ORIGINAL ARTICLE

Possible effects of melatonin on thymus gland after pinealectomy in rats

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Abstract

OBJECTIVES: The purpose of this study was to investigate the effects of pinealectomy and pinealectomy plus melatonin administration on thymus weight and histology in adult Wistar-albino rats.
METHODS: The animals were divided into three groups. Group I and Group II were designated as control (sham-pinealectomized) and pinealectomized rats, respectively. They received 10% ethanol (0.1 ml per day s.c.) alone. The rats in Group III were pinealectomized and daily injected with melatonin (3 mg/kg/0.1 ml 10% ethanol per day s.c.) commencing on the day seven after surgical operation. Injections were applied for two months.
RESULTS: The thymus atrophied and its weight decreased after pinealectomy ($p<0.001$). The cortico-medullary boundary could not be distinguished and in the thymus induced a loss of lymphoid elements, increased number of phagocytic macrophages and enlarged blood vessels. Melatonin prevented the thymic involution.
CONCLUSIONS: These results suggest that pinealectomy decreases thymus weight and that long-term administration of melatonin restores thymus weight to normal levels.

Introduction

Melatonin is a neurohormone released by the pineal gland. It is well known that melatonin is continuously synthesized and secreted during a period of continuous darkness, and that its synthesis and secretion are inhibited during a period of continuous light [1–3].

A series of experimental studies have suggested that there is a functional relationship between the pineal gland and the immune system. The first evidence of this was reported by Csaba and Barath [4]. The pineal gland, via its hormone melatonin, increases immune functions [5, 6]. It was shown that melatonin had a strong stimulating effect on the lymphocytes [7]. It was reported that the main activity of melatonin on immune functions in humans might be T lymphocytes and macrophages [8, 9]. The surgical and pharmacological inhibition of melatonin decreases humoral and cell-mediated immune responses [10–12]. Pinealectomy causes atrophy in the thymus gland [4] and a decrease

in interleukin-2 production and natural killer cell activity [13]. On the contrast the administration of melatonin occurs hyperplasia in the thymus gland [4] and an increase in the antibody response [14]. Both in vivo and in vitro studies have shown that the administration of melatonin inhibits programmed cell death in the thymus gland [11, 15].

The purpose of this study was histologically to examine the effect of melatonin on the thymus gland following pinealectomy in the rats.

Materials and methods

Adult male Wistar rats weighing about 180–200 g each (aged 8–10 wk, n= 15) were used in this study. The animals were maintained at constant temperature (21±2 °C) and humidity (50±5%) on a 12 h light/12 h dark cycle (light on from 07.00 h to 19.00 h). They were housed in plastic cages (five rats per cage) and fed with standard pellet food and tap water *ad libitum*. The animals were divided into three groups. Group I (n=5) and Group II (n=5) was designated as control (sham-operated) and pinealectomized rats, respectively. They received 10% ethanol (0.1 ml per day s.c.) alone. The animals in the Group III (n=5) were pinealectomized and injected with melatonin (3 mg/kg/0.1 ml 10% ethanol per day s.c.; Sigma Chemical, Poole, UK) commencing on the day seven after surgical operation. Two months after beginning the treatment the animals were killed by ether anaesthesia. The thymus gland of all rats was removed, dissected free from adjacent connective tissue and weighed. Organ weights were expressed as g/100 g body weight. One lobe taken randomly was fixed for 2 h in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) at 4 °C for electron microscopic examinations. The specimens were post-fixed in phosphate-buffered 1% osmium tetroxide. Then they were dehydrated in graded solutions of ethanol and embedded in Epon 812. Thin sections cut on ultramicrotome were stained with uranyl acetate and lead citrate. The other lobe taken for the light microscopic examinations was fixed with 10% neutral formaline, dehydrated in ethanol, embedded in paraffin. The specimens sectioned at 5–6 μm were stained with hematoxylin and eosin. All specimens were examined in Carlzeiss-900 electron microscope and BH2 Olympus light microscope.

Statistical analysis was performed for the values of thymus weight. Results were expressed as Mean±S.D. Statistical significant was determined by using analysis of variance (ANOVA). When this was significant, Tukey's test (Honestly Significant Difference, HSD) was made to localize differences between group means.

Results

As shown in Table I, there was statistically a significant difference in the thymus weight between the groups. The thymus weight in the pinealectomized rats was significantly lower than both control and melatonin-treated groups ($p<0.001$). The thymus weight in the melatonin-treated rats was higher than that of sham-pinealectomized rats. But, there was no statistically a significant difference when compared with those in the sham-pinealectomized rats.

