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EFFECT OF PINEALECTOMY AND MELATONIN ON VASOPRESSIN-POTENTIATED PASSIVE AVOIDANCE IN RATS

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The pineal indoleamine, melatonin, and the hypothalamic neuropeptide, vasopressin, facilitate passive avoidance behaviour in rats. The similarity of the effects suggests that interaction might occur between the two substances. Therefore, the effect of intraperitoneally applied vasopressin and/or melatonin on one-trial learning passive avoidance behaviour was studied in pinealectomized rats. Intraperitoneal treatment with 500 ng vasopressin 1 hr before the retention test increased passive avoidance latency of sham-operated rats. In pinealectomized rats, an identical amount of vasopressin was ineffective. In sham-operated rats, melatonin blocked the effect of vasopressin. It is concluded that vasopressin needs a regulated pineal function for developing effects in passive avoidance behaviour.

Key words: *pineal gland, melatonin, vasopressin, behaviour.*

INTRODUCTION

The effects of exogenous arginine-vasopressin (AVP) on memory processes have been studied extensively. The peptide facilitates consolidation and potentiates retrieval of passive avoidance response (1).

The pineal gland has been demonstrated to play a role in behavioural processes too. Melatonin (MEL), produced in the pineal gland, was shown to affect the active and passive avoidance behaviour of rats in a way highly similar to that of AVP (2).

The similarity of the effects of MEL and AVP suggests that interaction might occur between the two substances. Hence, the question arises whether, in the case of the pineal gland missing, the AVP effect on functions of the central nervous system is comparable to that observed in non-operated controls. Initial evidence indicative of such a relationship was obtained from changes demonstrated in the function of the hypothalamo-neurohypophyseal system

after pinealectomy which mainly related to the synthesis and release of neurohypophyseal hormones (3—5). Moreover, MEL was shown to modify the AVP content in the rat hypothalamo-neurohypophyseal system (4).

Therefore, the present study was designed to investigate, using a passive avoidance task, the role of the pineal gland and MEL as to the efficacy of AVP in the regulation of behaviour.

MATERIALS AND METHODS

Adult male Wistar rats (190—240 g b.w.) of an inbred strain (Mollegaard Breeding Company) were used. The rats were housed in cages measuring $18 \times 30 \times 50$ cm (3—4 rats per cage) under a 12/12 hr light-dark schedule (lights on at 6 a.m.) and at a temperature of 23°C. The animals received standard pelleted food (Altromin, Germany) and had free access to tap water.

Series A:

Fourty-four animals were divided into two groups: I — rats pinealectomized under hexobarbital (150 mg/kg b.w., intraperitoneally) anaesthesia following the procedure after Kuszak and Rodin (6); II — rats sham operated in a similar procedure, without removing the pineal gland.

After a recovery period of seven days, pinealectomized and sham operated rats were used for the experiments. The passive avoidance behaviour was studied by using one-trial learning paradigm in a step-through situation (7). The behavioural experiments were started at 9.00 ± 10 min. Briefly, rats were placed on a platform illuminated by a 100 W light bulb and attached to a dark compartment with a grid floor inside. Since rats prefer dark to light they normally entered within a time of about 10 sec. After the trial, rats were returned to their home cages and the platform as well as the grid floor were cleaned.

After an additional trial on the following day, an unavoidable footshock (current 2 mA, pulse duration 2 ms, interval 10 ms, train duration 3 s) was delivered through the grid floor in the dark compartment. About 23 hours after this single learning trial with footshock, the animals were injected i.p. (= 0.5 ml solution per rat) with 500 ng AVP (Arg8-vasopressin, Serva, Heidelberg/New York) dissolved in 0.9% saline or with 0.9% saline only in controls. The retention of passive aviodance behaviour was tested 24 hours after the learning trial with footshock (i.e., one hour following AVP or saline injections) by placing the rats on the platform and measuring the latency necessary to enter the dark compartment.

Series B:

Eighty-six rats were pinealectomized or sham-operated as in series A. In each of the two groups four further experimental subgroups were set up: 1 — rats injected i.p. with 500 ng AVP dissolved in 0.5 ml of 0.9% saline as well as with MEL (N-Acetyl-5-methoxytryptamine, Sigma, St. Louis) dissolved in 0.1 ml of the vehicle (2.2% ethanol in 0.9% saline) in a daily dose of 100 µg MEL per rat; 2 — rats similarly injected with 500 ng AVP and additionally treated with the vehicle solution (2.2% ethanol in 0.9% NaCl); 3 — rats injected i.p. with 0.5 ml of saline solution and with 0.1 ml of MEL solution (a daily dose of 100 µg MEL per rat); 4 — rats similarly injected with 0.5 ml of saline and 0.1 ml of vehicle solution.

