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Pineal Gland and Melatonin are Associated with Serum Element Metabolism in Rats

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ABSTRACT

Introduction: The aim of this study is to investigate the relationship of melatonin supplementation with serum element metabolism in pinealectomized rats.

Methods: The research was conducted on 32 adult male Spraque-Dawley rats. The study protocol was approved by the local animal ethics committee.
Animals were divided into four equal groups. Control (Group 1), Melatonin (Group 2), Pinealectomy "Px" (Group 3), Pinealectomy+melatonin (Group 4). Animals in groups 2 and 4 were given intraperitoneal (ip) melatonin support (4 weeks/day; 3 mg/kg melatonin). Animals in groups 3 and 4 underwent pinealectomy under general anesthesia. At the end of the applications, serum element levels were determined by atomic emission (µg/dl) in the blood samples taken from the sacrificed animals.

Results: While pinealectomy increased chromium and manganese levels in Group 3 ($p<0.001$), it caused significant suppression of magnesium, calcium and zinc levels ($p<0.001$). Melatonin supplementation in pinealectomized animals (Group 4) treated the impairments in the mentioned parameters.

Conclusion: The results of the current study show that the melatonin hormone secreted from the pineal gland has a regulatory effect on serum element metabolism. This study is the first to examine the relationship between the pineal gland and element metabolism as a whole.

Keywords: Elements metabolism, melatonin administration, pinealectomy, rat

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INTRODUCTION

One of the best-known functions of the melatonin hormone, which is synthesized from the amino acid tryptophan in the pineal gland, is the regulation of the light-dark cycle, that is, biological rhythms (1). The antioxidant activity of melatonin, which is also a powerful free radical scavenger, is also critical for the living organism (2). Pinealectomy refers to the surgical removal of the pineal gland. This procedure ensures that the hormone melatonin, secreted primarily by the pineal gland, is withdrawn from the systemic circulation (3). The withdrawal of melatonin from the systemic circulation causes deficiency in many physiological mechanisms controlled by this hormone, such as circadian rhythm, antioxidant activity, reproductive system and immune reactions (3). Therefore, pinealectomy is a frequently used method in experimental studies.

Highlights

- **Pinealectomy significantly disrupts serum element metabolism in rats.**
- **Melatonin support has a regulatory effect on serum element metabolism.**
- **This publication is the first study on element metabolism and melatonin.**

In addition to the known effects of melatonin, it has been reported that it may be related to elements in mammalian organisms (4). It is known that calcium ions play an active role in melatonin biosynthesis (5). It has been shown that the melatonin hormone can prevent the toxic effects caused by elements such as cadmium, chromium, lead, iron and copper in living organisms (4,6–8). Popovi et al. (9), based on the fact that melatonin decreases in old age, reported in their study on old rats that a decrease in Cu, Zn and Mn levels occurred in parallel with

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the suppressed antioxidant activity in the liver tissue. In publications investigating melatonin and element metabolism, publications related to zinc metabolism come to the fore. A striking example of the relationship between melatonin and zinc can be given as the reported treatment of decreased zinc levels in the kidney and pancreatic tissue of old rats with melatonin supplementation (10). The demonstration that decreasing blood zinc levels in pinealectomized rats is prevented by melatonin supplementation draws attention to a critical relationship between the pineal gland and zinc metabolism (11). Apart from treating the toxic effects of some elements, there are almost no studies on how the melatonin hormone affects the metabolism of elements other than zinc. The aim of this study is to investigate the relationship of melatonin supplementation with serum element metabolism in pinealectomized rats.

METHODS

Animal Material and Groups

The current study was conducted at the Experimental Medicine Research Institute of Yeditepe University on 32 adult male Spraque-Dawley rats. The study protocol was approved by the Experimental Animal Ethics Committee of the same institution (2009–21/12).

Animals were divided into four equal groups. Control (Group 1), Melatonin (Group 2), Pinealectomy "Px" (Group 3), Pinealectomy+melatonin (Group 4). Animals in groups 2 and 4 were given intraperitoneal (ip) melatonin support (4 weeks/day; 3 mg/kg melatonin). Animals in groups 3 and 4 underwent pinealectomy under general anesthesia.

Experimental Animals and Their Nutrition

Experimental animals were fed with standard rat chow (in pellet form) without any restrictions. Experimental animals were kept in an environment of 12 hours of darkness, 12 hours of light and standard room temperature (21±1 ºC). All injections were made between the same hours (09:00–10:00) in the morning.

At the end of the four-week studies, all animals were sacrificed and serum samples were taken to be used in the necessary analyses. The serum samples taken were stored at -80°C until analysis.

