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Inhibition of the vasopressin-enhancing effect on memory retrieval and relearning by a vasopressin V_1 receptor antagonist in mice

Béatrice Alescio-Lautier *, Hongwei Rao, Véronique Paban, Colette Devigne, Bernard Soumireu-Mourat

Laboratoire de Neurobiologie des Comportements, URA CNRS 372 – Université de Provence, I.B.H.O.P. – Traverse Charles Susini, 13388 Marseille Cedex 13, France

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Abstract

We have previously shown that [Arg^8]vasopressin bilaterally administered into the ventral hippocampus of mice at a dose of 0.025 ng/animal 10 min prior to the retention session, improved long-term retrieval processes and relearning of a Go-No-Go visual discrimination task. The purpose of the present study was to determine whether the vasopressin V_1 receptor antagonist, [β-mercapto-β,β-cyclopentamethylenepropionyl^1,O-Me-Tyr^2,Arg^8]vasopressin, d$(CH_2)_5$Tyr(Me)vasopressin), is able to block the behavioral effect of arginine-vasopressin in the ventral hippocampus. We first tested the effect of three doses of d$(CH_2)_5$Tyr(Me)vasopressin (0.025, 1, and 6.3 ng/animal) in the same experimental conditions as used for arginine-vasopressin. The results showed a dose-dependent deleterious effect of the vasopressin V_1 receptor antagonist on retrieval and relearning, suggesting the involvement of endogenous arginine-vasopressin in the ventral hippocampus for these memory processes. Second, we tested the ability of d$(CH_2)_5$Tyr(Me)vasopressin to block the enhancing effect of experimentally administered arginine-vasopressin. The antagonist was injected at a dose of 0.025 ng, which had no intrinsic effect on behavior, or at a dose of 1 ng, which had a weak deleterious effect on behavior, followed by administration of 0.025 ng of arginine-vasopressin. The results showed that even at the weakest dose (0.025 ng), d$(CH_2)_5$Tyr(Me)vasopressin blocked the enhancing effect of arginine-vasopressin on retrieval and relearning. Thus, as for other behaviors and structures, the antagonist microinjected into the ventral hippocampus prevents the enhancing effect of arginine-vasopressin on long-term retrieval and relearning. However, the exclusive involvement of the vasopressin V_1 receptors remain to demonstrate vis-à-vis oxytocin receptors.

Keywords: [Arg^8]Vasopressin; Vasopressin V_1 receptor antagonist; Ventral hippocampus; Retrieval; Relearning; Locomotor activity

1. Introduction

Previous studies have indicated that arginine-vasopressin, a 9-amino-acid peptide has a behavioral effect in several brain areas, in addition to playing a role in peripheral target organs. Indeed, it has been shown that central vasopressinergic systems are implicated in a variety of physiological and behavioral processes such as cardiovascular regulation (Buwalda et al., 1992; Mohr and Richter, 1994), thermoregulation (Banet and Wieland, 1985), grooming (Meisenberg, 1988), motor effects (Kruse et al., 1977; Kasting et al., 1980; Willcox et al., 1992), vocal behavior (Winslow and Insel, 1993), aggression and partner preference (Winslow et al., 1993), sexual behavior (Södersten et al., 1986) and flank marking (Ferris et al., 1984, 1988; Irvin et al., 1990). There is also substantial evidence that arginine-vasopressin functions as a neurotransmitter in the central nervous system, affecting learning and memory processes. Indeed, central administration of arginine-vasopressin affects several types of learned behavior such as avoidance conditioning (De Wied et al., 1984; Engelmann et al., 1992), social conditioning (Siegfried et al., 1984; Dantzer et al., 1987; Le Moal et al., 1987; Ferris et al., 1988) and appetitive conditioning (Sara et al., 1982; Alescio-Lautier et al., 1987, 1989;

* Corresponding author. Tel.: (33) 91 28 87 23; fax: (33) 91 98 26 97.