After a recovery period, both pinealectomized and sham operated rats were used for the experiments. The passive avoidance behaviour was studied as described for series A. The

behavioural experiments were started at 9.20±10 min. After the first contact of animals with the experimental situation, on the same day at 17.00 — 17.30 (60 — 30 min before lights off) rats were given the first injection of MEL or vehicle solution, respectively. On the following day, after an additional trial and unavoidable footshock delivered through the grid floor (learning trial), the second injection of MEL or vehicle solution was given in the late afternoon (at 17.00 — 17.30). About 23 hours after the single learning trial, the animals were treated with AVP or saline, respectively. The retention of passive avoidance behaviour was tested 24 hours after the learning trial as described for series A.

Data analysis

Significance of the differences between means was evaluted by non-parametric analysis of variance followed by Mann-Whitney U-test. Differences were considered significant if $p<0.05$.

RESULTS

Series A:

The behavioural data are summarized in *Fig. 1*. An unavoidable footshock (learning trial) caused prolonged avoidance latencies 24 hr later. Intraperitoneal application of 500 ng AVP in sham-operated rats resulted in a significant facilitation of the avoidance latency in comparison with the trial before footshock. After pinealectomy, an identical amount of AVP was ineffective.

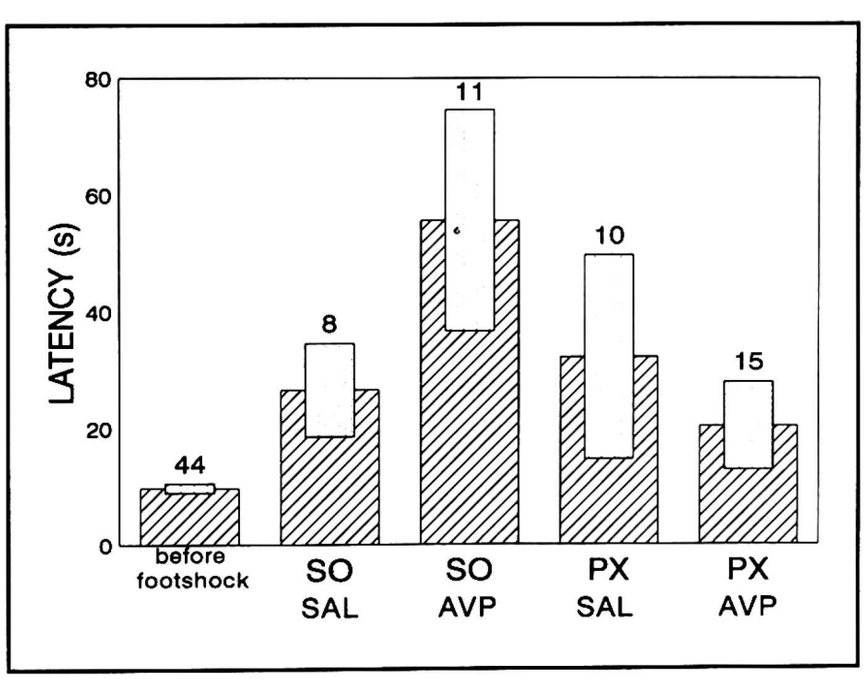

Fig. 1. Effect of arginine vasopressin (AVP) on passive avoidance latency in sham-operated (SO) or pinealectomized (PX) rats. Controls received 0.9% saline (SAL). The left column is the mean±SEM of the trial before footshock of all series, and each following column is the mean±SEM of the retention trial. The number of animals is indicated at the top of the respective bars. Difference significant ($p<0.05$): SO.SAL vs SO.AVP; SO.AVP vs PX.AVP.

Series B:

The results are shown in *Fig. 2*. In control experiments, the missing effect of AVP in pinealectomized rats was reproduced. Melatonin alone, when injected to saline-treated animals, did not significantly affect the passive avoidance

response whereas MEL injected into AVP-treated rats blocked the AVP-induced lengthening of the passive avoidance latency in sham operated animals. In pinealectomized rats, the passive avoidance response was not modified by MEL neither in saline-treated, nor in AVP-treated rats. Nevertheless, the passive avoidance latency was significantly prolonged in pinealectomized and MEL-AVP-treated as well as in pinealectomized and MEL-saline-treated rats when compared to sham-operated animals.