Experimental Applications

Melatonin application

After 40 mg of melatonin (Sigma M-5250) was dissolved in 3 ml of pure ethanol, this suspension was kept in the dark and closed in the freezer until the time of use. 0.1 ml of the stock solution was taken and 0.9 ml NaCl was added (3 mg/kg/day) and injected subcutaneously into the rats at 9:00 in the morning. Melatonin can be given to experimental animals in drinking water, by subcutaneous or intrapertoneal injection. In this study, subcutaneous administration of melatonin was preferred due to its subcutaneous absorbability and easy administration (12). Melatonin application was carried out at the same time for 4 weeks. In experimental studies, physiological dosage applications of melatonin are in the range of 1–3 mg/kg. However, in experimental studies, there have been melatonin doses applied from 1 mg/kg (13), 50–100 mg/kg and even up to 300 mg/kg (13). Especially in studies on tissue damage, high doses of melatonin are used to prevent oxidative stress (14). In the current study, a melatonin supplement dose was administered to animals at a rate of 3 mg/kg, which is considered a physiological dose.

Pinealectomy

Pinealectomy in experimental animals was performed under general anesthesia in accordance with the method determined by Kuszack and Rodin (15). (combination of ketamine hydrochloride "Ketalar, Parke-Davis" at a dose of 60 mg/kg and xylazine "Rompun, Bayer" at a dose of 5 mg/kg).

Biochemical Analysis

Serum element analysis on atomic emission device

Blood samples (2 ml) were taken by decapitation to determine the serum levels of zinc, lead, cobalt, molybdenum, chromium, sulfur, magnesium, manganese, sodium, potassium, phosphorus, copper, iron, calcium and selenium of the experimental animals. After the blood samples were centrifuged and their serum was separated, they were stored in plastic capped tubes at -80°C until the time of analysis.

Serum samples obtained from animals were determined in an inductively coupled plasma emission spectrophotometer (ICP-AES; Varian Australia Pty LTD, Australia). The results were given as μg/dl.

Statistical Methods

A computer package program was used to statistically analyze the findings obtained in the study. First, the arithmetic means and standard errors of the data obtained were calculated. Analysis of variance was applied to show differences between groups. The Least Significant Difference (LSD) Test was applied to determine the comparison of group averages in the statistically significant variance analysis results. Differences at $P < 0.05$ were considered significant.

RESULTS

Serum cobalt, molybdenum, nickel, lead, phosphorus, copper, iron and selenium levels of the study groups were not different from each other. The highest chromium, manganese and magnesium values in serum were obtained in Group 3 ($p<0.001$), and the same parameters for groups 1, 2 and 4 were not different from each other. The lowest potassium, sodium, sulfur and zinc values in serum were obtained in Group 3, which underwent pinealectomy ($p<0.001$). No significant difference was detected between the other groups in terms of the same parameters. Serum calcium levels of groups 2 and 4, which received melatonin applications, were significantly higher than groups 1 and 3 ($p<0.001$, Tables 1, 2, 3 and 4).

Table 1. Serum cobalt, molybdenum, chromium and nickel levels of study groups (μg/dl)

Groups	Cobalt	Molybdenum	Chrome	Nickel
Control (G1)	0.04±0.01	0.10±0.03	0.11±0.01^{B}	0.24±0.13
Melatonin (G2)	0.05±0.03	0.11±0.09	0.12±0.04^{B}	0.27±0.19
Px (G3)	0.04±0.02	0.11±0.09	0.28±0.14^{A}	0.24±0.18
Px+Melatonin (G4)	0.05±0.03	0.11±0.09	0.12±0.04^{B}	0.27±0.19

$P < 0.001$; A > B.
*Means with different letters in the same column are statistically significant.

Table 2. Serum manganese, magnesium, lead and phosphorus levels of the study groups (µg/dl)

Groups	Manganese	Magnesium	Lead	Phosphorus
Control (G1)	0.09±0.00^B	29.30±3.28^A	0.16±0.06	258.4±22.8
Melatonin (G2)	0.09±0.03^B	27.51±1.10^A	0.16±0.05	261.2±26.1
Px (G3)	0.19±0.11^A	16.44±3.99^B	0.17±0.08	261.9±25.7
Px+Melatonin (G4)	0.09±0.03^B	27.38±2.17^A	0.15±0.07	260.8±23.5

P <0.001; A > B.
*Means with different letters in the same column are statistically significant.

Table 3. Serum potassium, sodium, sulfur and calcium levels of study groups (µg/dl)

Groups	Potassium	Sodium	Sulfur	Calcium
Control (G1)	436.4±34.8^A	4164.3±174.0^A	995.7±56.6^A	118.74±24.95^B
Melatonin (G2)	437.2±25.1^A	4167.9±134.9^A	983.1±47.6^A	120.45±25.87^A
Px (G3)	312.5±32.4^B	3585.3±164.4^B	613.2±43.5^B	78.90±28.30^B
Px+Melatonin (G4)	435.7±34.6^A	4170.5±140.3^A	985.4±40.6^A	121.40±23.54^A

P <0.001; A > B.
*Means with different letters in the same column are statistically significant.

Table 4. Serum copper, iron, selenium and zinc levels of study groups (µg/dl)

Groups	Copper	Iron	Selenium	Zinc
Control (G1)	2.11±0.25	8.57±1.42	0.77±0.41	2.00±0.22^A
Melatonin (G2)	2.18±1.15	8.70±2.15	0.78±0.45	2.01±0.24^A
Px (G3)	2.13±0.30	8.57±1.65	0.72±0.48	1.38±0.21^B
Px+Melatonin (G4)	2.18±1.20	8.59±1.85	0.76±0.40	2.01±0.29^A

P <0.001; A > B.
*Means with different letters in the same column are statistically significant.