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Meck, 1987). Limbic structures are particularly involved in the behavioral effect of arginine-vasopressin. Numerous studies in rats have shown that the hippocampus, the amygdala and the septal area were the most effective structures for the effect of arginine-vasopressin on passive avoidance behavior (Kovács et al., 1979, 1986). Dantzer et al. (1988) showed that the administration of arginine-vasopressin in the lateral septum improves social recognition in rats. We have previously shown in mice that the hippocampus is involved in the enhancing effect of arginine-vasopressin on retrieval and relearning of appetitive conditioning in a Go-No-Go visual discrimination task (Alescio-Lautier et al., 1987, 1989), with more improvement when the peptide is injected into the ventral hippocampus than into the dorsal hippocampus (Metzger et al., 1989; Alescio-Lautier et al., 1993) as was previously shown by Kovács et al. (1986) for avoidance behavior.

The involvement of limbic structures in the behavioral effects of arginine-vasopressin is supported by anatomical (De Vries et al., 1985; Sofroniew, 1985; Caffé et al., 1987; Castel and Morris, 1988), electrophysiological (Joëls and Urban, 1982; Mühlethaler et al., 1982; Burnard et al., 1987; Chen et al., 1993; Armstrong et al., 1994) and autoradiographic (Chen et al., 1993; Johnson et al., 1993; Krémarik et al., 1993; Ferris et al., 1993; Tribollet et al., 1992; Insel et al., 1993) studies. The last group of studies has shown that the [Arg^8]vasopressin binding sites detected in the brain correspond to the vasopressin V_{1A} subtype receptors, and that the distribution of these binding sites can vary according to the species studied.

Behavioral studies using microinjection of a vasopressin V_1 receptor antagonist in the lateral ventricle of the brain, or in limbic structures such as the septum area and the amygdala or the anterior hypothalamus, have shown that the central effects of arginine-vasopressin on avoidance conditioning (De Wied et al., 1984), social recognition (Dantzer and Bluthé, 1992) and other behaviors (Ferris et al., 1988; Irvin et al., 1990; Willcox et al., 1992; Winslow et al., 1993) are mediated through vasopressin V_1 receptors.

We have also reported that the neutralization of the amount of bioavailable vasopressin in the ventral hippocampus by microinjection of arginine-vasopressin antisera prior to the retention session led to drastic impairment of both retrieval and relearning performance, suggesting that endogenous vasopressin in the ventral hippocampus is involved in retention processes (Metzger et al., 1993).

Considering our results (i.e., improving effect of exogenously administered arginine-vasopressin in the ventral hippocampus versus impairing effect of exogenously administered arginine-vasopressin antisera, also in the ventral hippocampus) and the fact that no study has been reported on the use of vasopressin V_1 receptor antagonists in the ventral hippocampus, the present study was an attempt to determine whether or not the improvement in retrieval and relearning following vasopressinergic treatment in the ventral hippocampus is mediated through vasopressin V_1 receptors as was reported in the literature for other behaviors. We first tested the effect of a microinjection into the ventral hippocampus of the vasopressin V_1 receptor antagonist, d(CH_2)$_5$Tyr(Me)vasopressin, at various doses. We then analyzed the ability of the vasopressin V_1 receptor antagonist to block the enhancing effect of arginine-vasopressin by the coadministration of the two peptides. Finally, in order to evaluate the hypothesis that motor changes contribute to the observed behavioral effect, all treatments in this study were tested for effects on locomotor activity.

2. Materials and methods

2.1. Animals

204 naive male BALB/c mice from the Iffa Credo Co. (St Germain sur l'Arbresle, France) were used when 8–9 weeks old. Until the day of the experiment, the mice had been housed 20 per cage with food and water available ad libitum on a 12–12 light-dark cycle (light on at 07:00 a.m.) in a vivarium at a temperature of 21–23°C. Behavioral studies were conducted between 08:00 a.m. and 12:00 p.m. 1 week before the beginning of the experiment, the mice were housed individually and adapted to daily handling.

