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# THE PINEAL AND OXYTOCIN SYNTHESIS

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The aim of this study was to investigate the effect of pineal removal on oxytocin synthesis in the hypothalamus using the colchicine method. To this end, rats were injected intracerebroventricularly (i.c.v.) with colchicine solution (5 μg/5 μl) or normal saline and decapitated 20 h later. The animals were either pinealectomized or sham-operated two or eight weeks before i.c.v. injection.
The oxytocin content in the hypothalamus was significantly higher in colchicine-treated rats whereas no significant differences were seen in the neurohypophysial hormone level between saline- or colchicine-injected animals. Thus, colchicine inhibited the hormonal transport but probably did not affect the function of the neurohypophysis. Two weeks after pinealectomy neither the oxytocin synthesis rate nor its neurohypophysial content were significantly different from control values. The oxytocin synthesis rate was increased markedly eight weeks after pineal removal. At that time, the neurohypophysial oxytocin content was reduced suggesting the increased secretion of the hormone. It is concluded that the pineal has an inhibitory impact on both oxytocin synthesis and release.

Key words: *oxytocin, pinealectomy, colchicine, hormone synthesis.*

## INTRODUCTION

Oxytocin is a peptide neurohormone which contributes to maintaining reproductory functions and/or body homeostasis when released systemically into the general circulation. When secreted centrally, it is considered to be a neurotransmitter/neuromodulator involved in the control of some autonomic functions (e.g., cardiovascular regulation during stress).

Stimuli that affect oxytocin release change the rate of the hormone synthesis and transport. Parturition, suckling, hypovolaemia, plasma hypertonicity and stress are known to alter the oxytocin gene expression (1—5). Activity of this gene may also be changed by some neuromodulators present in the magnocellular system, e.g., cholecystokinin (6) and histamine (7).

The pineal hormone melatonin is another agent known to alter the function of the rat hypothalamo-neurohypophysial system (HNS). The oxytocin content

in the HNS is modified by pinealectomy and/or melatonin treatment (8, 9) and shows a circadian rhythm (10) that may be disturbed by pinealectomy (11) or constant lighting (10). Pinealectomy attenuates vasopressin and oxytocin release into the blood under conditions of hyperosmotic stimulation; this event is accompanied by a decrease in the amount of Fos (a protein involved in regulation of gene expression) within neurones of the supraoptic nuclei (12). Therefore, we hypothesize that the pineal affects not only the oxytocin secretion but also its biosynthesis.

Oxytocin is synthesized in the rough endoplasmic reticulum within the perikarya of the supraoptic and paraventricular nuclei, packed into secretory granules and transported along the infundibular axons down to the posterior lobe of the pituitary. Intraneuronal transport may be inhibited by colchicine that impairs the neurotubular system without affecting the hormonal biosynthesis rate (13). As a result, the newly produced pool of oxytocin accumulates in the perikarya (14), progressively increasing with the passage of time (15). When compared with the hormone level in untreated animals, the accumulation of the hypothalamic oxytocin content in colchicine-injected rats over a constant period of time is considered to be an index of the neurohormonal biosynthesis (16).

The goal of the present paper was to study possible effects of the pineal on oxytocin synthesis in the rat hypothalamus using the colchicine method.

## MATERIAL AND METHODS

Male Wistar rats (250—350 g) housed under controlled lighting regime (a 12:12 light-dark cycle) and temperature, with food and water available ad libitum were used for the experiments. They were kept in cages with wire bar lids in order to provide an even illumination (2 — 3 rats per cage). The animals were divided into the following groups:

— pinealectomized under hexobarbital anaesthesia (0.2 g/kg b.w., i.p.). These rats were decapitated 2 weeks (group I) or 8 weeks (group II) after pinealectomy and pineal removal was verified post mortem in each animal;

— sham-operated; these animals were subjected to the same surgical procedure except that the pineal remained intact. These rats were decapitated 2 weeks (group III) or 8 weeks (group IV) after the surgery.

