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ORIGINAL ARTICLE

Effects of oxytocin and vasopressin on retrieval of passive avoidance response in melatonin-treated and/or pinealectomized male rats

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Abstract The role of the pineal gland and its hormone—melatonin—as to the impact of vasopressin (VP) and/or oxytocin (OT) on the regulation of behavior was studied, the passive avoidance task being chosen as an experimental model. The results showed that VP facilitated the avoidance latency during the first retention trial; after pinealectomy, however, VP was ineffective in this regard. Intraperitoneal application of OT was ineffective in modifying the passive avoidance latency when compared with respective saline-treated animals. Melatonin alone, when injected to sham operated animals 30 min before behavioral experiment, did not affect the passive avoidance response in SA- or OT-treated rats, but blocked the VP-induced lengthening of the passive avoidance latency in the first retention trial. In pinealectomized and OT-treated animals the passive avoidance latency during the second retention trial was severely diminished by melatonin when compared to respective control. We conclude that: a) VP needs a regulated pineal function for developing short-term effects on the passive avoidance response and b) the effect of OT on the avoidance latency in pinealectomized rats develops after melatonin treatment as a long-term effect.

Introduction

The influence of the pineal gland and its hormone melatonin (MLT) on the vasopressin (VP) and oxytocin (OT) release and their content in the hypothalamo-neurohypophysial system is now well established. Pinealectomy diminishes the neurohypophysial VP and OT content (Juszczak and Guzek 1983, 1988) while treatment with MLT modifies these hormones' release, depending on a dose used, both *in vivo* (Juszczak and Stempniak 1997; Bojanowska and Forsling 1997) and *in vitro* (Juszczak et al. 1992, 1995; Yasin et al. 1993).

Studies concerning the effects of MLT on memory processes have shown that this hormone affects the active and passive avoidance behavior (Kovacs et al. 1974; Datta and King 1977) as well as the open field behavior (Golus and King 1981) in the rat. In this regard, the neurohypophysial neuropeptides are known to be effective: VP facilitates the consolidation as well as intensifies the retrieval of passive avoidance response (Bohus et al. 1978; Bohus 1996) while OT usually has an opposite to VP effects on these processes (Bohus et al. 1978; De Wied et al. 1991).

Although the pineal gland and MLT have been demonstrated to play a role in some behavioral processes, such aspects of pineal-neurohypophysial relationships are still poorly understood. Recently, it was shown that the effect of VP on the passive avoidance response depends upon intact pineal function (Juszczak et al. 1996a) and that MLT plays some role in this respect. Thus, the aim of the present investigations was to study the role of MLT as to the impact of OT/VP on the regulation of behavior, the passive avoidance task being chosen as an experimental model.

Materials and methods

Animals

Three-month old male Wistar rats were housed under a 12/12 hr light-dark schedule (lights on from 6 a.m.) and at a room temperature. The animals received standard pelleted food and had free access to tap water. They were sham operated or pinealectomized under light pentobarbital (50 mg/kg) anaesthesia following the procedure described by Kuszak and Rodin (1977). Sham operation consisted of an identical surgical trauma, including ligature and resection of the superior sagittal sinus, but without removing the pineal gland (Table 1).

Experimental design (Table 1). One hundred and three rats, both pinealectomized (PX) or sham operated (SO), were used for the experiments after a recovery period of about three weeks. In both groups six further experimental subgroups were set up:

1) rats injected intraperitoneally (ip) with 500 ng of OT (Oxytocin, Sigma) dissolved in 0.9% saline as well as with MLT (N-Acetyl-5-methoxytryptamine, Sigma) solution dissolved in the vehicle (VEH; 1.0% ethanol in 0.9% saline) in a daily dose of 100 μg MLT (= 0.1 ml solution, subcutaneously [sc]) per rat; 2) rats injected ip with 500 ng of VP (Arg8-vasopressin, Serva) dissolved in 0.9% saline as well as with MLT solution; 3) rats similarly injected with 500 ng of OT and additionally with the VEH in a daily dose of 0.1 ml solution per rat; 4) rats similarly injected with 500 ng VP and additionally with the VEH; 5) rats injected with saline and with MLT solution; 6) rats injected with saline and VEH (control groups).