2.2. Behavioral procedure

2.2.1. Visual discrimination task

The animals were trained in a successive visual discrimination test with food reinforcement, as described in detail (Alescio-Lautier and Soumireu-Mourat, 1986). Briefly, the experimental device consisted of two separate alleys, one white and one black. As the sessions progress, the animals must discriminate between the alley in which they are always reinforced and the alley in which they are never reinforced. A session includes six reinforced trials (Go trials) and six non-reinforced trials (No-Go trials) in random order. The initial learning period consisted of three daily sessions. Performance was measured in terms of an animal's running times. Learning is manifested by a decrease in running times in Go trials and increase in running times in No-Go trials. The retention session took place 24 days after the last learning session. The performance in the first Go trial and in the first No-Go trial are considered to reflect the level of retrieval, and performance on the subsequent Go and No-Go trials, to reflect the level of relearning. In this respect, the

test series always began with either a Go trial followed by a No-Go trial or vice-versa.

2.2.2. Locomotor activity

To test the hypothesis that a motor change might contribute to the observed behavioral effect of the treatment, locomotor activity was measured in a grey plexiglas open field (60 × 60 × 10 cm) with the floor marked in 10 × 10-cm squares. A daily session lasting 10 min was held on 2 successive days. At the beginning of the session, the animal was placed in the middle of the field and the time taken to reach the side was recorded. The number of square crossings, rearings, defecations, and groomings were then measured for 10 min.

2.3. Surgical procedure

Surgery took place 48 h after the end of the initial training in visual discrimination or 1 week before the beginning of the locomotor activity test. The animals were anesthetized with a solution of Imalgène 500 (0.01%) and Rompun xylazine (0.1%) (0.3 ml/animal) and then placed in a stereotaxic instrument. Cannulae were implanted bilaterally into the ventral hippocampus at the following coordinates: 3.0 mm posterior to the bregma, ±3.1 mm lateral to the midline, and 3.3 mm below the surface of the skull.

2.4. Intra-hippocampal injection

Arginine-vasopressin and the vasopressin V_1 receptor antagonist, d(CH_2)$_5$Tyr(Me)vasopressin, were obtained from the Sigma Chemical Co. Arginine-vasopressin was diluted in NaCl (0.9%) and d(CH_2)$_5$Tyr(Me)vasopressin was first dissolved in 0.01 M acetic acid and then diluted in 0.9% NaCl. The peptides or their respective vehicles (0.9% NaCl or 0.9% Na Cl + 0.01 M acetic acid) were microinjected into the ventral hippocampus in a volume of 0.3 μl. The dose for arginine-vasopressin was 0.025 ng/animal and corresponds to the intra-hippocampal dose in mice behaviorally active on retention of the visual discrimination task. The doses for d(CH_2)$_5$Tyr(Me)vasopressin were 0.025, 1, or 6.3 ng/animal. Coadministration of arginine-vasopressin (0.025 ng) and d(CH_2)$_5$Tyr(Me)-vasopressin (0.025 or 1 ng) was done with consecutive injections, the vasopressin V_1 receptor antagonist being given first. In this case, the vehicle consisted of coadministered 0.9% NaCl and 0.9% NaCl + 0.01 M acetic acid, as control for non-specific effects of the injection procedure.

Nine groups of 12 animals were set up for each test, corresponding to the following treatments: vehicle 1 (NaCl 0.9%), arginine-vasopressin (0.025 ng), vehicle 2 (NaCl 0.9% + 0.01 M acetic acid), d(CH_2)$_5$Tyr(Me)-vasopressin (0.025, 1, or 6.3 ng), vehicle1/vehicle 2, d(CH_2)$_5$Tyr(Me)vasopressin (0.025 or 1 ng)/arginine-vasopressin (0.025 ng) (/ indicates a 5-min interval between injections). All injections were given bilaterally, 10 min prior to either the retention session of the visual discrimination task or the second session of the locomotor activity test.

2.5. Histological control experiments

Histological assessment was done at the end of the experiment, i.e. 22 days after cannula implantation, in order to evaluate cannula placement. Each animal was deeply anesthetized with sodium pentobarbital and then perfused intracardially using 0.9% NaCl followed by 10% formol-NaCl solution, pH 7.0. The brains were removed and post-fixed in the same fixative for several days. Transverse sections of 40 μm were subsequently cut, mounted, and stained with cresyl violet.

