Effects of Pinealectomy and Melatonin Supplementation on Elements Metabolism in Rat Testicular Tissue

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Informed Consent: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Main Points

Taken together, the results of our study highlight the important relationship between pineal gland and testicular element metabolism.

This study is the first to investigate pineal gland and testicular element metabolism as a whole.
ABSTRACT

Objective: The aim of this study was to investigate how pinealectomy and melatonin application affect elements metabolism in rat testicular tissue.

Methods: The study was carried out on 32 adult male Spraque-Dawley rats. Animals were divided into 4 equal groups. Group 1: Control, Group 2: Melatonin, Group 3: Pinealectomy, Group 4: Pinealectomy+Melatonin. Group 2 and 4 animals received daily 3mg/kg intraperitoneal (ip) melatonin supplementation for 4 weeks. The pineal glands of Group 3 and 4 animals were removed under general anesthesia. At the end of the applications, testicular tissue samples were taken from the animals sacrificed under general anesthesia. Elemental determinations (µg/gram/tissue) were performed in testicular tissue samples using the atomic emission method.

Results: The highest cobalt, molybdenum, nickel, manganese, phosphorus, and sodium levels (p<0.001) and the lowest potassium levels in the testicular tissue were obtained in the pinealectomy group (group 3) (p<0.001). Magnesium and selenium values in testicular tissue were highest in the pinealectomy group (group 3) (p<0.001), and were higher in the pinealectomy+melatonin group (group 4) than in group 1 (control) and group 2 (melatonin) (p<0.001). Testicular zinc levels were highest in group 2, where melatonin was administered, and lowest in group 3, which was the pinealectomy group (p<0.001).

Conclusion: The findings obtained as a result of the study show that pinealectomy significantly disrupts element metabolism in the testicular tissue of rats, and melatonin supplementation may have a regulatory effect on testicular elemental metabolism.

Keywords: Pinealectomy; melatonin administration; testicular elemental metabolism; rat.

INTRODUCTION

Melatonin (MEL) is a hormone produced mainly by the pineal gland in the brain, which is defined as a neuroendocrine organ [1]. MEL, which plays a role in the sleep-wake cycle, is also a powerful antioxidant that clears free radicals directly and indirectly [2]. MEL, which also serves as a control mechanism that prevents the early onset of puberty, is closely related to the reproductive system [3]. High concentrations of the MEL lead to a delay in biochemical reactions that regulate puberty in both girls and boys [4]. The results of some reports show that MEL can also be found in testicular tissue [5]. This situation occurs when MEL crosses the blood-testis barrier [5, 6]. The strong antioxidant effect of melatonin has also been shown to prevent testicular tissue damage caused by chemotherapeutic agents [7, 8]. MEL also has a protective effect on the testicles against local inflammatory processes and lipid peroxidation [9]. Therefore, it is recommended that melatonin can be used in the treatment of infertility [10]. As a result, MEL, as an endogenous antioxidant, emerges as a strong candidate for the treatment of functional disorders caused by testicular tissue damage in the male reproductive system [11]. In addition to its known effects, there are limited publications on how MEL affects element metabolism in testicular tissue [11]. In these limited publications, it is mostly related to zinc metabolism, which is an important trace element [12]. The relationship between melatonin and elements is either the prevention of oxidative stress caused by some toxic elements in the testicular tissue with MEL support [13] or the combined application of some antioxidant elements with MEL in the
prevention of oxidative stress [11, 14]. Although the effects of the pineal gland, an important neuroendocrine gland, on the male reproductive system are known, there is almost no research on how it affects the element metabolism in the testicular tissue. This study focused on the relationship between the pineal gland and testicular tissue.

MATERIALS AND METHODS
Animal Material and Groups
This study was carried out at Yeditepe University Experimental animals were obtained from the Experimental Medicine Research Institute of the same university. The study was conducted on Spraque-Dawley adult male rats. The study protocol was approved by the local ethics committee of the same center (21122009). A total of 32 male rats were used in the study and the animals were divided into 4 equal groups.

Group 1, Control: The group in which no application is made.

Group 2, Melatonin: Animals in this group were provided with melatonin support. MEL was given intraperitoneally (ip) (3 mg/kg/day for 4 weeks)

Group 3, Pinealectomy: The pineal glands of the animals in this group were removed under general anesthesia in order to create melatonin deficiency.