Experimental procedure

The passive avoidance behavior was studied by one trial learning paradigm as described by Ader et al. (1972) with minor modifications (Juszczak et al. 1996a). The behavioral experiments started at 08.30. Rats were placed on an illuminated platform with the head facing away from the opening to the dark compartment of the box and were allowed to enter a dark compartment with a grid floor inside (the first contact of animals with the experimental situation). On the following day, after an additional trial, an unavoidable footshock was delivered through the grid floor. About 23 hours after single learning trial with footshock, the animals were injected (= 0.5 ml per

Table 1. Experimental design and number of animals (n) per group

Sham operation (SO) Subgroups	Vehicle (VEH)-treated rats	Melatonin (MLT)-treated rats	Pinealectomy (PX) Subgroups	Vehicle (VEH)-treated rats	Melatonin (MLT)-treated rats
Saline (SA)-injected rats	SO-VEH-SA (8)	SO-MLT-SA (10)	(SA)-injected rats	PX-VEH-SA (8)	PX-MLT-SA (8)
Oxytocin (OT)-injected rats	SO-VEH-OT (9)	SO-MLT-OT (9)	(OT)-injected rats	PX-VEH-OT (9)	PX-MLT-OT (9)
Vasopressin (VP)-injected rats	SO-VEH-VP (7)	SO-MLT-VP (9)	(VP)-injected rats	PX-VEH-VP (9)	SO-MLT-VP (8)

rat) with OT or VP dissolved in 0.9% saline or with 0.9% saline alone, respectively. Thirty minutes later, the injection of MLT or VEH solution was given. The retention of passive avoidance behavior was tested 24 hours after learning trial with footshock (first retention), i.e., 60 minutes following OT/VP or saline injections and 30 minutes after MLT or VEH injections, by measuring the latency necessary for the rat to enter the dark compartment. The same procedure for passive avoidance test was repeated on the next day, i.e., 48 hours after learning trial with footshock (second retention).

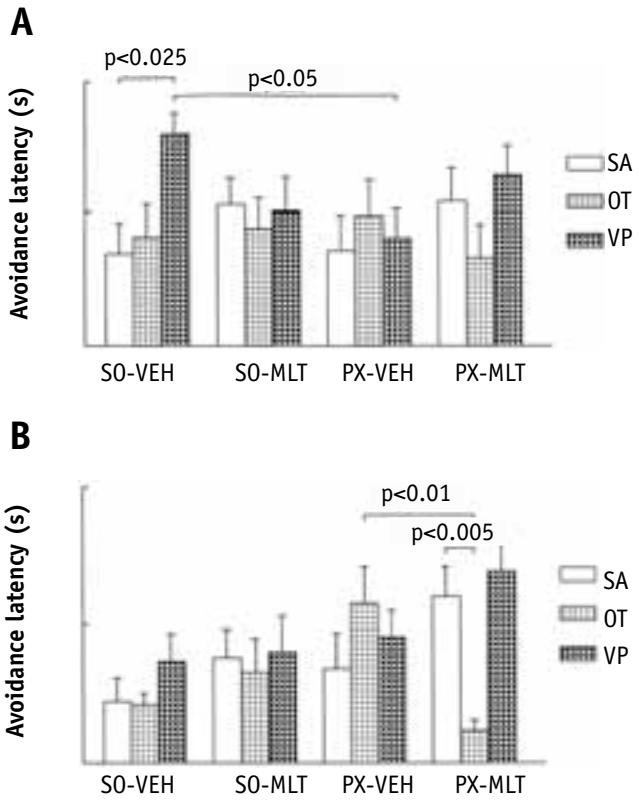
Statistical evaluation of the results.

Significance of the differences between means was evaluated by non-parametric ANOVA followed by Mann-Whitney U-test, using $p<0.05$ as the minimal level of significance.

Results

In sham-operated rats VP facilitated the avoidance latency during the first (but not during the second) retention trial; after pinealectomy, however, VP was ineffective in this regard. On the other hand, ip application of OT was ineffective in modifying the passive avoidance latency (during the first and second retention trial) when compared with respective saline-treated animals (Fig. 1A, B). Melatonin alone, when injected to sham operated animals 30 minutes before behavioral experiment, did not affect the passive avoidance response in SA- or OT-treated rats, whereas similarly injected into VP-treated rats, it blocked the VP-induced lengthening of the passive avoidance latency in the first retention trial. Melatonin, when injected to sham-operated rats, did not affect the passive avoidance response during the second retention trial in OT/VP/SA-injected rats. However, in pinealectomized and OT-treated animals the passive avoidance latency during the second retention trial was severely diminished by MLT when compared to respective control (Fig. 1B).

Figure 1. The effect of melatonin (MLT) on the passive avoidance latency in sham operated (SO) or pinealectomized (PX) as well as oxytocin (OT)-, vasopressin (VP)- or saline (SA)-injected rats during the first (A) and the second (B) retention test (mean ± S.E.M.; number of animals: n = 7-10).



Discussion

The effects of VP and/or OT on behavior and memory processes

The lengthening of avoidance latency, as found in this study one hr after VP administration in pineal-intact animals, was not unexpected: such effect of VP on passive avoidance behavior has already been reported (Bohus et al. 1978; Bohus 1996; Juszczak et al. 1996a). Moreover, similarly to previous observations (Juszczak et al. 1996a) the effect of VP on passive avoidance behavior could not be seen in pinealectomized animals one hr after VP injection, i.e., during the first retention trial. During the second retention trial (i.e., 25h after VP administration), however, the influence of VP on avoidance latency could be seen neither in sham-operated nor in pinealectomized animals. Therefore, the present findings seem to suggest that exogenous VP exerts short-term effect on the passive avoidance behavior and that an intact pineal gland is needed for developing such effect.