2.6. Statistical analysis

Retention test data were analyzed by a repeated measure multivariate analysis of variance (MANOVA) for the trial-by-trial measure (relearning) to determine the main effect of time (chronological series of trials) and its interaction effect with treatment and reinforcement (Go and No-Go trials). ANOVAs for treatment × reinforcement were also done for each trial. When significant treatment effects were obtained, they were analyzed by means of post-hoc Newman-Keuls t-tests. $P < 0.05$ was accepted as statistically significant. Locomotor activity test measures were analyzed by ANOVA, also followed by post-hoc Newman-Keuls t-tests for the significant effects.

3. Results

3.1. Visual discrimination

First, the three vehicle groups (vehicle 1, Fig. 1; vehicle 2, Fig. 2; veh 1/veh 2, Fig. 3) were analyzed in a MANOVA in order to determine whether the presence of acetic acid in the vehicle or the double-volume injection caused any differences in performance. The analysis revealed no difference between groups regardless of the factor considered (time: $F(5,62) = 0.75$, $P = 0.42$; treatment × time interaction: $F(10,124) = 1.005$, $P = 0.45$; reinforcement × time interaction: $F(5,62) = 2.08$, $P = 0.08$; treatment × reinforcement × time interaction: $F(10,124) = 0.48$, $P = 0.91$). Subsequent ANOVAs on each trial showed no effect of the vehicle treatment, in any trial ($F(2,66) \leq 1.76$, $P \geq 0.18$, no treatment × reinforcement interaction ($F(2,66) \leq 1024$, $P \geq 0.30$), but a reinforcement effect ($F(1,66) \geq 6.03$,

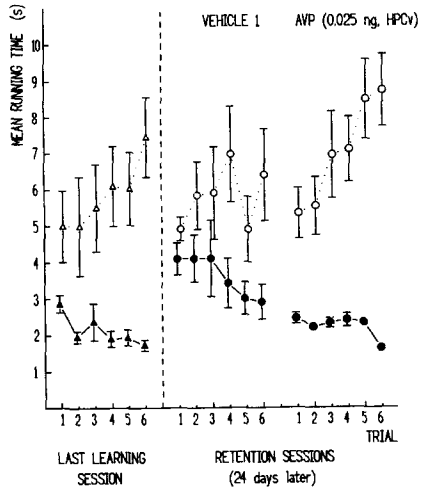

Fig. 1. Effects of bilateral microinjection of arginine-vasopressin (0.025 ng) into the ventral hippocampus on the retention of a Go-No-Go visual discrimination task. Left: trial-by-trial analysis of mean Go (▲) and No-Go (△) running times during the last learning session, indicating the level of initial learning. Right: trial-by-trial analysis of mean Go (●) and No-Go (○) running times during retention session with arginine-vasopressin (AVP)-injected mice and corresponding controls: vehicle. Vehicle (NaCl) or peptide was injected 10 min prior to the retention session.

$P \leq 0.02$). In general, the analyses (MANOVA and ANOVAs) indicated that the three groups performed in the same manner and were able to discriminate between Go and No-Go trials despite the 24-day time lapse after the initial training. However, separate ANOVAs for each group revealed that the difference

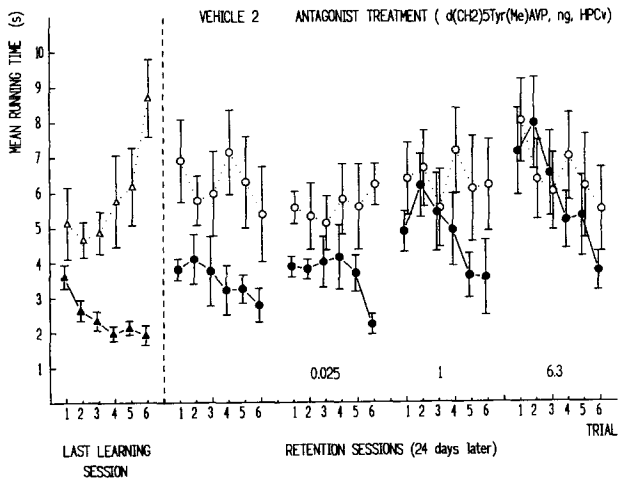

Fig. 2. Effects of bilateral microinjection of $d(CH_2)_5$Tyr(Me)vasopressin (0.025, 1 or 6.3 ng) into the ventral hippocampus on the retention of a Go-No-Go visual discrimination task. Left: trial-by-trial analysis of mean Go (▲) and No-Go (△) running times during the last learning session indicating the level of initial learning. Right: trial-by-trial analysis of mean Go (●) and No-Go (○) running times during retention session with $d(CH_2)_5$Tyr(Me)vasopressin-injected mice and corresponding controls: vehicle. Vehicle (NaCl + acetic acid 0.1 M) or $d(CH_2)_5$Tyr(Me)vasopressin was injected 10 min prior to the retention session.