Half of rats in each group was randomly injected intracerebroventricularly (i.c.v.) with either 5 µl colchicine vehicle, i.e., 0.15 M NaCl (group A) or 5 µg/5 µl colchicine (Sigma, USA) solution (group B). To this end, a stainless steel cannula was inserted into the lateral ventricle through a hole located 1.5 mm laterally and 1.5 mm posteriorly to the crossing of the sagittal and coronal sutures and lowered 4 mm below the skull surface under light hexobarbital anaesthesia. The cannula was kept in the position by means of a special mount. The i.c.v. infusions were given via polyethylene tubing attached to a 10 µl Hamilton syringe (Hamilton Company, Reno, NV) filled with appropriate solution. The duration of the infusion was 30 s. The cannula was removed one minute after the injection; the hole was immediately sealed with bone wax and the wound was sutured. Then the animals were returned to their cages and provided with food and water. The rats were decapitated 20 h after colchicine or saline injection at 9.30—10.00 a.m. The brain with the pituitary was removed from the skull, 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 (17) and homogenized in 0.5% acetic acid. Oxytocin content in the samples was determined by radioimmunoassay (for detailed description see (17)).

### *Statistical analysis*

The difference in the mean hypothalamic hormone content of the colchicine- and saline treated rats subjected to the same experimental procedure was used for calculation of the oxytocin synthesis rate. To estimate the synthesis rate over a 1-hour period, the calculated difference was divided by 20 (animals were decapitated 20 hours after the injection). The synthesis rate calculated in such a way cannot be analysed statistically. Therefore, the level of significance was estimated by comparing the mean hypothalamic hormone content in pinealectomized versus sham-operated rats (see *Table 1*). When a significant difference was found between some groups, the synthesis rates for these groups were also recognized to be significantly different. The two-way Wilcoxon test was used to estimate the statistical significance of the results, i.e., to evaluate the significance of difference in the mean hypothalamic/neurohypophysial oxytocin content in the groups compared.

## RESULTS

The oxytocin content in the hypothalamus of colchicine-injected rats was markedly increased as compared with saline-treated animals subjected to the same experimental procedure (*Table 1*). No significant differences were found, however, in the neurohypophysial oxytocin levels in the respective groups of animals (*Fig. 1*).

*Table 1.* Effects of pinealectomy on hypothalamic oxytocin content in animals injected with colchicine.

| HYPOTHALAMIC OXYTOCIN CONTENT (ng per hypothalamus) | | | |
|---|---|---|---|
| Group of animals | A: normal saline | B: colchicine | Statistical significance A vs B |
| I — Pinealectomized 2 weeks after the surgery | $18 \pm 1$ ($n = 10$) | $44 \pm 5$ ($n = 11$) | $p < 0.01$ |
| II — Pinealectomized, 8 weeks after the surgery | $16 \pm 1$ ($n = 11$) | $67 \pm 8$ ($n = 8$) | $p < 0.01$ |
| III — Sham-operated, 2 weeks after the surgery | $19 \pm 2$ ($n = 10$) | $48 \pm 8$ ($n = 8$) | $p < 0.01$ |
| IV — Sham-operated, 8 weeks after the surgery | $16 \pm 1$ ($n = 10$) | $45 \pm 7$ ($n = 8$) | $p < 0.01$ |
| Statistical significance: | | | |
| I vs III | NS | NS | |
| II vs IV | NS | $p < 0.05$ | |
| III vs IV | NS | NS | |