Oxytocin is considered to be an amnesic neuropeptide (Bohus et al. 1978); however, there are also reports which contradict such a suggestion (see: Engelman et al. 1996). The treatment of neonatal rats with OT did not change the latency times in the learning trial or the 24h retention trial in the passive avoidance test (Boer et al. 1994). After ip administration OT had no effect on learning in animals under conditions of normal water balance, whereas in thirsty rats it caused a clear amnesic effect (Schulz et al. 1976). In the present experiment, no effect of exogenous OT on the avoidance latency in rats with intact pineal gland could be seen in the first or the second retention trial. However, the passive avoidance latency was severely diminished by OT in pinealectomized and MLT-treated animals during the

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second, but not during the first retention trial. Similarly, the long-lasting (48h) effect of OT on self-stimulation in the rat could be seen after its intracerebroventricular application (Schwarzberg et al. 1978). These results may suggest that exogenous OT, possibly because of its effect on the release of endogenous OT (Moss and Richard 1989), requires some time before any changes in behavior and/or memory processes are developed. Moreover, some possible, more complex interactions between neurohypophysial peptides involved in memory processes (VP and/or OT) and the pineal gland function cannot be excluded. Indeed, it has been shown that the behavioral effects of VP could be seen only when the function of an other endocrine glands (i.e., levels of respective hormones in blood plasma) was within the physiological range (Schwarzberg and Pross 1992; Schwarz-berg et al. 1996).

The effects of melatonin on VP/OT-modified memory processes

Melatonin has been found to modulate the release of both posterior pituitary hormones under physiological (i.e., normal) conditions (Juszczak and Stempniak 1997; Bojanowska and Forsling 1997) as well as under some pathological states such as dehydration (Juszczak et al. 1986), hypertonic saline injection (Juszczak et al. 1996b) or stress (Juszczak 1998). Melatonin was also shown to modify the VP and OT release *in vitro* from the posterior pituitary (Juszczak et al. 1992, 1995) as well as from the hypothalamus (Yasin et al. 1993). Since both pinealectomy and MLT (Juszczak et al. 1986; Juszczak and Guzek 1988; Yasin et al. 1993) modify the VP and OT secretion from the hypothalamus, the main hypothesis of this study was that MLT plays an important role in mechanisms concerning the pineal-dependent effects of VP and/or OT on retrieval of the passive avoidance response. Melatonin was injected during the day, i.e., 30 minutes before the first retention trial in the passive avoidance test, according to the results of the *in vitro* experiments, which showed that the vasopressinergic and oxytocinergic neurons were most responsive to exogenous MLT during the light phase of the light, dark:cycle (Yasin et al. 1996). Melatonin did not affect the passive avoidance response (this study), but when injected to sham-operated and VP-treated animals it blocked (similarly to pinealectomy) the VP-induced lengthening of the response. However, in pinealectomized rats MLT modified the passive avoidance response neither in saline-treated nor in VP-treated rats. On the contrary, in pinealectomized and OT-treated animals the passive avoidance latency, in the second retention trial, was severely diminished by MLT when compared to respective control (this study).

The mechanisms of MLT action on the vasopressinergic and oxytocinergic neurons are still not clear. Melatonin receptors have been demonstrated in several brain areas of male rat with high levels of binding over the suprachiasmatic nuclei (SCN) and pars tuberalis of the pituitary, but were described neither over the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei nor in the neurohypophysis (Morgan et al. 1994; Williams et al. 1995). After systemic administration, MLT crosses the blood-brain barrier and accumulates in the hypothalamus both in cytosolic and nuclear fractions (Menendez-Pelaez and Reiter 1993). Exogenous MLT may, therefore, modify VP/OT effects on memory processes via MLT receptors localized in the SCN; indeed, the SCN neurons project directly to the PVN. It is therefore possible that the neural input originating in the SCN and reaching the PVN affects, at least in part, the release of VP and OT within the brain: such a course of events is conceivable as to both normal and pinealectomized animals. In addition, MLT may act via MLT receptors localized in some nuclei of the limbic system (Morgan et al. 1994; Williams et al. 1995), which are known to participate in learning and memory functions (Bohus 1996). However, more studies are necessary to evaluate the role of the pineal in consolidation and retrieval processes.

On the basis of the present results we conclude that: a) VP needs a regulated pineal function for developing short-term effects on the passive avoidance response in the rat (which is in agreement with our previous studies) and b) the effect of OT on the avoidance latency in pinealectomized rats develops after MLT treatment as a long-term effect.

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