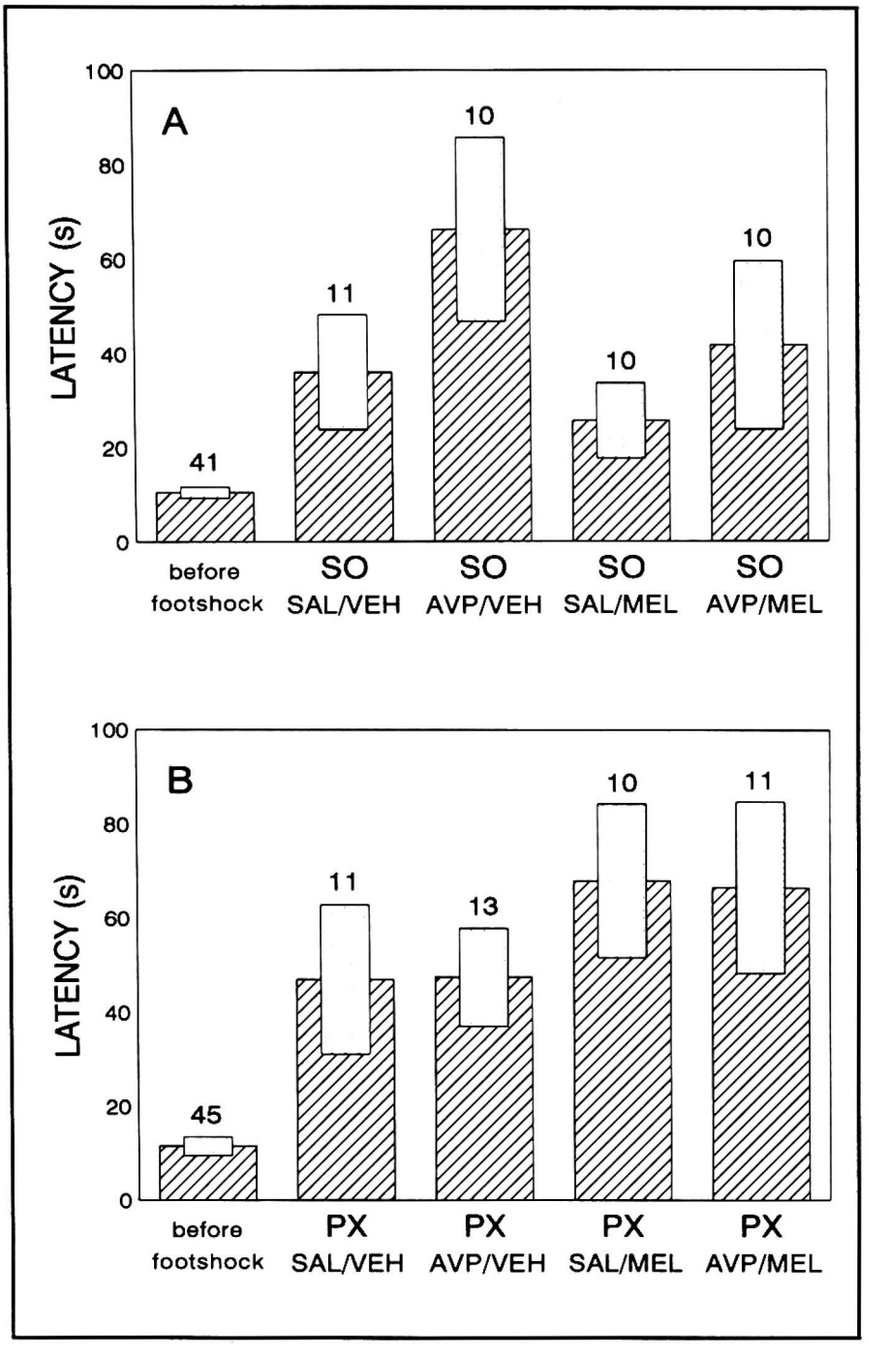

Fig. 2. Effect of arginine vasopressin (AVP) and melatonin (MEL) on passive avoidance latency in (A — top panel) sham-operated (SO) or (B — bottom panel) pinealectomized (PX) rats. Controls received 0.9% saline (SAL) and/or vehicle solution: 2.2% ethanol in 0.9% saline (VEH). The left column is the mean ± SEM of the trial before footshock of all series, and each following column is the mean ± SEM of the retention trial. The number of animals is indicated at the top of the respective bars. Difference significant ($p < 0.05$): SO.SAL/VEH vs SO.AVP/VEH; SO.AVP/VEH vs SO.AVP/MEL; SO.SAL/MEL vs. PX.SAL/MEL; SO.AVP/MEL vs PX.AVP/MEL.

