# Melatonin and the Synthesis of Vasopressin in Pinealectomized Male Rats (44566)

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*Abstract.* **The pineal hormone, melatonin, is known to modify, under different experimental conditions, neurohypophysial hormone secretion in the rat. The aim of this study was to investigate the effect of melatonin on the vasopressin biosynthesis rate in the hypothalamus of either pinealectomized or sham-operated rats, using the colchicine method. To estimate whether colchicine affects the function of the neurohypophysis in these animals, the neurohypophysial and plasma vasopressin levels were also measured.The vasopressin synthesis rate was increased after pineal removal, when compared with sham-operated animals, and melatonin strongly inhibited the rise in the hormone synthesis due to pinealectomy. After pineal removal plasma vasopressin concentration was significantly elevated, and melatonin attenuated this effect. On the contrary, the neurohypophysial vasopressin content was significantly decreased after pinealectomy, but it was not further modified by melatonin.Thus, melatonin suppresses the synthesis and secretion of vasopressin in pinealectomized rats. The present results confirm our previous reports as to the inhibitory impact of the pineal on both vasopressin synthesis and release and suggest that melatonin may mediate the effect of the pineal gland on vasopressinergic neuron activity.**

[P.S.E.B.M. 2000, Vol 225:207–210]

Growing evidence suggests the influence of the pineal gland and its hormone—melatonin—on neurohypophysial hormone secretion. Pinealectomy as well as melatonin have been found to modify the vasopressin and/or oxytocin release from the hypothalamo-neurohypophysial system both *in vivo* (1–3) and *in vitro* (4–6). Melatonin was also reported to modify the neurohypophysial hormone secretion in response to several physiological or pathological stimuli such as suckling, parturition, hypovolaemia, plasma hyperosmolality, or stress (1–3, 7, 8), all of which are known to change hormone biosynthesis and secretion (9–11). Moreover, a reduced Fos (a protein product of the immediate early gene c-*fos*) production in the supraoptic nuclei was found to accompany the pinealectomy-induced diminution of neurohypophysial hormone secretion into the blood in response to hyperosmotic stimulation (12). Therefore, the main hypothesis underlying this study was that melatonin possibly affects not only the vasopressin secretion into the blood, but also its biosynthesis in the hypothalamus.

Vasopressin is synthesized within the perikarya of magnocellular neurons of the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei. The newly produced pool of the hormone is then transported along axons of the magnocellular neurons toward the neurohypophysis (13, 14). If the axonal transport is blocked, the newly synthesized vasopressin accumulates within the hypothalamus. Colchicine was found to induce a complete blockade of transport of the newly formed secretory material from the perikarya to the axonal terminal in the neurohypophysis (13, 15). Intracerebroventricular (icv) administration of a small amount of colchicine results in accumulation of vasopressin within the perikarya, increasing progressively with the passage of time (13–15). The accumulation of vasopressin over a constant time period in the hypothalamus of colchicine-injected rats in comparison with untreated animals is considered to be an index of the neurohormonal biosynthesis rate (14, 15).

This work has been supported by the State Committee for Scientific Research (Poland), grant no. 4.P05 A.104.12 (contract no 546/P05/97/12).
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Received November 23, 1999. [P.S.E.B.M. 2000, Vol 225]
Accepted July 11, 2000.

0037-9727/00/2253-0207$15.00/0

Therefore, to test the hypothesis underlying this study (i.e., that melatonin possibly affects the vasopressin biosynthesis in pinealectomized male rats) the colchicine method was applied.

## Materials and Methods

Male rats of the Wistar strain were housed at room temperature in a controlled light:dark cycle (lights on from 0600 to 1800), with food and water available *ad libitum*. Rats were sham-operated (SO) or pinealectomized (PX) according to the procedure described by Kuszak and Rodin (16). On the next day after surgery, the following subgroups of animals were selected: (i) rats injected intraperitoneally (ip), once daily for 14 days, with vehicle solution (VEH; 1% ethanol in 0.9% sodium chloride; 100 μl/100 g body wt; subgroups: SO-VEH, PX-VEH); (ii) rats similarly injected with melatonin (MLT; N-acetyl-5-methoxytryptamine; Sigma Chemical Co., St. Louis, MO) dissolved in the vehicle (50 μg MLT in 100 μl of solution per 100 g body wt; subgroups: SO-MLT, PX-MLT). All injections were made approximately 1 hr before lights off (i.e., about 1700 hr). Melatonin was injected at the end of light phase of the light:dark cycle because the endocrine response to such injection was found to be the most pronounced, due to the diurnal rhythm of MLT receptors (17, 18). Moreover, to avoid differences in vasopressin (AVP) levels due to the diurnal rhythm of hormone synthesis and release (19), all animals were sacrificed at the same time of day.