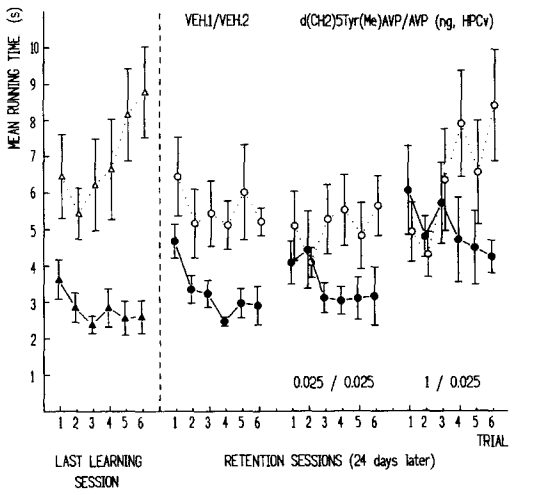

Fig. 3. Effects of bilateral microinjection of $d(CH_2)_5$Tyr(Me)-vasopressin (0.025, or 1 ng) followed by bilateral microinjection of arginine-vasopressin (0.025 ng) into the ventral hippocampus on the retention of a Go-No-Go visual discrimination task. Left: trial-by-trial analysis of mean Go (▲) and No-Go (△) running times during the last learning session, indicating the level of initial learning. Right: trial-by-trial analysis of mean Go (●) and No-Go (○) running times during retention session with $d(CH_2)_5$Tyr(Me)vasopressin- and arginine-vasopressin-injected mice and corresponding controls: double volume injection of vehicle (veh/veh). Injections of vehicle or peptides were performed 15 min prior to the retention session. The two injections were separated by a 5-min interval.

between Go and No-Go trials was not reached for all trials, since a level of discrimination appeared only at the end of the retention session (i.e. starting from the third or fourth trial, depending on the vehicle group). This level of discrimination corresponds to partial forgetting, as previously described for intact mice (Alescio-Lautier and Soumireu-Mourat, 1986) and mice receiving vehicle (Metzger et al., 1993; Alescio-Lautier et al., 1993).

When arginine-vasopressin was injected 10 min before the retention session, an improvement in both retrieval and relearning was observed compared to the vehicle group (Fig. 1). MANOVA for the retention performance of these two groups revealed a treatment × time interaction ($F(5,40) = 2.50$, $P < 0.05$), a reinforcement × time interaction ($F(5,40) = 7.03$, $P < 0.0001$), but no treatment × reinforcement × time interaction ($F(5,40) = 1.01$, $P = 0.43$). Subsequent ANOVAs on each trial revealed a significant group × reinforcement interaction for the first and the last two Go and No-Go trials ($F(1,44 \geq 7.06$, $P \leq 0.02$). This improvement in retrieval and relearning after arginine-vasopressin treatment, which was characterized by an enhancement of performance at the beginning and end of the session, is consistent with previously published data (Metzger et al., 1993).

$d(CH_2)_5$Tyr(Me)vasopressin induced a dose-dependent impairment of performance (Fig. 2). At the dose

