

## Melatonin secretion profile after experimental pineal gland compression in rats\*

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### Abstract

**OBJECTIVES:** Pineal gland hormone, melatonin, is a current issue of interest for accumulating data concerning its diverse physiological functions. The disturbances in melatonin secretion are observed in different pathological conditions involving pineal regions, but it is not ascertained if those disturbances present any clinical implications.

The aim of this work was to examine whether pineal gland compression changes melatonin secretion.

**SETTING AND DESIGN:** The experiment was carried out on adult rats, divided into four equal groups: (i) control (no surgery was performed), (ii) sham-operated, (iii) with sham pineal gland compression and (iv) with pineal gland compression performed by cotton piece application.

**METHODS:** The profile of melatonin secretion was assessed in blood samples collected five times daily, every second day, starting from 8 to 14 day following surgery.

**RESULTS:** We found that surgery itself significantly increased night melatonin secretion in comparison to controls. By contrast, in pineal-gland compressed rats, melatonin secretion was lower than in control group, suggesting that the influence of pineal compression overcame that induced by operation stress.

**CONCLUSIONS:** In conclusion, we presume that pineal gland compression (like in case of some tumors) results in decrease of the concentration of blood melatonin, that may possibly result in decreased protective action of the indoleamine.

## Introduction

Melatonin, the hormone produced and released by pineal gland, is a key regulator of circadian rhythm, core body temperature and pubertal development. It is secreted rhythmically under the control of light/dark cycle and hypothalamo-pituitary axis [1]. The production of melatonin increases at night and decreases during the day; and even dim light put on at night can inhibit melatonin production [2]. Melatonin secretion was reported to be attenuated in severe sleep disorders and in some of bipolar affective diseases [3, 4, 5]. Recently, accumulating data suggest that melatonin may also contribute to free radicals scavenging, stress reactions and, via modulating linoleic acid metabolism, tumor growth [2, 5, 6, 7]. The changes in melatonin secretion in various pathological conditions, however, are not fully clarified. Among them, the lesions concerning pineal region seem to be of special interest.

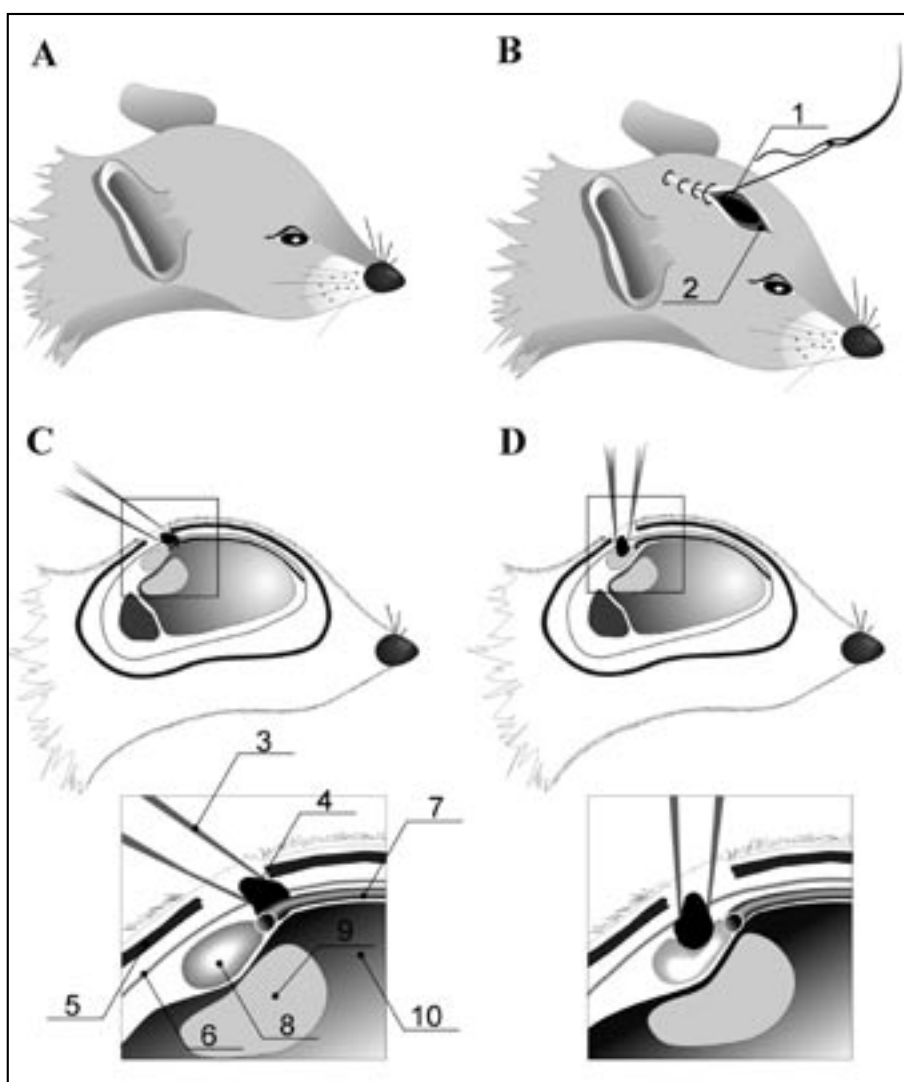
Tumors of the pineal region are not very common, but the frequency of recognition still grows due to the progress in novel radiological techniques, especially computed tomography and magnetic resonance imaging [8]. These tumors are diverse in histology and natural history, therefore, different treatment should be applied in individual cases. It is established that some of them, like pineocytoma, need aggressive surgical treatment; others, like germinomas, are radiosensitive and could be treated mainly with radiotherapy [9, 10, 11]. Non-germinomatous germ-cell tumors are considered to be treated first by chemotherapy,