Table 1. Mean±SD weight of the thymus in control, pinealectomized, and pinealectomized plus melatonin-injected rats.

Groups	n	Mean±SD
Control	5	41.16±2.05
Pinealectomized	5	34.98±2.16*
Pinealectomized+Melatonin	5	46.62±2.61

*$p<0.001$ compared to the control group.

The thymus tissue of control groups showed normal structure at the light and electron microscopic levels (Fig. 1 and 6).

Under the light microscopy, pinealectomy induced a loss of lymphoid elements from the thymic cortex. Lymphocytes with pyknotic nuclei were abundant in the cortex. The cortico-medullary boundary could not be distinguished. After pinealectomy, an increase in number of phagocytic macrophages (Fig. 2), enlarged blood vessels (Fig. 3) and colloid accumulation in the some lobules (Fig. 4) were observed in the thymus. In general, normal histology reappeared in the thymus gland of melatonin-treated rats, although some lobules maintained signs of lymphocyte depletion. Hypertrophy was seen in the epithelial cells both in the cortex and the medulla. There were clear vesicles in the cytoplasm of epithelial cells (Fig. 5).

In the ultrastructural examinations, altered medium to large-sized lymphoid cells were observed in the thymic cortex after pinealectomy. There were numerous macrophages containing abundant phagocytic materials which included degenerated nuclei, electron dense bodies, etc. (Fig. 7). The thymus gland of melatonin-treated rats appeared ultrastructurally similar to control groups and contained mitotic figures (Fig. 8).

Fig. 1. Light micrograph of the thymus in control rats. C, cortex; M, medulla. Hematoxylin and eosin stains, x 20.
Fig. 2. Numerous large macrophages (M) and degenerated lymphocytes (arrows) seen in the thymic cortex after pinealectomy. Toluidine blue, x 100.
Fig. 3. Enlarged blood vessels (v) seen in the thymus of pinealectomized rats. Hematoxylin and eosin stains, x 10.
Fig. 4. Colloid accumulation (c) observed in the thymus of pinealectomized rats. Hematoxylin and eosin stains, x 10.
Fig. 5. Hypertrophied epithelial cells including clear vesicles (arrows) observed in thymic cortex after melatonin administration. Toluidine blue, x 100.
Fig. 6. Electron micrograph of the thymic cortex in control rats. L, lymphocyte; E, epithelial cell. Lead citrate and uranyl acetate, original magnification, x 3000.
Fig. 7. Degenerated lymphocytes (arrows) and macrophage cytoplasm containing phagocytic materials (M) in the thymus of pinealectomized rats. Lead citrate and uranyl acetate, original magnification, x 3000.
Fig. 8. Mitotic figure (MF) seen in thymic cortex of melatonin-treated rats. L, lymphocyte. Lead citrate and uranyl acetate, original magnification, x 7000.
(Publisher's note: Figures 83% of original size)

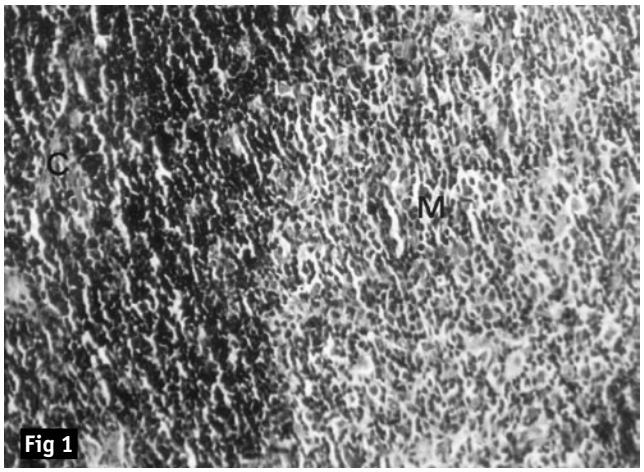
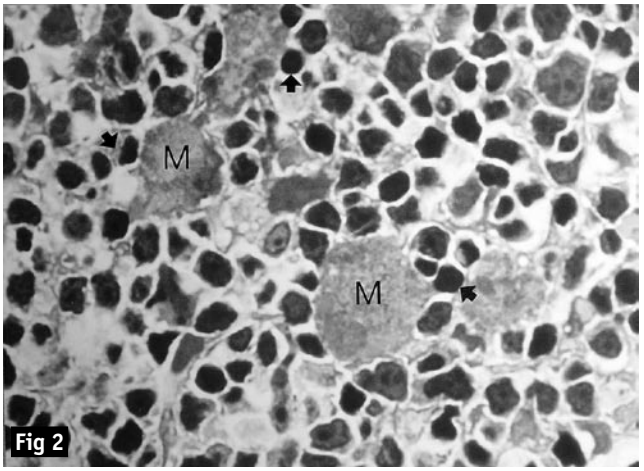