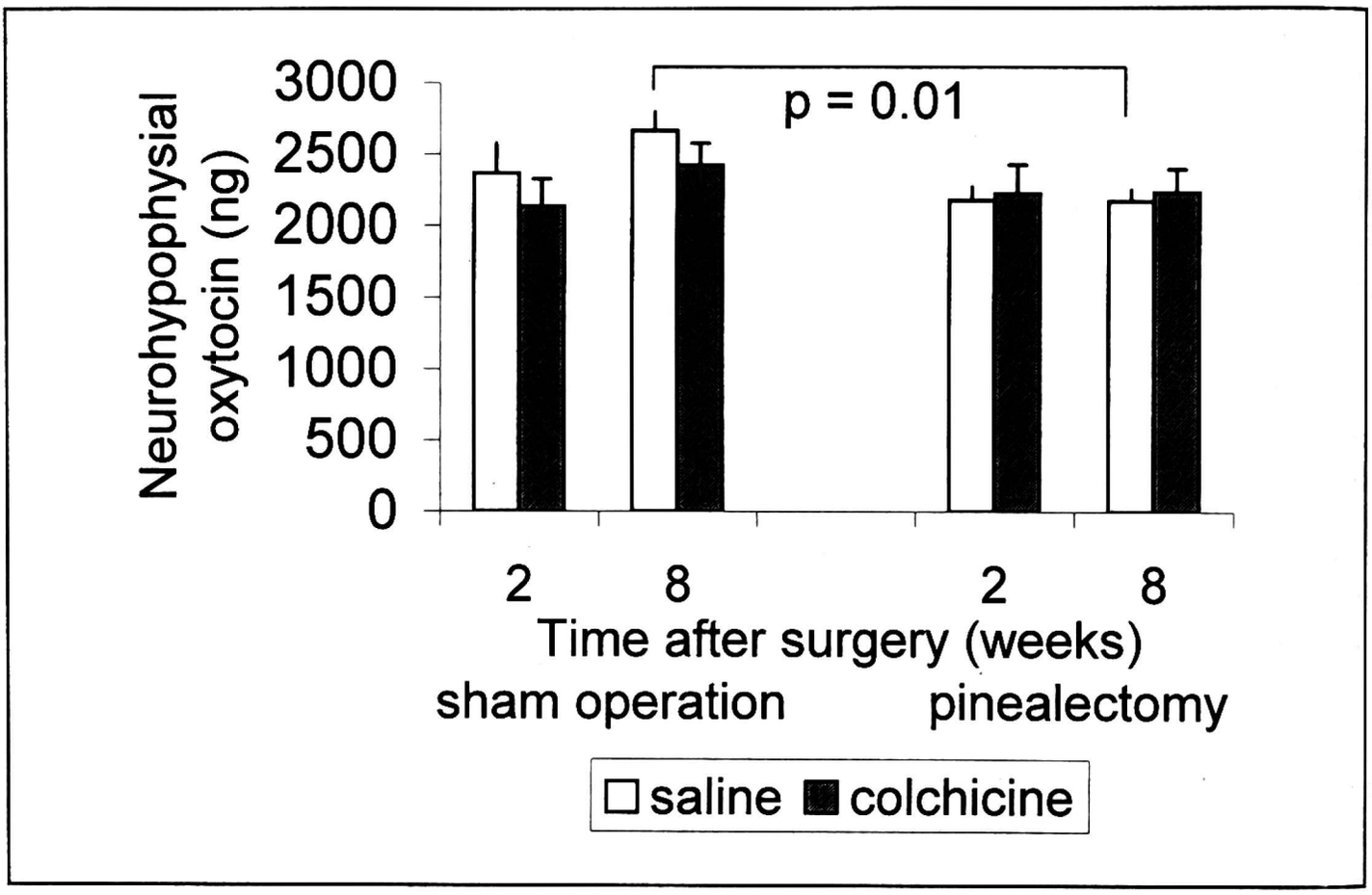


*Fig. 1.* The effect of pineal removal on the neurohypophysial oxytocin content in saline- or colchicine-injected rats (mean ± SEM).