The day before decapitation, rats were randomly injected intracerebroventricularly (icv) with either 5 μl colchicine vehicle (i.e., 0.9% sodium chloride) or 5 μg/5 μl colchicine (Sigma, Chemical Co., St. Louis, MO) solution. The effectiveness of the colchicine method for studying the AVP biosynthesis in the hypothalamus has been discussed previously (15, 20).

Animals were decapitated 20 hr after colchicine or saline injection at 0830–0900 AM. The brain with the pituitary was removed from the skull, and the neurointermediate lobe was separated and homogenized in 0.25% acetic acid. A block of tissue containing the hypothalamus was dissected from the brain as described previously (1) and homogenized in 0.5% acetic acid. The trunk blood was collected, and AVP was extracted from the plasma using C18 Sep-Pak columns (Waters Associates Ltd., Northwick, Essex, UK). The hormone content in the samples was determined by radioimmunoassay.

**Radioimmunoassay (RIA).** The hypothalamic and neurohypophysial AVP content as well as plasma hormone concentration were determined by double-antibody specific RIA. Anti-AVP antibodies were raised by Dr. Monika Orlowska-Majdak (Department of Physiology, Institute of Physiology and Biochemistry, Medical University of Lodz). The antibody titer was 1:24,000 (final dilutions), and the lower limit of detection for the assay was 1.25 pg AVP/tube (3, 8). For standard curve preparation as well as for iodination with $^{125}I$, using the chloramine-T method, the AVP ([Arg$^8$]-Vasopressin) from Peninsula Laboratories Europe Ltd. was used. The intra-assay coefficient of variation for the AVP assay was 2.7% (all samples within the experiment were tested in the same RIA to avoid interassay variability).

**Statistical Evaluation of the Results.** The AVP levels were finally expressed in nanograms (ng) for whole hypothalamus or neurohypophysis and in picograms (pg) per 1 ml of plasma. By use of the Kruskal-Wallis analysis of variance by ranks (one-way ANOVA) test, the null hypothesis was rejected ($P < 0.001$) for each set of data (all subgroups). Thereafter, the statistical significance of differences between means (of two subgroups compared) was determined by the Mann-Whitney "U" test, using $P < 0.05$ as the minimal level of significance.

The difference in the mean hypothalamic hormone content of colchicine- and saline-injected rats, subjected to the same experimental procedure, was used for calculation of the AVP biosynthesis rate. To estimate the hormone biosynthesis rate over a 1-hr period, the calculated difference was divided by 20 (animals were decapitated 20 hr after the colchicine or saline injection) as described previously (14, 20). The synthesis rate calculated in such a way cannot be analyzed statistically. Therefore, the level of significance was estimated by comparing the mean hypothalamic hormone content in pinealectomized and melatonin- or vehicle-treated rats versus sham-operated and melatonin- or vehicle-treated rats (Table I). When a significant difference was found between some subgroups, the synthesis rates for these subgroups were assumed to be significantly different.

## Results

In both sham-operated and pinealectomized rats treated with the vehicle (subgroups SO-VEH and PX-VEH), the hypothalamic AVP content was significantly higher in col-

**Table I.** Hypothalamic Vasopressin Content in Sham-Operated (SO) or Pinealectomized (PX) as well as Vehicle (VEH)- or Melatonin (MLT)-Treated Male Rats

| Subgroups of Animals | Saline-Injected (*a*) | Colchicine-Injected (*b*) | Statistical Significance (*a* vs *b*) |
|---|---|---|---|
| 1) SO-VEH | 70.0 ± 5.0 ($n = 10$) | 88.0 ± 5.5 ($n = 10$) | $P < 0.05$ |
| 2) PX-VEH | 38.3 ± 6.6 ($n = 10$) | 78.0 ± 7.9 ($n = 9$) | $P < 0.005$ |
| 3) SO-MLT | 62.7 ± 7.5 ($n = 9$) | 90.2 ± 7.8 ($n = 9$) | $P < 0.05$ |
| 4) PX-MLT | 70.1 ± 7.6 ($n = 10$) | 79.7 ± 8.2 ($n = 9$) | NS |
| Statistical significance | | | |
| 1 vs 2 | $P < 0.005$ | NS | |
| 1 vs 3 | NS | NS | |
| 2 vs 4 | $P = 0.01$ | NS | |
| 3 vs 4 | NS | NS | |

