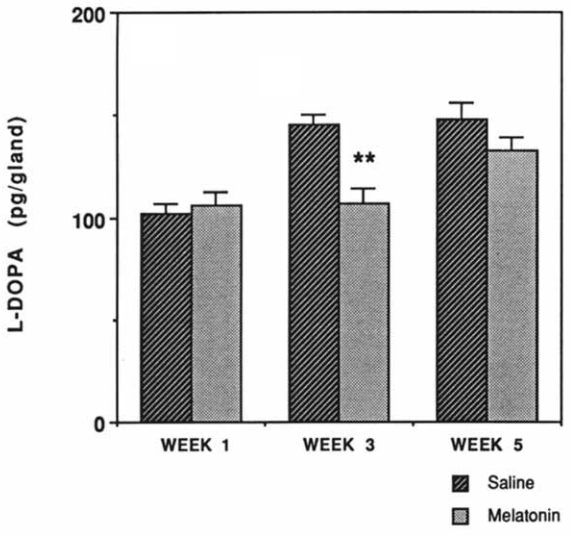

FIG. 1. L-DOPA accumulation in posterior pituitary of control and melatonin-treated hamsters. Effects of melatonin administration for 1, 3, and 5 weeks on the accumulation of L-DOPA in the neurointermediate lobe of the pituitary. Data points represent Mean ± SE (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$).

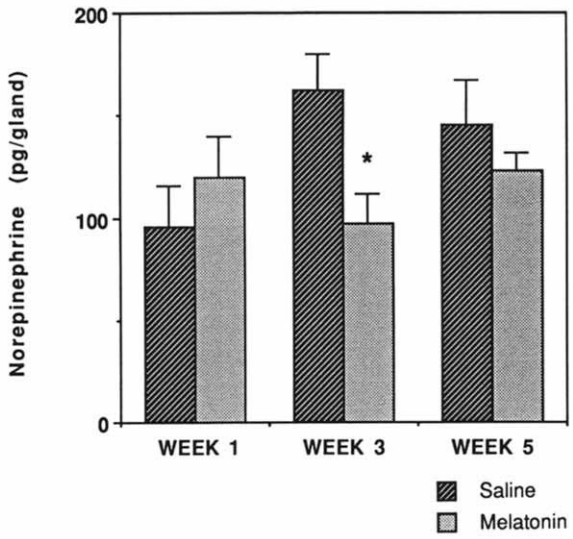

FIG. 2. Norepinephrine content of posterior pituitary of control and melatonin-treated hamsters. Effects of melatonin administration for 1, 3, and 5 weeks on content of norepinephrine in the neurointermediate lobe of pituitary. Data points represent Mean ± SE (*$p < 0.05$).

Statistical Analysis

All of the data was subjected to analysis of variance (ANOVA). ANOVA (treatment × week) was used to analyze the data on L-DOPA accumulation after NSD-1015 administration and the content of monoamines (DA and NE). The data were then subjected to Student's t tests. Statistical significance was considered as a p value of less than 0.05. The following levels of significance were distinguished: $p < 0.05$; $p < 0.01$; $p < 0.001$.

RESULTS

L-DOPA Accumulation

Melatonin treatment was demonstrated to produce an overall inhibitory effect on the accumulation of the catecholamine precursor, L-DOPA, after administration of NSD-1015 ($F = 10.18$; $p < 0.01$; Fig. 1). The greatest inhibition of TH activity (as reflected by the decreases found in the accumulation of L-DOPA after NSD-1015), was observed in those animals treated with melatonin for 3 weeks ($t = 4.396$; $p < 0.001$). No significant effect was observed at 5 weeks.

NE Content

Melatonin treatment significantly decreased NE content but according to ANOVA this effect was time-dependent ($F = 3.55$; $p < 0.05$; Fig. 2) The greatest inhibition of NE was evident during week 3 ($t = 2.703$; $p < 0.05$). No significant decrease was observed at 5 weeks.

DA Content

Melatonin administration significantly decreased DA content after NSD-1015 in the posterior pituitary ($F = 9.42$; $p < 0.01$; Fig. 3). This effect was demonstrated at 3 weeks and significant at 5 weeks of treatment ($t = 3.222$; $p < 0.01$).

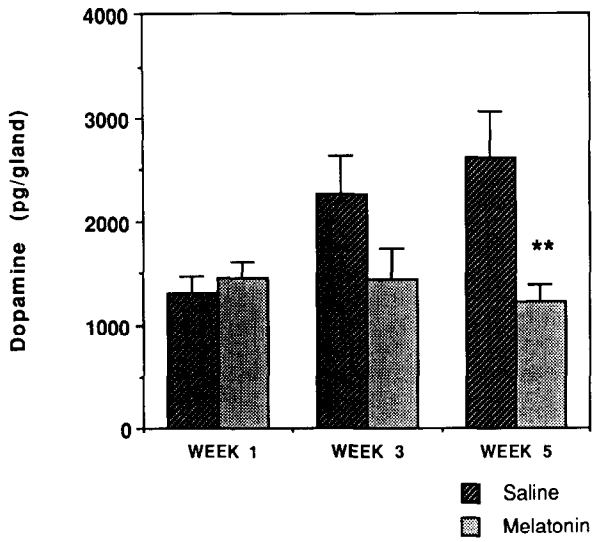

FIG. 3. Dopamine content of posterior pituitary of control and melatonin-treated hamsters. Effects of melatonin administration for 1, 3, and 5 weeks on content of dopamine in the neurointermediate lobe of pituitary. Data points represent Mean ± SE (**$p < 0.01$).