of 0.025 ng, no effect of the vasopressin V_1 receptor antagonist was observed compared to the vehicle group. MANOVA of the performance of these two groups revealed no interaction effect, whatever the factor considered, and subsequent ANOVAs for each trial showed that the treatment × reinforcement interaction did not reach statistical significance for any trial. Thus, at this dose, d(CH_2)$_5$Tyr(Me)vasopressin did not affect retrieval and relearning performance. Statistical analyses comparing the vehicle group with groups receiving each of the other two doses of the vasopressin V_1 receptor antagonist (i.e. 1 or 6.3 ng) led us to the same conclusion as for the previous comparison. However, separate ANOVAs performed on each d(CH_2)$_5$Tyr(Me)vasopressin group (1 and 6.3 ng) revealed no statistical difference between Go and No-Go trials, across the session, indicating that the animals were not able to discriminate between the two alleys. However, the same analysis for the performance after vasopressin V_1 receptor antagonist treatment at the 0.025 ng dose showed a statistically significant difference between Go and No-Go trials for the first and the last two trials ($F(1,22) \geq 4.80$, $P \leq 0.04$), suggesting that this group was able to weakly discriminate between the two alleys. Looking at behavioral performance in Fig. 2, we can see that Go-trial performance was affected by the last two doses. This impairment was reflected by a large increase in Go running times at the beginning of the session, and became more pronounced as the dose increased. This was confirmed by ANOVAs of Go trials only for each group showing a significant effect of the treatment in Go trials 1 and 2 ($F(3,44 \geq 4.70$, $P \leq 0.007$). A post-hoc Newman-Keuls t-test ($P < 0.05$) confirmed that the deleterious effect of the treatment concerned the two higher doses of the vasopressin V_1 receptor antagonist. There was no statistically significant effect for No-Go running times for any group. However, with the highest dose, No-Go running times decreased over the session. The latter observation, together with the Go running times led us to conclude that at the highest dose of the vasopressin V_1 receptor antagonist, both retrieval and relearning performance were drastically impaired.

The comparison between retention performance following arginine-vasopressin treatment (Fig. 1) or d(CH_2)$_5$Tyr(Me)vasopressin treatment (Fig. 2) revealed numerous differences, with the most pronounced found for the two highest doses of the vasopressin V_1 receptor antagonist. ANOVAs of the Go running times of the arginine-vasopressin group and d(CH_2)$_5$Tyr(Me)vasopressin 1 ng group revealed a treatment effect for the first four Go trials ($F(1,22) \geq 6.76$, $P \leq 0.02$), and for all Go trials pooled ($F(1,22) \geq 6.67$, $P \leq 0.02$) when the arginine-vasopressin group and the d(CH_2)$_5$Tyr(Me)vasopressin 6.3 ng group were compared. For No-Go running times, ANOVAs showed a treatment effect for the last No-Go trial ($F(1,22) = 10.21$, $P < 0.005$), whatever dose (1 or 6.3 ng) of the vasopressin V_1 receptor antagonist the arginine-vasopressin treatment was compared with. It is interesting to note that animals treated with arginine-vasopressin had gradually increased No-Go running times over the session, whereas animals treated with the vasopressin V_1 receptor antagonist had gradually decreased No-Go running times. The significance of this was confirmed by MANOVA, showing a treatment × time interaction ($F(5,18) = 3.32$, $P < 0.03$). We can conclude that, compared with arginine-vasopressin, d(CH_2)$_5$Tyr(Me)vasopressin at the highest two doses tested altered both retrieval and relearning, and induced, as previously concluded from comparison with the vehicle, a marked deleterious effect for the highest dose on both Go and No-Go running times.

Keeping in mind the results obtained with the different doses of d(CH_2)$_5$Tyr(Me)vasopressin, we chose the smallest two doses (i.e. 0.025 and 1 ng) to assess the ability of the vasopressin V_1 receptor antagonist to block the enhancement effect of arginine-vasopressin

Table 1
Effects of arginine-vasopressin or d(CH_2)$_5$Tyr(Me)vasopressin and their respective controls on locomotor activity and other behavioral reactions