Group 4, Pinealectomy+Melatonin: Animals whose pineal glands were removed under general anesthesia were provided with MEL support via ip (3 mg/kg/day for 4 weeks).

Experimental Animals and Their Nutrition
Experimental animals were fed standard rat chow and tap water without dietary restrictions. At the end of the experimental phases, the animals were sacrificed and testicular tissue samples were taken for elemental analysis.

Sacrifice of Animals and Collection of Tissue Samples
After the completion of the experimental stages of the study, all animals were sacrificed under general anesthesia and serum samples were taken. General anesthesia was administered to all animals (with intramuscular administration of a combination of Ketalar (60 mg/kg), Parke-Davis and xylazine (5 mg/kg) "Rompun, Bayer") to avoid animal suffering. Testicular tissue samples taken from animals were stored at -80°C until analysis.

Experimental Applications
MEL Application: Melatonin was commercially available (Sigma M-5250). Stock solution was prepared by dissolving melatonin in pure ethanol. A dose of 3 mg/kg MEL was prepared daily from the stock solution. MEL was injected into the peritoneal cavity of the animals in Groups 2 and 4 daily for 4 weeks [15].
**Pinealectomy**

Two methods attract attention in preventing the functions of the pineal gland in rats. The first of these is to suppress the function of the pineal gland by creating strong artificial light at night. The second method is to surgically remove the pineal gland. Surgical method was preferred in the current study. Pinealectomy was performed under general anesthesia in accordance with the method determined by Kuszack and Rodin [16]. A 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 was used to perform pinealectomy operations.

The head of the experimental animal was placed in the stereotactic device. After the top of the skull skin was shaved, a 1.75 cm medial incision was made longitudinally, reaching the occipital protrusion. The periosteum of the bones where the sagittal and lambdoid sutures were located was scraped down to the temporal muscles. Then, with a dental drill, the skull bone was cut rostrocaudally, approximately 1.25 cm rectangularly and 0.75 cm mediolaterally. The bone fragment on the superior sagittal vein, transfer sinuses, and confluent sinus was held from the rostral angle and lifted. The dura was cut from the mediorostral edge of the transfer sinus along the lateral edges of the sagittal vein. Two ligatures were placed on the superior sagittal vein with 6-0 atraumatic silk, 1 mm apart. The sagittal sinus was cut between the two ligatures, and its posterior part was cut following the dissection of the dura until the pineal gland was exposed. The pineal gland was removed from the anterior side by grasping the stem with a thin-tipped forceps. Then, both ends of the sagittal vein were tied together and the skull skin was sutured with 5-0 silk.

**Biochemical Analysis**

**Elements Analyzes in Testicular Tissue**

Testicular tissue samples taken from experimental animals were placed in polyethylene capped tubes washed with NHO3 and deionized water to prevent contamination. Samples were stored at -80 C until the day of analysis. Then, the tissue samples were crushed into powder in a mortar and the wet weight of the tissue was recorded. Concentrated H2SO4 and concentrated HNO3 were added (gram tissue /ml H2SO4 / ml HNO3 = 1 / 1 / 10). It was kept in a closed system microwave oven (CEM – Marsx5) at 170 psa pressure and 200C for 20 minutes. Then, the final volume was filled to 25 ml with deionized water and the samples were read by waiting for a maximum of half an hour. Analysis process, S. Ü. It was carried out on the Atomic Emission (ICP – AES Varian Australia Pty LTD, Australia) device located in the Soil Department of the Faculty of Agriculture. Results were calculated as µg/gram tissue.

**Statistical Analysis**

Statistical evaluation of the results was made with SPSS 22.0 statistical software. Statistical analysis was performed with a computer package program. The following tests were applied respectively.

1. Arithmetic means and standard errors of all parameters were calculated.

2. Analysis of variance was applied to determine the differences between groups.

3. Least Significant Difference (LSD) Test was used to compare group averages in the variance analysis results that were found to be statistically significant. Differences at P<0.05 were considered significant.
RESULTS

No significant difference was detected between chromium, lead, calcium, sulfur, copper and iron parameters in the testicular tissue of the study groups.

The highest levels of cobalt, molybdenum, nickel, manganese, phosphorus and sodium in the testicular tissue were obtained in the pinealectomy group (group 3; *p<0.001*). Group 3 also had the lowest potassium levels (*p<0.001*).