and surgery is only an adjuvant therapy [12]. Unfortunately, it is usually very difficult to predict tumor biology on the basis of neuroimaging and clinical features. There is also a marked group of pineal cysts with no recommended therapy. The differential diagnosis between the simple cysts and neoplasm is often very difficult and uncertain.

Any tumor of pineal region can compress pineal gland, but the influence of such compression on melatonin secretion is not ascertained. Our previous works revealed no correlation between melatonin secretion and histological type of tumor [13]. However, there are some reports that pineal parenchymal tumors can stimulate secretion of melatonin while in other lesions, like teratoma, hypothalamic hamartoma or pineal cysts, very low values of melatonin are found [5, 14].

Finally, pineal gland can be compressed indirectly, by increased intracranial pressure [15]. This was shown in cases of intracranial tumors with marked midline shift [16]. As decreased level of melatonin was recently reported to promote some neoplasms growth, it seemed to be of special importance to clarify if a compression of pineal gland influences melatonin secretion [17, 18].

**Fig.1.** Schematic presentation of procedures performed in individual groups: **A** – no surgery, **B** – sham operation (skin incision and trepanation above the sinus confluence only without any penetration below the level of dura mater; 1 – trepanation hole, 2 – the wound; **C** – in group III the cotton piece was placed under the dura mater, in front of pineal gland, without compressing it; enlarged box shows detailed topography of pineal gland and the cotton piece localization: 3 – surgical forceps, 4 – cotton piece, 5 – skull bone, 6 – dura mater, 7 – sinus confluens, 8 – pineal gland, 9 – tectum, 10 – brain; **D** – in group IV the cotton piece was placed exactly on the dorsal surface of pineal gland, to compress it. In the box – the detailed topography of the gland and the cotton piece.



## Methods

The study was carried out on 40 male Sprague-Dawley rats weighting 150–200 g each, according to the European Council Directive regarding care and use of laboratory animals and they were approved by the Ethics Committee of the Medical University of Silesia. During the whole experiment, the rats were housed in individual cages, at the room temperature of  $21 \pm 1^\circ\text{C}$ , stable air humidity and a 12-h light/dark cycle. Animals had free access to both standard rodent laboratory food and tap water. All the operations were performed between 8:00 a.m. and 10:00 a.m. Animals were randomized into four groups of ten rats each, namely:

- I. control group (no surgery was done)
- II. sham surgery group
- III. sham pineal gland compression group
- IV. pineal gland compression group

In group II – IV the animal were anesthetized using intraperitoneal injection of Ketamine (100 mg/kg) and 2% solution of Xalazin. In group II we performed skin incision and trepanation above the sinus confluence only, without any penetration below the level of dura mater (sham surgery). This group was constituted to eliminate influence of surgery-induced stress on melatonin secretion when considering the experiment results. In group III and IV, we modified Hoffman and Reiter [19] method to reach the pineal gland. In group IV, pineal gland compression was performed by implantation of the 3 mm diameter, ball-shaped piece of cotton to the region of the gland. In order to verify the influence of cotton implanted intracranially on the results obtained in this study, in group III (sham pineal gland compression) we put a piece of cotton of the same