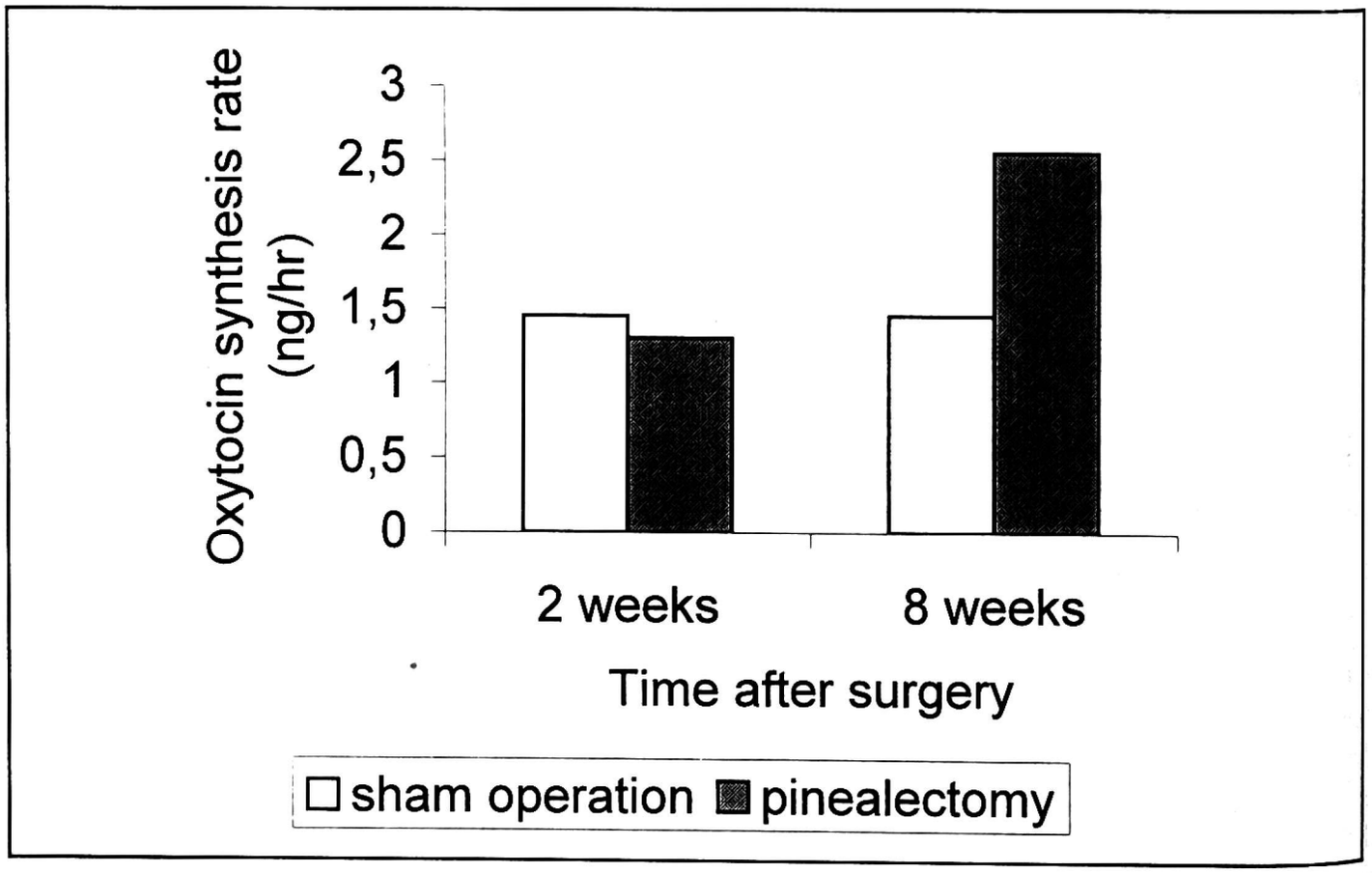


*Fig. 2.* Oxytocin synthesis rate in rats decapitated two or eight weeks after pinealectomy. Since the synthesis was calculated as the difference in the mean hypothalamic vasopressin content (accumulated within a 20-hr period after injection) between colchicine- and saline-treated rats subjected to the same experimental procedure, standard deviations cannot be shown.