*Note.* Values are expressed in ng per whole hypothalamus; *n* is number of animals per subgroup; mean ± SEM. NS: not significant.

chicine-injected as compared with saline-injected animals subjected to the same experimental procedure (ANOVA, $P = 0.0001$). Similarly, in sham-operated MLT-treated rats (subgroup SO-MLT) the hypothalamic AVP content was increased after colchicine injection (Table I). In control animals (subgroup SO-VEH) injection of colchicine resulted in a diminution of the neurohypophysial AVP content (ANOVA, $P = 0.0001$); however, no significant differences were seen in other (i.e., SO-MLT, PX-VEH, PX-MLT) subgroups (Fig. 1). Plasma AVP concentration was significantly increased after pinealectomy, when compared with sham-operated animals (ANOVA, $P = 0.0001$); this effect was markedly reduced by MLT (Fig. 2).

Present results confirm the usefulness of the colchicine method for the evaluation of the AVP biosynthesis rate in the rat. In the control animals, (i.e., vehicle-treated rats) the AVP biosynthesis rate was higher after pineal removal (1.99 ng/hr) when compared with sham-operated (0.9 ng/hr) animals. However, the rise in AVP biosynthesis rate due to pinealectomy was attenuated (0.48 ng/hr) by the treatment with MLT (Fig. 3).

## Discussion

Diurnal rhythmic variations of the AVP levels in the hypothalamus were described in the rat; the hypothalamic AVP content has been found to increase over the light phase and decrease during the night, reaching the lowest level in the morning (19). Melatonin production and secretion by the pineal also exhibit day/night variations (i.e., they increase during the night and fall during the day) (17). Therefore, it seemed reasonable to suppose that MLT possibly exerts an inhibitory influence on AVP biosynthesis in the hypothalamus. Indeed, the present experiment showed, for the first time, that MLT decreases the AVP biosynthesis in the hypothalamus of pinealectomized rats. In saline-injected rats, the neurohypophysial AVP content was diminished while plasma AVP concentration was increased 2 weeks after pinealectomy; this finding suggests the accelerated secretion of AVP into the blood in pineal-deprived animals. In

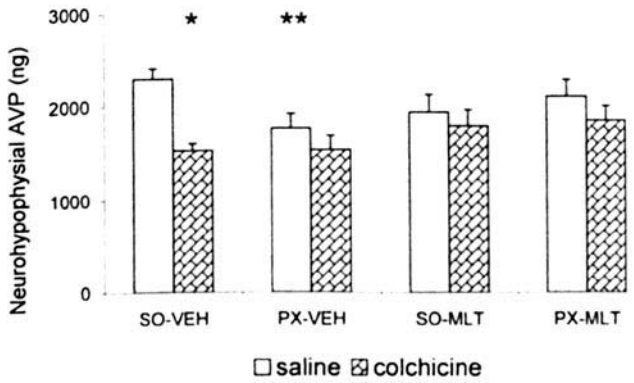


**Figure 1.** The effect of colchicine on neurohypophysial vasopressin (AVP) content in sham-operated (SO) or pinealectomized (PX) and vehicle (VEH)- or melatonin (MLT)-injected rats (mean ± SEM; $^{*}P < 0.001$, $^{**}P < 0.05$ both significantly different versus SO-VEH saline).