DISCUSSION

The data presented show that AVP needs a regulated pineal function for developing effects on passive avoidance. The prolonged avoidance latencies after AVP applied 1 hr prior to the retention test in sham-operated rats suggest that this peptide facilitates memory retrieval (8). In this context, MEL is

assumed to play an essential role. Indeed, Reiter (9) reviewed MEL as an ubiquitously acting hormone: "regulator of regulators". This indoleamine, produced in and released from the pineal gland in a rhythmic manner with peak MEL levels occurring at night, has been found to modulate the release of anterior as well as posterior pituitary hormones. A MEL-induced decrease of AVP content in the hypothalamo-neurohypophyseal system has been demonstrated in rats under conditions of a normal water balance and following dehydration or haemorrhage (4, 10, 11). However, while the stimulatory action of MEL on AVP release from the isolated posterior pituitary of intact (12) as well as of sham-operated or pinealectomized rats (13) has been described, in other experiments MEL produced a dose-dependent inhibition of AVP release from the rat hypothalamus *in vitro* (14).

Melatonin receptor binding sites have been detected in the brain, especially in the median eminence/pars tuberalis region and the paraventricular nuclei of the hypothalamus (15). However, the striking number of MEL binding sites in the rat pars tuberalis (16) were found to be the highest at the end of the day and the lowest at the end of the night. Due to up regulation of MEL receptors at the end of the light phase and also on the ground of mostly nocturnal secretion of MEL, this hormone was administered at the end of the light phase (17). When given approximately 16 hrs prior to electric shock and (on the next day) 16 hrs prior to testing the latency of passive avoidance response, MEL inhibited the AVP-dependent prolongation of the passive avoidance latency.

There are some data indicating a possible modulatory role of AVP for the regulation of MEL synthesis in the rat (18). Electrophysiological and pharmacological findings point to an inhibitory influence of AVP upon pineal MEL synthesis (19). Moreover, it is now well documented that AVP is present in the rat pineal gland (20). In this context, it may be interjected that some fibres originating from vasopressinergic neurons located in hypothalamic paraventricular nuclei reach directly the pineal gland (21), and AVP receptors of V_{1a} type have been identified in the rat pineal gland (22).

In the present experiment, AVP did not modify the retrieval of passive avoidance response in pinealectomized rats. A similar finding, relating to another agent was reported previously: removal of the pineal gland in the goldfish reduced the stimulatory effect of beta-endorphin on shoaling behaviour, and attenuated the day-night rhythm in the effects on beta-endorphin on shoaling (23). In searching for the reasons why the effects occured, interaction between substances of the pineal gland and neuroactive peptides cannot be ruled out. Interaction between different hormones has been known for some time. The problem of peptide-peptide interactions was reviewed in the literature (24). It has been shown that some neuropeptides can reverse or increase the action of another peptide, i.e., oxytocin inhibited the effect of AVP in behavioural experiments (25).

CONCLUSION

The present results support the hypothesis of complex interactions between the pineal and some neuropeptides involved in memory retrieval processes. The missing effect of AVP in both pinealectomized as well as MEL-pretreated animals suggests that, under these circumstances, MEL injection does not function as a simple substitution, i.e., it does not restore the homeostatic situation prevailing before the pinealectomy. It is concluded that the effect of AVP on passive avoidance response depends upon intact pineal function for developing effects in passive avoidance behaviour. However, further work is required to elucidate the respective mechanisms.