Two weeks after pineal removal the oxytocin synthesis rate was 1.3 ng/h and did not differ significantly from the control value (1.45 ng/h). Changes in the oxytocin synthesis rate due to pinealectomy were shown, however, in rats decapitated eight weeks after the surgery, where a significant increase up to 2.55 ng/h could be seen (*Fig. 2*). In these animals, the neurohypophysial oxytocin content was markedly reduced (*Fig. 1*).

## DISCUSSION

There are numerous reports as to interactions between the pineal gland and the HNS (9, 11, 12, 18) and it seems that melatonin mediates the effects of the pineal on this system (19, 20). However, melatonin receptors — although widely distributed in several brain structures (21) — were found neither in supraoptic and paraventricular nuclei nor in the posterior pituitary of rodents (22, 23). It might be hypothesized that melatonin affects the magnocellular neurones indirectly, i.e., *via* some neurotransmitter (neuromodulator) systems, e.g., acetylcholine, dopamine or prostaglandins, which is suggested from both *in vitro* (24) and *in vivo* studies (25). On the other hand, a direct effect of melatonin on vasopressinergic and oxytocinergic neurones cannot be excluded. Owing to its lipophilicity, melatonin penetrates readily through membranes of virtually each cell and accumulates in the cell nucleus (26) where it selectively associates with chromatin (27). These events could contribute to melatonin-induced effects on cells that do not have membrane binding sites for the hormone. Yet, melatonin might bind to a nuclear receptor and change gene activity just as steroid hormones do.

To estimate the effect of the pineal on the synthesis of neurohypophysial hormone we have employed the colchicine method. I.c.v. injection of 5 μg colchicine resulted in a significant increase of oxytocin content in the hypothalamus of both pinealectomized and sham-operated rats similar to that obtained by other authors (15, 16). The oxytocin biosynthesis rate calculated in non-pinealectomized controls also resembles values reported by others (15, 16). Because the neurohypophysial oxytocin levels were not affected by colchicine treatment (i.e., they were not significantly different from the respective values in saline-injected rats), it seems that colchicine did not affect the secretory activity of the neurohypophysis.

We have shown that the oxytocin synthesis rate was increased eight weeks after pinealectomy. In contrast to the accelerated synthesis of the hormone, the hypothalamic oxytocin content was not affected and the neurohypophysial oxytocin stores were significantly reduced in control (i.e., saline-injected) pinealectomized animals. This suggests that the oxytocin release was increased and surpassed the effect of augmented synthesis of the hormone. Similar results

were obtained by Juszczak *et al.* (28) who found that pineal removal decreased oxytocin content in the neurohypophysis of the Syrian hamster exposed to long days (i.e., relatively low melatonin concentrations) for twelve weeks. On the other hand, in these animals, oxytocin mRNA level, although relatively high, was not significantly different from that found in control animals (28). The detailed analysis of results reported by Juszczak *et al.* (28) shows, however, that the standard error of the mean for the mean level of oxytocin mRNA in animals exposed to long days was apparently larger than in controls. Therefore, the authors suggested that such considerable variation could have precluded changes in oxytocin synthesis due to manipulations of photoperiod. If this is the case, these results would be consistent with observations made in our study where a clear increase of the oxytocin synthesis rate was shown in pinealectomized rats. Gender differences as well as different experimental procedure employed in both studies (i.e., surgical pinealectomy versus "functional" pinealectomy) could also account for the lack of statistically significant results as to the effect of the pineal on oxytocin synthesis reported by Juszczak *et al.* (28).

It is also of interest that, in the present study, the effects of pineal removal on oxytocinergic neurones were seen after a relatively long period of time (i.e., eight weeks). This is consistent with results reported by Forsling (11) who found that the plasma oxytocin concentration was increased eight, but not two weeks after pinealectomy. This finding also supports our hypothesis as to increased secretory activity of the neurohypophysis in pinealectomized rats.

In conclusion, pinealectomy was shown to increase oxytocin synthesis suggesting that the pineal has an inhibitory impact on oxytocinergic neurones. Hence, the data provide new evidence for possible functional connection between the pineal and the hypothalamo-neurohypophysial system.

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