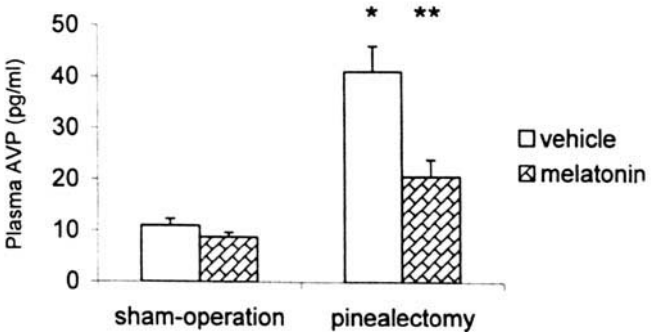


**Figure 2.** The effect of melatonin on plasma vasopressin (AVP) concentration in sham-operated or pinealectomized male rats (mean ± SEM; * significantly different versus vehicle sham-operation, $P < 0.001$; **significantly different versus vehicle pinealectomy, $P < 0.01$).

addition, MLT was found to inhibit the secretion of AVP in pinealectomized animals. Taken together, these results support the hypothesis that the pineal gland exerts an inhibitory impact on both AVP biosynthesis and secretion and that MLT plays an important role in pineal-dependent activity of the vasopressinergic neurons.

The mechanism(s) underlying modification of the AVP production and secretion by the pineal and its hormone, MLT, are still not clear. After systemic administration, MLT crosses the blood-brain barrier, and the hypothalamus as well as the anterior pituitary seem to be its target areas (17, 21). Exogenous MLT may modify AVP biosynthesis *via* specific membrane receptors. However, MLT receptors have been found in several brain areas of the male rat with high levels of binding over the suprachiasmatic nucleus (SCN) and pars tuberalis of the pituitary, but neither over the hypothalamic SON and PVN nuclei nor in the neurohypophysis (18, 22). It is possible that MLT modifies AVP biosynthesis acting *via* receptors localized in the SCN; neural input originating in the SCN and reaching the PVN (23) and SON (24) may affect, at least in part, the biosynthesis of AVP. Indeed, neuronal activation and Fos protein production in the rat SON have been noted to be diminished after pinealectomy (12).

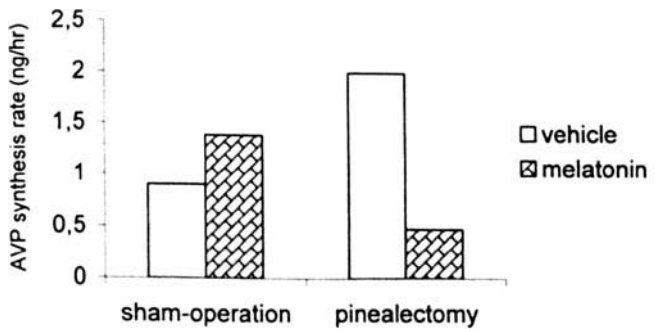


**Figure 3.** The effect of melatonin on the vasopressin (AVP) synthesis rate in sham-operated or pinealectomized male rats. Since the synthesis rate was calculated as a difference between two means (i.e., the difference between the mean hypothalamic AVP content, accumulated within a 20-hr period after injection, in colchicine- and saline-injected rats subjected to the same experimental procedure), standard errors could not be calculated.

Another possibility is that MLT modifies activity of vasopressinergic neurons by a direct action on the genome. Indeed, subcutaneous injection of MLT results in a rapid increase of its content in the nuclear fraction of a number of tissues including hypothalamus, without interaction with specific membrane receptors (21, 25). Such a mode of MLT action is consistent with the evidence that MLT has been found to be a specific ligand for the brain-specific nuclear receptors RZR, both α- and β-subtypes (26).

Melatonin alters the metabolism of some catecholamines in the hypothalamus and in the neurointermediate lobe (27, 28). Therefore, by its direct effect on the hypothalamic neurons and/or indirect (i.e., by modified neurotransmission in the brain), MLT could influence the synthesis and/or release of AVP. In fact, acetylcholine, dopamine, and prostaglandins were found to be involved in MLT-mediated inhibition of neurohypophysial hormone secretion both *in vivo* (29) and *in vitro* (30).

In summary, we conclude that the pineal gland exerts an inhibitory impact on both AVP biosynthesis and secretion and that MLT mediates these effects. However, identification of the mechanism (5) of MLT action on the hypothalamic SON and PVN magnocellular neurons will require further investigation.

The authors wish to thank Professor Jan W. Guzek (Department of Pathophysiology, Medical University of Lodz) for his helpful comments on the manuscript, and Dr. Bozena Stempniak (Department of Pathophysiology, Medical University of Lodz) for her assistance with the radioimmunoassay technique.

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