Again, the highest magnesium and selenium values in testicular tissue were detected in group 3 (*p<0.001*). The same parameters were higher in group 4 than in groups 1 and 2 (*p<0.001*). Testicular zinc levels were highest in group 2, where MEL was applied, and lowest in group 3, where pinealectomy was applied (*p<0.001; Table 1-4).

Table 1. Cobalt, Molybdenum, Chromium and Nickel Levels in Testicular Tissue of Study Groups (μg/g)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cobalt</th>
<th>Molybdenum</th>
<th>Chrome</th>
<th>Nickel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>0.14±0.04B</td>
<td>0.30±0.17B</td>
<td>0.41±0.09</td>
<td>0.98±0.10B</td>
</tr>
<tr>
<td>Melatonin (G2)</td>
<td>0.15±0.09B</td>
<td>0.29±0.09B</td>
<td>0.40±0.07</td>
<td>0.98±0.20B</td>
</tr>
<tr>
<td>Px (G3)</td>
<td>0.24±0.10A</td>
<td>0.80±0.47A</td>
<td>0.38±0.04</td>
<td>2.09±1.30A</td>
</tr>
<tr>
<td>Px+Melatonin(G4)</td>
<td>0.14±0.06B</td>
<td>0.28±0.13B</td>
<td>0.37±0.04</td>
<td>1.02±0.50B</td>
</tr>
</tbody>
</table>

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

Table 2. Manganese, Magnesium, Lead and Phosphorus Levels in Testicular Tissue of Study Groups (μg/g)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Manganese</th>
<th>Magnesium</th>
<th>Lead</th>
<th>Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>0.54±0.07B</td>
<td>87.28±12.29C</td>
<td>0.11±0.04</td>
<td>275.2±99.8B</td>
</tr>
<tr>
<td>Melatonin (G2)</td>
<td>0.53±0.05B</td>
<td>84.60±14.57C</td>
<td>0.12±0.04</td>
<td>278.4±86.4B</td>
</tr>
<tr>
<td>Px (G3)</td>
<td>0.75±0.07A</td>
<td>128.83±8.76A</td>
<td>0.11±0.05</td>
<td>133.6±53.5A</td>
</tr>
<tr>
<td>Px+Melatonin(G4)</td>
<td>0.52±0.05B</td>
<td>110.00±8.30B</td>
<td>0.11±0.05</td>
<td>273.9±57.0B</td>
</tr>
</tbody>
</table>

*Means with different letters in the same column are statistically significant (*P<0.001*). (A> B>C)
Table 3. Potassium, Sodium, Sulfur and Calcium Levels in the Testicular Tissue of the Study Groups (μg/g)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Potassium</th>
<th>Sodium</th>
<th>Sulfur</th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>1360.7±87.0^B</td>
<td>1237.5±78.4^A</td>
<td>865.7±79.3</td>
<td>74.41±17.95</td>
</tr>
<tr>
<td>Melatonin (G2)</td>
<td>1372.3±94.9^A</td>
<td>1241.6±82.5^B</td>
<td>855.8±78.6</td>
<td>82.43±20.20</td>
</tr>
<tr>
<td>Px (G3)</td>
<td>980.9±57.4^B</td>
<td>1987.6±97.8^A</td>
<td>864.9±80.3</td>
<td>86.54±30.42</td>
</tr>
<tr>
<td>Px+Melatonin(G4)</td>
<td>1356.5±93.6^A</td>
<td>1258.9±92.2^B</td>
<td>849.3±69.6</td>
<td>83.10±25.59</td>
</tr>
</tbody>
</table>

*Means with different letters in the same column are statistically significant (P<0.001).