DISCUSSION

In our study, we obtained the highest serum chromium, manganese and magnesium values in Group 3, which underwent pinealectomy. It has been shown that high doses of chromium cause tissue damage (16), and similarly, manganese accumulates in large amounts in tissues, causing toxic effects and increasing oxidant damage (17). The increased chromium and manganese levels we obtained after pinealectomy in our study can be considered as a striking result that should be emphasized. Consistent with our study, Cemek et al. (18) reported that chromium and manganese levels, which cause lipid peroxidation when accumulated in blood and tissues, decreased with melatonin application and, as a result, tissue damage was prevented. In our study, the increased chromium and manganese levels we obtained in pinealectomy rats (Group 3) probably occured as a result of the decrease in melatonin concentration as a result of the removal of the pineal gland. The chromium and manganese levels of Group 2, in which only melatonin was applied, and in Group 4, in which we applied exogenous melatonin after pinealectomy, were not different from the controls. This finding can be presented as evidence that the increased chromium and manganese levels we obtained in Group 3, the pinealectomy group, resulted from melatonin deficiency. Similarly, in our study, we obtained the highest magnesium levels in the pinealectomy group (G3). It has been shown that there is an increase in serum magnesium levels in chicks with pineal glands removed, but serum magnesium is not important in reflecting body magnesium status (19). The increased magnesium levels we obtained in Group 3 in our study are compatible with the report of Turgut et al. (19).

In our study, the lowest serum potassium, sodium, sulfur and zinc values were obtained in Group 3, which underwent pinealectomy. Sodium and potassium ions take an active role in melatonin biosynthesis and are also involved in the mechanism of action of this hormone by facilitating its entry into the cell (3). The decreased potassium and sodium values we obtained in our study show that the interaction between melatonin and these substances is not unidirectional. The report that there was a significant decrease in serum sodium levels of rats after pinealectomy (20) also supports this idea. The pinealectomy group (Group 3) also had the lowest sulfur values. We could not find any literature in which we could directly compare this finding during our scans. However, some studies, albeit limited, point out that the pineal gland may have a regulatory effect on elemental metabolism in blood and tissues (4,19). In our study, the potassium, sodium and sulfur values of Group 2, in which we applied only melatonin, and Group 4, in which we applied melatonin after pinealectomy, were not different from the control group in which no application was made. Therefore, it can be concluded that the decreased potassium, sodium and sulfur levels we obtained in pinealectomized animals (Group 3) may be due to melatonin deficiency and that there may be an important relationship between the pineal gland and serum regulation of these substances.

The pineal gland is the richest region in the brain in terms of zinc (21). For this reason, there are many publications on the relationship between the pineal gland and zinc. In the study conducted by Mocchegiani et al. (22), pinealectomy resulted in a decrease in body zinc in animals, and application of melatonin (100 µg/mouse) to pinealectomized mice for

1 month changed the body zinc pool in mice from negative to positive value. Discontinuing melatonin administration to the same mice for 1 month led to the reappearance of the negative zinc pool. The results of the above-mentioned study provide critical information regarding the relationship between the pineal gland and zinc. At the same time, this report is parallel to the decreased zinc levels we obtained in pinealectomized animals (Group 3) in our study. In our study, decreased serum zinc levels in pinealectomized animals (Group 3) were treated with melatonin support. This therapeutic effect of melatonin was seen both in Group 2, where we applied melatonin, and in Group 4, where we applied melatonin after pinealectomy.

The findings of our study regarding the zinc parameter are also compatible with the study of Mocchegiani et al. (22).

In our study, melatonin applications resulted in an increase in serum calcium levels in groups 2 and 4. Cemek et al. (14) reported that melatonin application increased calcium levels in various tissues of rats. Similarly, Pawlak et al. (23) showed that melatonin application and stimulation of testicular cells of both control and diabetic animals resulted in an increase in calcium levels. The increased serum calcium we obtained with melatonin application is also compatible with the findings of the researchers whose reports are presented above.

There is no study investigating the combined relationship of melatonin with elemental metabolism in serum. However, it is known that metallothioneins, a protein that binds metal elements, are affected by melatonin (24). Decreased metallothionein levels in pinealecomy are reversed by melatonin support (24). It has been reported in many studies that melatonin can prevent tissue damage and tissue toxicity by increasing metallothionein gene expression, especially by binding metal elements that cause toxic effects (24–26). Metallothionein gene expression was not determined in the current study. However, it can be said that metallothioneins may be an important mediator in the effect of melatonin on changes in serum element levels.

As a result taken together, the results of our study show that pinealectomy significantly disrupts serum element metabolism in rats, and melatonin application may have a regulatory effect on body element metabolism. The current study is the first to evaluate the metabolism of trace elements and pinealectomy and melatonin application as a whole.

Ethics Committee Approval: The study protocol was approved by the Experimental Animal Ethics Committee of Yeditepe University Experimental Medicine Research Institute (2009–21/12).

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Conflict of Interest: The authors declared that there is no conflict of interest.

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