Fig 1



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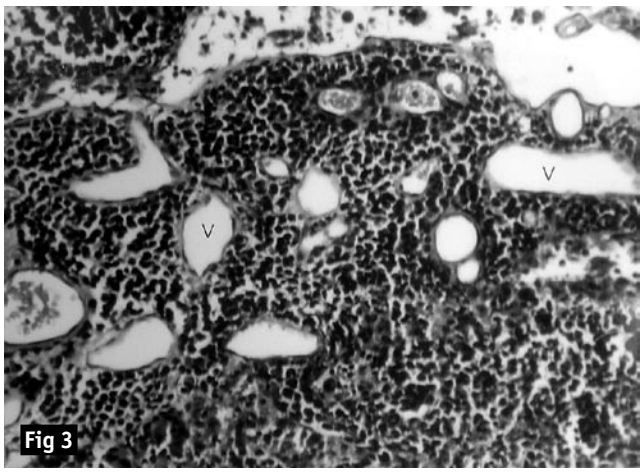

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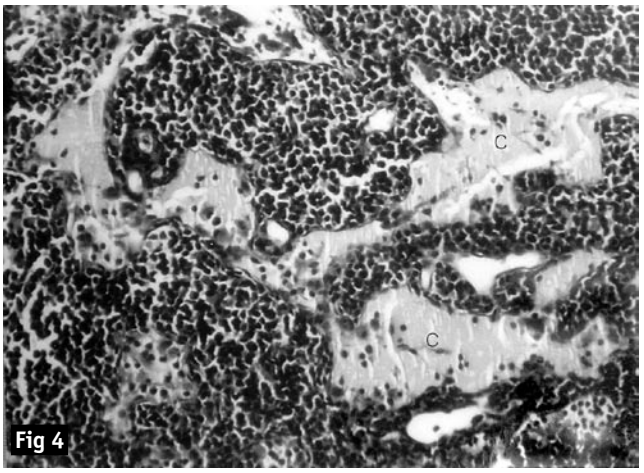


Fig 4

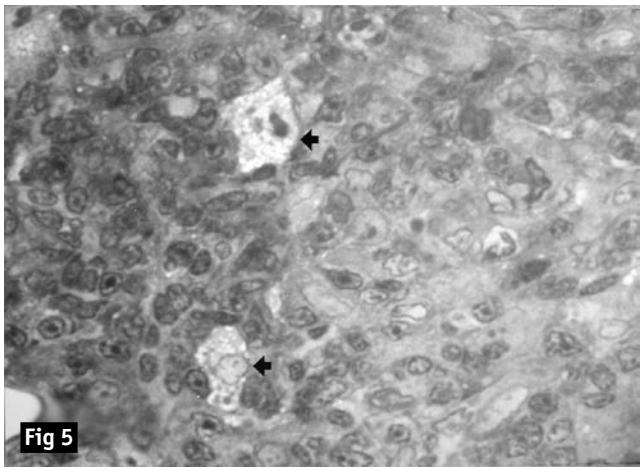
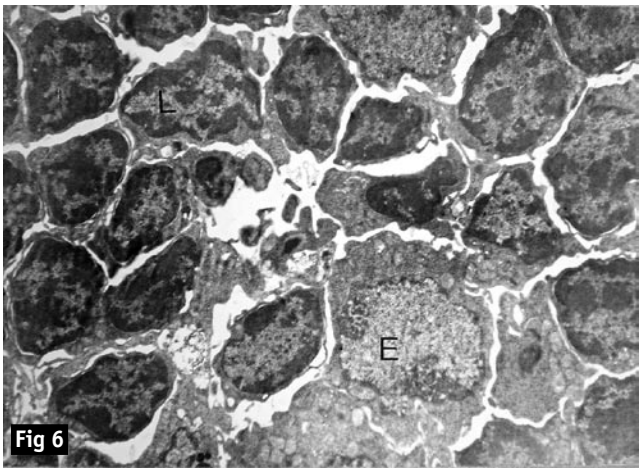