Dose (ng/animal)	Time to reach side latency (s) (mean ± S.E.M.)	Square crossing (mean ± S.E.M.)	Rearing (mean ± S.E.M.)	Defecation (mean ± S.E.M.)	Grooming (mean ± S.E.M.)
Arginine-vasopressin					
Vehicle 1	13.54 ± 4.04	73.33 ± 3.50	15.67 ± 4.38	0	0.08 ± 0.08
0.025	11.75 ± 2.43	58.67 ± 3.82 [a]	9.41 ± 2.0	0.33 ± 0.19	0
d(CH_2)$_5$Tyr(Me)vasopressin					
0.025	15.3 ±4.20	73.30 ± 2.79	11.5 ±2.40	0.66 ± 0.28	0.17 ± 0.11
1	10.14 ± 2.50	75.42 ± 5.11	17.42 ± 2.68	0.58 ± 0.29	0.16 ± 0.11
6.3	10.62 ± 0.98	76.33 ± 2.59	16.67 ± 0.62	0.41 ± 0.15	0.41 ± 0.1
d(CH_2)$_5$Tyr(Me)vasopressin + arginine-vasopressin					
Veh 1/veh 2	10.8 ±3.95	74.7 ±1.91	13.75 ± 2.78	0.25 ± 0.13	0.58 ± 0.19
0.025/0.025	8.96 ± 2.63	75.75 ± 4.59	13.58 ± 3.05	0.25 ± 0.18	0.33 ± 0.14
1/0.025	13.16 ± 1.36	76.67 ± 3.58	15.42 ± 1.88	0.33 ± 1.14	0.33 ± 0.14

[a] Represents Newman-Keuls t-test comparisons of peptide and vehicle control values ($P < 0.05$).

on retrieval and relearning. The results are presented in Fig. 3 and show that d(CH_2)$_5$Tyr(Me)vasopressin was able to block the enhancing effect of arginine-vasopressin on retrieval and relearning, even for the weakest dose (0.025 ng). ANOVAs of the performance following vasopressinergic treatment (Fig. 1) and d(CH_2)$_5$Tyr(Me)vasopressin (0.025 ng) treatment prior to arginine-vasopressin treatment (Fig. 3) revealed a treatment × reinforcement interaction for the first and last two trials of the session ($F(1,44) \geq 7.19$, $P \leq 0.01$), confirming the ability of the vasopressin V_1 receptor antagonist to block the effect of arginine-vasopressin. A similar analysis comparing effects of d(CH_2)$_5$Tyr(Me)vasopressin treatment (0.025 ng) alone (Fig. 2) and followed by arginine-vasopressin (Fig. 3), showed no statistically significant difference for any trial. When the vasopressin V_1 receptor antagonist was injected at a dose of 1 ng and was followed by arginine-vasopressin (Fig. 3), the results obtained in the retention session were similar to those obtained when the vasopressin V_1 receptor antagonist was administered alone at the same dose (Fig. 2), with no significant difference between the performance of these two groups.

3.2. Locomotor activity test

The results obtained for the different measures are presented in Table 1.

Arginine-vasopressin had no effect on the measures considered except for square crossings. The peptide induced a decrease in locomotor activity that was statistically significant at $P < 0.05$ (Newman-Keuls t-test). As above for behavioral performance, d(CH_2)$_5$Tyr(Me)vasopressin blocked the effect of arginine-vasopressin on square crossings since the locomotor activity of the animals receiving the double injection was similar to that of animals injected with the vehicle, and different from that of animals injected with arginine-vasopressin (Newman-Keuls t-test, $P < 0.05$). No other effects could be observed, whatever the treatment.

4. Discussion

We have shown in this study that an intra-hippocampal injection of the vasopressin V_1 receptor antagonist, d(CH_2)$_5$Tyr(Me)vasopressin, blocks the enhancing effect of centrally administered arginine-vasopressin on the retrieval and relearning of a visual discrimination task by mice.

In accordance with previous work (Metzger et al., 1993; Alescio-Lautier et al., 1993), arginine-vasopressin injected into the ventral hippocampus improves retrieval performance, and consequently relearning, compared to animals that received only the vehicle. This result is in agreement with that reported by Kovács et al. (1986) who had also shown the involvement of the ventral part of the hippocampus in the improving effect of arginine-vasopressin on the retention of passive avoidance behavior in rats. Locomotor activity was affected by vasopressinergic treatment, since square crossing was significantly reduced after the injection of arginine-vasopressin. However, this reduction of locomotor activity does not seem to be responsible for the enhancing effect of arginine-vasopressin on retention performance, since treated animals were able to perform quickly on Go trials, which, combined with slow running times on No-Go trials, led to a performance better than that of the vehicle group. It is interesting to note that this peptide-related reduction in activity seems to depend on what test was used to measure square crossing. Indeed, in previous studies where square crossings were recorded in a circular runway (Metzger et al., 1989) or in one of the alleys of the visual discrimination task (Metzger et al., 1993, 1994), no effects were found on locomotor activity after vasopressinergic treatment according to the same protocol and at the same dose as used here. However, reduced locomotor activity following central treatment with arginine-vasopressin in infant rats was also reported by Winslow and Insel (1993), who observed a decrease in the number of grid cells crossed after an intracerebroventricular injection of arginine-vasopressin (500 or 1000 ng).