(A> B)

Table 4. Copper, Iron, Selenium and Zinc Levels in Testicular Tissue of the Study Groups (μg/g)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Copper</th>
<th>Iron</th>
<th>Selenium</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>2.97±0.54</td>
<td>22.50±2.30</td>
<td>1.71±0.53^C</td>
<td>23.17±4.70^B</td>
</tr>
<tr>
<td>Melatonin (G2)</td>
<td>2.77±0.18</td>
<td>23.20±3.15</td>
<td>0.70±0.35^B</td>
<td>29.63±2.85^A</td>
</tr>
<tr>
<td>Px (G3)</td>
<td>2.55±0.86</td>
<td>22.68±2.70</td>
<td>4.70±1.66^A</td>
<td>11.45±1.58^C</td>
</tr>
<tr>
<td>Px+Melatonin(G4)</td>
<td>2.74±0.11</td>
<td>23.40±2.55</td>
<td>1.80±0.62^B</td>
<td>24.62±2.54^B</td>
</tr>
</tbody>
</table>

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

DISCUSSION

The highest levels of cobalt, molybdenum, nickel, manganese, magnesium, selenium, and phosphorus in testicular tissue were obtained in the pinealectomy group (group 3). In Med-line searches, we could not find a publication with which we could directly compare our study in terms of the effect of the pineal gland on the element metabolism in the testicular tissue. Publications on the effects of the pineal gland and MEL on the metabolism of body elements other than testicular tissue are also very limited. It has been reported in a few publications that MEL has a regulatory effect on elemental metabolism in the body [17, 18]. Consistent with this, it has been shown that body element metabolism is impaired in rats after pinealectomy [19]. The high levels of cobalt, molybdenum, nickel, manganese, magnesium, selenium, and phosphorus in the testis that we obtained in our study show that MEL deprivation after pinealectomy significantly changes the element metabolism in the testicular tissue and strongly supports the findings of the researchers whose reports are presented above. The fact that MEL application after pinealectomy (group 4) returns the levels of the mentioned elements to control values shows the regulatory effect of MEL on the element metabolism in testicular tissue. Again, the highest sodium levels and lowest potassium levels in testicular tissue were obtained in the pinealectomy group (group 3). This finding we obtained in the testicular tissue is evidence that the sodium-potassium balance is disrupted in the testicular tissue after pinealectomy and is compatible with the reports of Mogulkoc and Baltaci [20] who reported that pinealectomy caused a deterioration in the
fluid-electrolyte balance in rats. In our study, we obtained the highest zinc level in testicular tissue in group 2, where MEL was applied, and the lowest zinc level in group 3, where pinealectomy was applied. Zinc, which is critical in the reproductive system [21], is the only metal found in almost every enzyme class [22]. The high concentration of zinc in both the testicles and glands of the male reproductive system is evidence of its critical importance in the reproductive system [23]. In rats, zinc deficiency causes an atrophic picture in the seminiferous tubules. Consistent with this, it also causes a deficiency in spermatogenesis [24]. Zinc is also critical in the physiological functions of sperm. Zinc is necessary for the integrity of the sperm membrane, regulation of sperm tail motility, and coordination of the spiral movements of the sperm tail [25].

There are also important relationships between zinc and MEL, which play an important role in testicular tissue and the male reproductive system [24]. While MEL increases the absorption of zinc, an important trace element, from the digestive system [26], on the contrary, pinealectomy results in zinc deficiency in the body [27, 28]. In our study, the low zinc levels we obtained after pinealectomy (group 3) or the high zinc levels we obtained after MEL application alone (group 2) are not only evidence of a significant and positive relationship between the pineal gland and zinc, but are also compatible with the studies whose findings are presented above. Defined as metal binding proteins, metallothioneins (MT) are low molecular weight proteins that play an important role in protecting against metal toxicity [29, 30]. The presence of specific binding sites for MEL in the intestines suggests that the pineal gland may be a fundamental mechanism in regulating the absorption of elements in the digestive system [31, 32]. In our study, the altered elemental metabolism in the testicular tissue obtained by pinealectomy or MEL application may be related to the altered elemental absorption in the gastrointestinal tract.

Taken together, the results of our study showed that pinealectomy significantly disrupts elemental metabolism in the testicular tissue of rats, and MEL application may have a regulatory effect on testicular elemental metabolism. The current study is the first to consider elemental metabolism in the testicular tissue combined with the pineal gland and MEL. The results of the current study are at a level that will provide additional contributions to the known relationship between the pineal gland and the male reproductive system in terms of element metabolism.

Limitations
The limiting factor in the current study is that the relationship between the pineal gland and testicular tissue could not be demonstrated with various melatonin doses and application times. Addressing this gap in future studies may provide us with new and critical information.

CONCLUSION
Taken together, the results of our study highlight the important relationship between pineal gland and testicular element metabolism. This study is the first to investigate pineal gland and testicular element metabolism as a whole in rats.
REFERENCES