REFERENCES

1. Bohus B, Kovacs GL, De Wied D. Oxytocin, vasopressin and memory: opposite effects on consolidation and retrieval process. *Brain Res* 1978; 157: 414—417.
2. Kovács GL, Gajari I, Telegdy G, Lissak K. Effect of melatonin and pinealectomy on avoidance and exploratory activity in the rat. *Physiol Behav* 1974; 13: 349—355.
3. Juszczak M, Guzek JW. The content of vasopressin and oxytocin in the hypothalamus and neurohypophysis of pinealectomized male rats. *Acta Physiol Pol* 1983; 34: 41—46.
4. Juszczak M, Guzek JW. Hypothalamic and neurohypophysial vasopressin and oxytocin in melatonin-treated pinealectomized male rats. *J Pineal Res* 1988; 5: 545—552.
5. Demaine ByC, Forsling ML, Kelestimur H, Stoughton RP. Effects of pinealectomy on daily rhythms of neurohypophysial hormone release in the rat. *J Physiol* 1990; 423: 12P.
6. Kuszak J, Rodin M. A new technique of pinealectomy for adult rats. *Experientia* 1977; 33: 283—284.
7. Ader R, Weijnen JAWM, Moleman P. Retention of a passive avoidance response as a function of intensity and duration of electric shock. *Psychon Sci* 1972; 26: 125—128.
8. Kovacs GL, Telegdy G. Role of oxytocin in memory and amnesia. *Pharmac Ther* 1982; 18: 375—395.
9. Reiter RJ. Melatonin: that ubiquitously acting pineal hormone. *NIPS* 1991; 6: 223—227.
10. Juszczak M, Guzek JW, Lewy A. The influence of melatonin on the content of vasopressin and oxytocin in the hypothalamus and neurohypophysis in euhydrated and dehydrated male rats. *J Pineal Res* 1986; 3: 199—211.
11. Juszczak M, Klopotowska A, Stempniak B, Guzek JW. Hypothalamic and neurohypophysial vasopressin and oxytocin content as influenced by haemorrhage in melatonin-treated male rats. *Patol Pol* 1993; 44: 55—59.
12. Lemay A, Brouillette A, Denizeau F, Lavoie M. Melatonin- and serotonin-stimulated release of vasopressin from rat neurohypophysis *in vitro*. *Mol Cell Endocrinol* 1979; 14: 157—166.
13. Juszczak M, Stempniak B, Guzek JW. Melatonin, pinealectomy, and release of neurohypophysial hormones: *in vitro* studies. *J Pineal Res* 1992; 12: 1—6.
14. Yasin SA, Costa A, Besser GM, Hucks D, Grossman A, Forsling ML. Melatonin and its analogs inhibit the basal and stimulated release of hypothalamic vasopressin and oxytocin *in vitro*. *Endocrinology* 1993; 132: 1329—1336.
15. Weaver DR, Rivkees SA, Reppert SM. Localization and characterization of melatonin receptors in rodent brain by *in vitro* autoradiography. *J Neurosci* 1989; 9: 2581—2590.

16. Gauer F, Masson-Pevet M, Skene DJ, Vivien-Roels B, Pevet P. Daily rhythms of melatonin binding sites in the rat pars tuberalis and suprachiasmatic nuclei; evidence for a regulation of melatonin receptors by melatonin itself. *Neuroendocrinology* 1993; 57: 120—126.
17. Reiter RJ. The melatonin rhythm: both a clock and a calendar. *Experientia* 1993; 49: 654—664.
18. Reus S, Stehle J, Schroder H, Vollrath L. The role of the hypothalamic paraventricular nuclei for the regulation of pineal melatonin synthesis: new aspects derived from the vasopressin-deficient Brattleboro rat. *Neurosci Lett* 1990; 109: 196—200.
19. Schroder H, Reuss S, Stehle J, Vollrath L. Intra-arterially administered vasopressin inhibits nocturnal pineal melatonin synthesis in the rat. *Comp Biochem Physiol* 1988; 89A: 651—653.
20. Gauquelin G, Gharib C, Krasnov IB et al. Presence of neurohypophyseal hormones in the retina, the pineal and harderian glands of the rat. Modifications induced by environmental factors. In: Recent progress in posterior pituitary hormones, S Yoshida, L Share (eds). Amsterdam, Elsevier Science Publishers, 1988, pp. 293—301.
21. Korf HW, Wagner U. Evidence for a nervous connection between the brain and the pineal organ in the guinea pig. *Cell Tissue Res* 1980; 209: 505—510.
22. Ostrowski NL, Lolait SJ, Young III WS. Cellular localization of vasopressin V1a receptor messenger ribonucleic acid in adult male rat brain, pineal, and brain vasculature. *Endocrinology* 1994; 135: 1511—1528.
23. Kavaliers M, Day-night rhythms of shoaling behavior in goldfish: opioid and pineal involvement. *Physiol Behav* 1989; 46: 167—172.
24. Schwarzberg H, Pross M. Neuropeptides in the cerebrospinal fluid and regulation of behaviour. *Progr Brain Res* 1992; 91: 455—457.
25. Schwarzberg H, Unger H. Aenderung der Reaktionszeit von Ratten nach Applikation von Vasopressin, Oxytocin und Na-Thioglykolat. *Acta Biol Med* Germ 1970; 24: 507—516.

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