When the vasopressin V_1 receptor antagonist was given in the ventral hippocampus, a dose-dependent effect opposite to that observed following arginine-vasopressin treatment was obtained for both retrieval and relearning. Indeed, at the same dose as that used for vasopressinergic treatment (0.025 ng), d(CH_2)$_5$Tyr(Me)vasopressin had no apparent intrinsic effect, whereas at higher doses (1 or 6.3 ng), a dose-dependent deleterious effect appeared. This dose-dependent deleterious effect following d(CH_2)$_5$Tyr(Me)vasopressin treatment affected both Go and No-Go running times. This result suggests the involvement of endogenous hippocampal arginine-vasopressin in retrieval and relearning. Our previous findings are consistent with this assumption, since it was shown that the neutralization of the amount of bioavailable arginine-vasopressin in the ventral hippocampus by the microinjection of vasopressin antisera prior to the retention session led to drastic impairment of both retrieval and relearning performance (Metzger et al., 1993). In the locomotor activity test, none of the measures studied were affected by the antagonist. Thus, secondary effects cannot be responsible for the deleterious effect on visual discrimination observed at the highest two doses of the vasopressin V_1 receptor antagonist. The opposite behavioral effects of arginine-

vasopressin and of d(CH_2)$_5$Tyr(Me)vasopressin treatment have been reported by several authors for other species, structures, and behaviors (Irvin et al., 1990; Ferris et al., 1988; Dantzer et al., 1987; 1988; Le Moal et al., 1987; Engelmann et al., 1992; Winslow et al., 1993).

d(CH_2)$_5$Tyr(Me)vasopressin injection (0.025 ng) into the ventral hippocampus, which had no apparent intrinsic effect on retention performance, associated with vasopressinergic treatment (0.025 ng), blocked the enhancing effect of arginine-vasopressin on retrieval and relearning. Similar results have been reported by Winslow and Insel (1993) regarding vocal behavior in infant rats. These authors reported that a central injection of d(CH_2)$_5$Tyr(Me)vasopressin at the same dose as arginine-vasopressin, which had no effect on vocal behavior, attenuated the effects of arginine-vasopressin. The reduction in locomotor activity that we have observed after vasopressinergic treatment is also blocked by d(CH_2)$_5$Tyr(Me)vasopressin, indicating that both effects of arginine-vasopressin, i.e. on retrieval and relearning and on locomotor activity, are mediated by the same hippocampal vasopressin receptors. However, these two effects can be distinct since we have previously shown that the enhancement of retrieval and relearning by arginine-vasopressin involves shorter running times. The reported effects of the peptide on other behaviors when injected in the septal area, in the anterior hypothalamus, or in the lateral ventricle, i.e. selective aggression and partner preference in prairie voles (Winslow et al., 1993), social recognition in rats (Dantzer and Bluthé, 1992), flank-marking behavior in rats (Irvin et al., 1990), and passive avoidance conditioning in rats (De Wied et al., 1991) were also antagonized by d(CH_2)$_5$Tyr(Me)vasopressin. However, De Wied et al. (1991) reported that the vasopressin V_2 receptor antagonist and the oxytocin receptor antagonist were almost as effective as a vasopressin V_1 receptor antagonist to block the effect of arginine-vasopressin on passive avoidance behavior. According to these results and since the ventral hippocampus is known to express both vasopressin and oxytocin receptors, the exclusive involvement of vasopressin V_1 receptors in mediation of the behavioral effects of arginine-vasopressin remains questionnable after the present study. Thus the determination of the receptor involved in the ventral hippocampus in the effect of arginine-vasopressin on retrieval and relearning needs further study particularly regarding the possible involvement of oxytocin receptors.

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