Testes Weight

As expected after several weeks of melatonin administration (or short-photoperiod exposure) following 5 weeks of melatonin treatment, there was evidence of testes involution. Melatonin administration significantly decreased testes weight at 5 weeks of treatment ($t = 3.214$; $p < 0.01$; Fig. 4). There were no significant differences in testes weight after 3 weeks of treatment.

DISCUSSION

Previous studies in which photoperiod was manipulated led to evidence showing that melatonin inhibited catecholamine synthesis in the mediobasal hypothalamus (1) of hamsters. The current study provides data suggesting that melatonin may be a major regulator of catecholamine metabolism in the posterior pituitary (neurointermediate lobe).

The occurrence in the present investigation of a concomitant decrease in the accumulation of L-DOPA ($p < 0.001$) and NE content ($p < 0.05$) in the posterior pituitary after 3 weeks of melatonin treatment is consistent with the interpretation that melatonin is inhibiting TH activity in noradrenergic neurons that enter this lobe. Noradrenergic fibers of the posterior pituitary have been demonstrated to include those with central as well as those with peripheral (from sympathetic neurons of the superior cervical ganglia) origin (2). The lack of a significant melatonin-induced inhibition of TH activity or a decrease in NE content after 5 weeks of melatonin administration suggests that compensatory mechanisms that serve to enhance TH activity could have been activated before this time.

The present study provides strong evidence that melatonin treatment progressively decreased DA content of the posterior pituitary (Fig. 3). After 5 weeks of melatonin administration, the DA content of the neurointermediate lobe was reduced to less than 50% of controls ($p < 0.01$). While data showing a melatonin-induced decrease in testicular weight (Fig. 4) verified the effectiveness of melatonin in the present experiment, a physiological role for a melatonin-induced decrease in THDA in modulation of the hormones of the posterior pituitary (e.g., oxytocin, vasopressin) remains to be determined.

The lack of a significant difference in the in situ activity of TH after 5 weeks at a period when differences in DA content of control and melatonin-treated hamsters were greatest, suggests that the melatonin-induced decrease in DA stores in the posterior pituitary were not due to an inhibition of DA synthesis in THDA axons. Melatonin-induced alterations in concentrations of circulating gonadal hormones could, however, influence TH activity. Since all the animals of this study were treated with the decarboxylase inhibitor, NSD-1015, before sacrifice, the tissue DA content measured would not include newly synthesized DA. Increases in DA content of controls, between Week 1 and Week 5 of treatment, apparently reflect age-related changes in the ability to store DA.

It has been reported that melatonin is a potent inhibitor of DA release in selected tissues including retina and hypothalamus (3,16). The present data suggest that melatonin may be modulating DA release in THDA axons. Melatonin has been reported to decrease impulse flow in certain regions of the rat brain including amygdala and rostral hypothalamus (9). If melatonin is inhibiting neuronal firing in TIDA and THDA systems it would be expected to decrease DA synthesis and/or release. Studies of the differential effects of evening versus morning melatonin administration on DA metabolism may be important because morning injections of melatonin are ineffective in inducing reproductive changes (10,13).

Studies that show a high concentration of melatonin binding sites in the median eminence/pituitary complex (7,14,15) supported the concept that DA axons of the A12 arcuate region of the hypothalamus are subject to melatonin regulation. One alternative explanation for these data is that melatonin acts on neurons which synapse on dopaminergic neurons distributed from the A12 region of the hypothalamus. Changes in the amount of circulating prolactin reaching this region reportedly could also influence DA turnover of A12 axons (5). Since both tuberoinfundibular and tuberohypophysial DA neurons take origin from the arcuate nuclei of the mediobasal hypothalamus, the data suggested the presence of a physiologically important site of action for melatonin within this region.

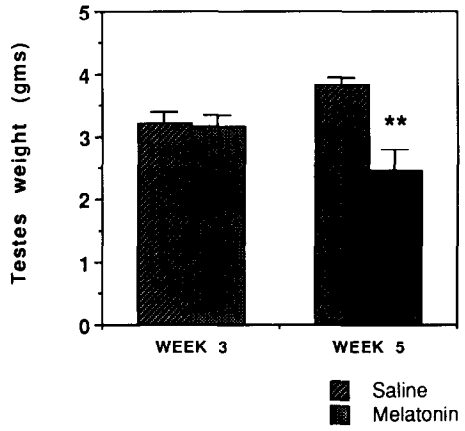

FIG. 4. Effects of melatonin administration on testes weights of male Syrian hamsters at 3 and 5 weeks of treatment. Data points represent Mean ± SE (**$p < 0.01$).

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