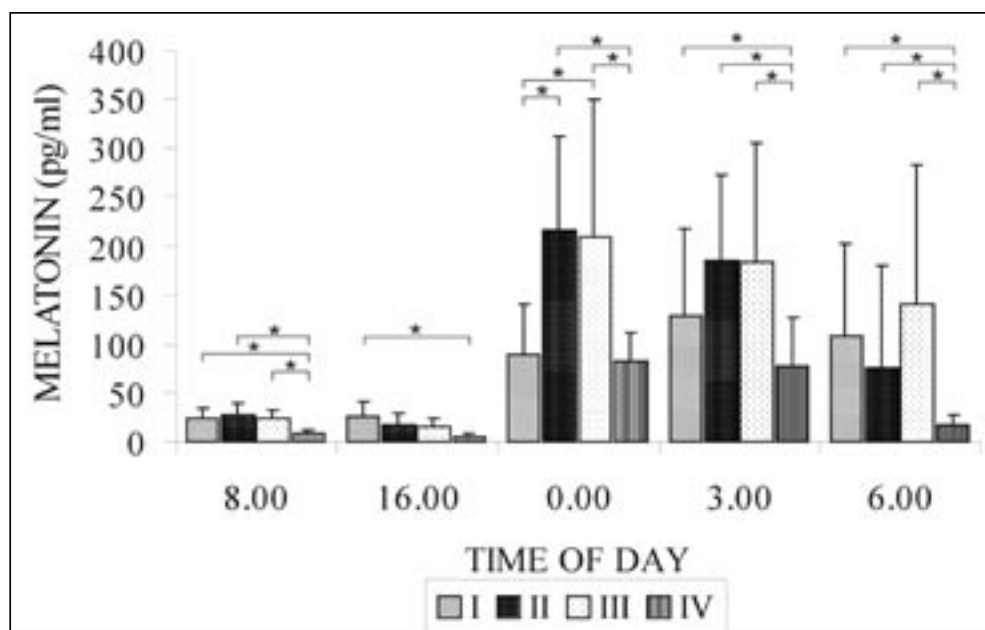
volume to the subdural space, avoiding the compression of pineal gland [Figure 1]. The cotton piece was marked with meningeal clips and their localization was confirmed by X-ray examination.

The blood samples of 0.4 ml were collected at 8.00 a.m., 04.00 p.m., 0.00 a.m., 3.00 a.m. and again 6.00 a.m. from all animals for 7 days. In groups II–IV, first blood samples were collected from the 8 to 14 day after surgery. Animals were anesthetized by halothane inhalation and blood samples were taken by the left cardiac ventricle puncture according to the method described previously by Dauchy et al. [2]. Each animal was tapped once a day every second day to minimize mortality and procedure-induced stress. The samples were immediately centrifuged and the serum stored at  $-20^\circ\text{C}$  until analysis. The sampling at 0.00 a.m. and 3.00 a.m. was performed under the red light (15 W), to not to introduce extra illumination that could disturb melatonin secretion. Serum melatonin concentrations were measured in duplicate by commercially available radioimmunoassay kits.

Data are presented as means  $\pm$  SD and compared by use one-way analysis of variance ANOVA followed by Tukey post-hoc test. Statistical significance was set at  $P < 0.05$ .

## Results

The number of failures in our study was relatively low (less than 20%). No wound inflammation symptoms were observed. Results of melatonin concentrations for each group are presented at Figure 2. In all groups we found melatonin level arranged in characteristic curve with night peak secretion. The values, however, differed in individual groups. We found that pineal gland compression resulted in significantly



**Fig.2.** Histogram showing plasma melatonin concentration in individual groups (I – control group, II – sham surgery group, III – sham pineal gland compression group, IV – pineal gland compression group) at 8.00, 6.00, 0.00, 3.00 a.m. and 4.00 p.m. Asterisks show significant differences ( $p < 0.05$ ) in melatonin concentration between groups at given time-point. Data as shown as mean  $\pm$  SD.

lower night and early morning (3.00 a.m. and 6.00 a.m.) melatonin secretion in comparison to other groups. At midnight, compressed pineal gland secreted the same amount of melatonin as controls. Day (8.00 a.m. and 4.00 p.m.) melatonin secretion was lower in group IV than in control. Interestingly, in the group with surgical procedure not involving pineal gland as well as group with false pineal gland compression, the concentration of plasma melatonin at night and in the morning (0.00 a.m., 3.00 a.m., 6.00 a.m., 8.00 a.m.) was significantly higher than in IV group. At midnight, secretion (0.00 a.m.) of melatonin in these groups was also markedly increased in comparison to control.