Fig 5



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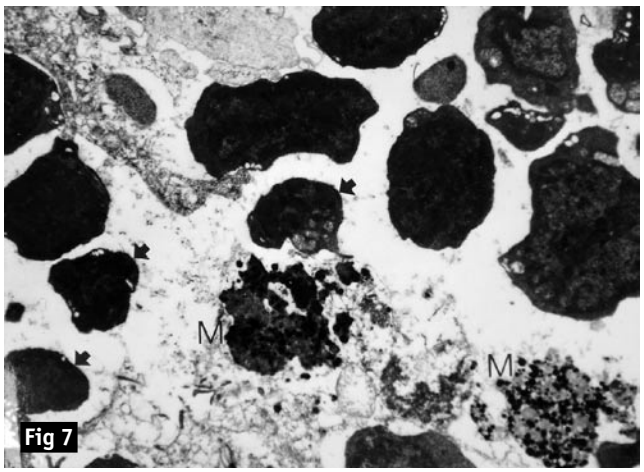

Fig 7

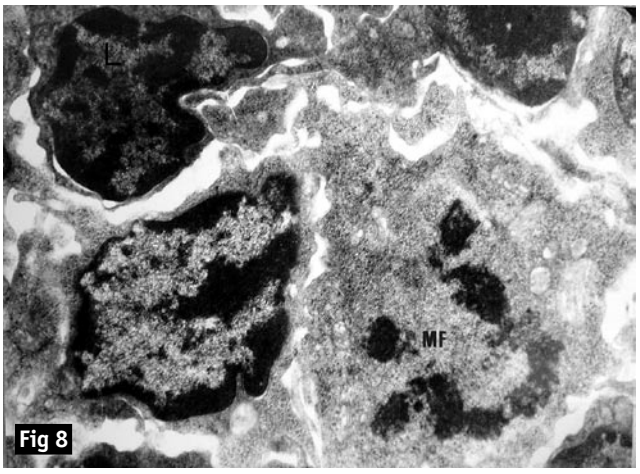

Fig 8

Discussion

Melatonin has an important role in the regulation of immune functions [16], but little information is available about structural changes that it causes in lymphoid organs.

The experimental studies showed that melatonin could affect the morphology of lymphoid tissues, particularly the thymus gland which is a central organ of the immune system, as well as immunoenhancing effect [4, 17, 18].

Csaba and Barath [4] have demonstrated that melatonin treatment causes thymic hyperplasia while pinealectomy induces thymic atrophy. Studies in aging have shown that melatonin treatment or pineal grafting in old mice prevents the thymic involution, and protects thymocytes from apoptosis [11, 18]. Provinciali et al. [11] have reported that the thymus weight and thymocyte number increase two-fold and three-fold respectively in 25–26 month-old mice, treated with melatonin or pineal grafting 8 months before, compared with untreated animals. Sainz et al. [15] have reported that the pineal neurohormone melatonin decreases by 35% the percantage of apoptotic cells induced by glucocorticoids in cultured thymocytes of 25 day old rats. It was reported that the drastic involution of the thymus induced by acute stress could be prevented by melatonin [19]. Similarly, we observed that lymphocytes degenerated after pinealectomy, and melatonin treatment prevented this both in light and electron microscopic levels.

Mahmoud et al. [17] have reported that the large thymic weight gain, an increase in the number of lymphocytes, an increase in mitotic activity of epithelial cells, hypertrophy in epithelial cells included the clear vesicles, and enlarged perivascular connective spaces, is seen during continuous darkness. In contrast, during the period of continuous light they observed the large thymic weight loss, a decrease in the number of cortical lymphocytes, and numerous pyknotic lymphocyte nuclei. Our study showed resemblance to above results, except the increased phagocytic macrophages, enlarged blood vessels, and colloid accumulation observed in the thymus after pinealectomy. Also we did not observe any mitotic activity in epithelial cells. The mitotic activity was available in cortical lymphocytes.

The thymic weight gain, increase in mitotic activity of lymphocytes, and the hypertrophy of epithelial cells seen in this study after melatonin treatment may be explained by the action of melatonin on both lymphocytes and epithelial cells. It is admitted that the clear vesicles observed in epithelial cells indicate the secretory activity of these cells, as reported by previous studies [15, 20, 21].

Previous studies have demonstrated that melatonin receptors are present in the thymus membranes of adult rats. It was found that melatonin receptors present on circulating lymphocytes and also on thymocytes and splenocytes [3, 6, 19]. It was reported that maximum binding was observed in newborn rats; there after, binding decreased progressively during the first weeks of life and exhibited the lowest values in adult rats [20].

In conclusion we suggest that pinealectomy causes thymic involution and that melatonin treatment following pinealectomy restores thymus weight to normal levels by binding receptors in the thymus.

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