## Discussion

Our model of experimental pineal gland compression was stated to confirm the hypothesis that tumors compressing the pineal gland diminish melatonin secretion. We paid special attention to not to destroy the gland so that we might obtain an experimental model of situation present in cyst or benign tumor of pineal gland region. We found that pineal gland compression resulted in the significant decrease in morning melatonin secretion in comparison to all the other groups. In the animals that underwent sham operations (II and III groups) the level of melatonin was markedly increased at night and in the morning. This may be due to stress induced by anesthesia and surgery. The reports on melatonin secretion changes in answer to stressors are conflicted. Nishimura et al. [20] reported normal melatonin secretion in 6 patients that underwent major surgical procedures, whereas Dempsey and Cahndler [21], analyzing 15 patients with intrasellar tumors, found alterations in melatonin production caused by hospitalization and operations. These discrepancies may result from different procedures of sampling and different time-lapse of the studies. Our results seem to support the thesis that melatonin secretion increases in an answer to stressors. Moreover, the increase of melatonin secretion in our experiment was more prominent at night, suggesting a specific way of regulation. The role of melatonin in stress phenomenon, however, requires further studies.

In animals with compressed pineal gland, although they underwent surgical procedure of severity similar to rats from II and III groups, we found decreased secretion of melatonin. It was significantly lower even than in control group during whole observation period, besides at midnight. This suggests that pineal gland compression caused the disturbances in melatonin secretion strong enough to overcome the influence of stress. Several explanations can be considered; among them, it cannot be excluded that compression caused at least partial destroy of pineal gland. The existing studies on the melatonin secretion in cases when pineal gland destruction occurs are ambiguous Vorkapic et al. [14] as well as Neuvelt at al. [11] revealed low levels of melatonin in pineal parenchymal tumors that destroyed the gland. Neuvelt and Levy [22] showed

that lack of melatonin in serum after pineal tumor surgery proved the completeness of pinealectomy and tumor removal. Webb and Puig-Domingo [5] showed low values of melatonin in lesions causing destruction or compression of pineal gland, like teratoma or hypothalamic hamartoma. On the other hand, in meningitis and some tumors invading pineal gland, the increased level of melatonin was reported [16]. It is likely that the changes in melatonin secretion depend on the time of compression leading to pineal gland destruction: initially, the increasing compression of pineal gland increases the melatonin concentration in blood by releasing the melatonin stored earlier, with the resulting decrease of melatonin secretion caused by the progressing destruction of the gland parenchyma [23]. In order to minimize stress reaction influence on our experiment, we started to measure melatonin blood concentrations few days after the surgery and presumably omitted the first phase mentioned above. Nevertheless, our investigations indicate that in the animal model, the direct pineal gland compression causes a significant reduction of melatonin secretion both during the day and during the night time, with preserved in tendencies but changed in values, diurnal rhythm. This rhythm was reported by Grimoldi et al. [24] to be conserved in benign tumors and depressed in more aggressive lesions like pineoblastoma, lymphoma or some germinomas.

Interestingly, in the rats with compressed pineal gland, the decrease in melatonin secretion was observed during a day, especially marked in the morning. The differences between the groups IV and II as well as III were also very distinct in the morning. The secretion of melatonin decreases physiologically during a day, mainly due to inhibitory influence of optic system on pineal gland [5]. The mechanisms in such a deep decrease in compressed pineal gland, however, remain elusive. The topography of pineal gland differs from in humans and rats. In rats, pineal gland is located superficially on the dorsal surface of the brain [25]. Application of a cotton piece and resulting pineal gland compression could evolve some disturbances in blood supply of the structures regulating melatonin secretion. Distance between pineal gland and some optic pathway formations is close, so we also cannot exclude their direct compression that could have resulted in melatonin secretion disturbances. Other possible explanation is that compression somehow disconnected pineal gland from its regulatory structures. Commentz and Helmke [26] revealed decreased melatonin values in a case of hypothalamic hamartoma. They speculated that it was caused by the interrupted connection between the suprachiasmatic nucleus and pineal gland. It is commonly accepted that ablation of the suprachiasmatic nucleus in rats eliminates both inhibitory and stimulatory input to the melatonin rhythm generating system [27].

The consequences of decreased melatonin secretion caused by pineal gland compression are not known. Very few reports show some sleep disturbances and other psychiatric symptoms in patients with extremely

low levels of melatonin [4]. Concerning the new insight into melatonin role, however, we presume that these consequences can be diverse and, sometimes, of special clinical importance. In some malignant tumors the decreased level of melatonin was reported, but the background of this finding is not clear [17, 18]. Based on the experimental data, the role of melatonin deficiency in malignant tumors pathogenesis should be considered.

## Conclusion

Our results showed that pineal gland compression (like in case of some tumors) results in decrease of the concentration of blood melatonin, that may possibly result in decreased protective action of the indoleamine.

It sheds new light on possible biochemical symptoms of pineal region tumors as well as pathology of melatonin secretion. Further studies should be undertaken to elucidate the consequences and therapeutic implications of disturbed melatonin secretion in pineal gland lesions.

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