Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology, Biochemistry and Behavior 78 (2004) 309–317
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Effect of pretraining administration of NC-1900, a vasopressin fragment analog, on memory performance in non- or CO_2-amnesic mice

Tomoaki Sato[a,*], Koh-ichi Tanaka[a], Toyonori Teramoto[a], Yoshiko Ohnishi[a], Kenji Hirate[b], Masahiro Irifune[c], Takashige Nishikawa[a]

[a]*Department of Applied Pharmacology, Kagoshima University Graduate School of Medical & Dental Sciences, Sakuragaoka-8, Kagoshima 890-8544, Japan*
[b]*Research Laboratories, Nippon Chemiphar Co., Ltd. Saitama 341-0005, Japan*
[c]*Department of Anesthesiology, Hiroshima University Dental Hospital Kasumi, Minami, Hiroshima 734-8533, Japan*

Received 25 December 2003; received in revised form 1 April 2004; accepted 5 April 2004
Available online 12 May 2004

Abstract

In the present study, we investigated the facilitative effect of NC-1900, a new arginine vasopressin (AVP_{1-9}) fragment analog, on memory performance in eight-arm radial maze or passive avoidance (PA) tasks in nonamnesic and amnesic (PA tasks only) mice. In the radial maze, all injections (subcutaneous) were given daily 60 min before each trail. NC-1900 (1 ng/kg)-treated animals showed enhancement of performance, and AVP_{4-9} (1 μg/kg), an AVP_{1-9} fragment, had similar effects, although the effective dose was 1000-fold higher. In the PA task, all drugs were administrated subcutaneously 60 min before the acquisition trial (Acq.), and the amnesic mice were exposed to CO_2 just after the Acq. NC-1900 (1 ng/kg) enhanced the memory performance of nonamnesic mice and ameliorated CO_2-induced amnesia. AVP_{4-9} (1 μg/kg) had a similar effect, although only at higher doses, while AVP_{1-9} (0.1–1 μg/kg) had no effect. The facilitating effect of NC-1900 on nonamnesic mice was inhibited by coinjection [Pmp^1-$Tyr(Me)^2$]-AVP (Pmp,Tyr-AVP; 1 μg/kg), a V_{1A} antagonist, but not by OPC-31260, a vasopressin$_2$ (V_2) antagonist. The effect of NC-1900 on CO_2-induced amnesia was also decreased by coinjection of Pmp,Tyr-AVP or [deamino-Pen^1, $Me\text{-}Tyr^2$]-AVP (10 μg/kg), both of which are V_1 antagonists. These results suggested that NC-1900 has a more potent effect on facilitation of memory via the V_{1A} receptor than AVP_{4-9} in non- and CO_2-amnesic conditions.

Keywords: NC-1900; AVP_{4-9}; Vasopressin; Memory; CO_2; Amnesia

1. Introduction

AVP_{1-9} has been suggested to play an important role in memory formation, and it has been confirmed that AVP_{1-9} facilitates learning and memory processes in several animal models (De Wied, 1971; Paban et al., 2003). Although the dissociation of the memory effect and the classical endocrine effects of AVP_{1-9} have been reported by Lande et al. (1971), these studies are often been criticized on the basis that AVP_{1-9}-induced memory facilitation may not actually involve an improvement of memory itself, but rather a change in performance as a result of alterations in attention, motivation, arousal, or peripheral effects (Ettenberg et al., 1983). De Wied et al. (1984) reported that central administration of AVP_{1-9} promoted passive avoidance (PA) behavior and that central administration of a vasopressin antagonist blocked the PA response, but not the peripheral effect of systemically administered AVP_{1-9}. They also showed that AVP_{4-8}, a desglycinamide derivative of a peptide fragment of AVP_{1-9}, improved the latency of the task in the absence of a pressor effect. Thereafter, a carboxy-terminal 4–9 sequence [pGlu–Asn–Cys(Cys)–Pro–Arg–Gly–NH_2; AVP_{4-9}] that was generated by aminopeptidase was discovered. AVP_{4-9} reportedly has a more potent facilitative effect on performance in PA (Burbach et al., 1983) and other memory tasks (Dietrich and Allen, 1997a,b; Mishima et al., 2001), and does not have peripheral effects, such as antidiuretic or pressor effects (Burbach et al., 1983; Dietrich and Allen, 1997a; Mishima et al., 2001).

NC-1900 (pGlu–Asn–Ser–Pro–Arg–Gly–NH_2) is a newly synthesized AVP_{4-9} analog in which the cysteine residue of AVP_{4-9} is replaced with a serine residue (Hori et al., 2002; Mishima et al., 2003). Hirate et al. (1997) reported

* Corresponding author. Tel.: +81-99-275-6162; fax: +81-99-275-6168.
E-mail address: tomsato@dentb.hal.kagoshima-u.ac.jp (T. Sato).

0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.04.002

that NC-1900 ameliorates cyclohexamide-induced memory impairment of PA behavior, and Hori et al. (2002) showed an ameliorating effect of NC-1900 on spatial memory impairment induced by transient forebrain ischemia in rats. We also revealed that the new peptide improved learning and memory impairment and cell damage to cultured cerebrocortical neurocytes induced by glutamic acid (Sato et al., 1999). In addition, it has been reported that NC-1900 inhibits glycine-induced Cl^- currents in the CA1 region of isolated rat hippocampus and that the inhibition is due to activation of protein kinase A (Omura et al., 1999). Most recently, Mishima et al. (2003) showed that the new derivative improves scopolamine-induced amnesia in a radial maze, a spatial memory task, and concluded that the improvement was not caused by an increase in the release of acetylcholine, but rather through the activation of V_{1A} receptors at postsynaptic cholinergic nerves and by interaction with postsynaptic M_1 receptors.

Previous studies in animal models of amnesia induced by scopolamine, cyclohexamide, or ischemia have demonstrated a facilitative effect of NC-1900 on memory performance; however, the effect of NC-1900 on amnesia induced by anoxia/hypoxia, such as that induced by CO_2 amnesia, has not yet been investigated. Therefore, in the present study, we examined the effects of NC-1900 and vasopressin receptor (V) antagonists using a CO_2-induced amnesia model, and compared the effects with those previously established for AVP_{1-9} and AVP_{4-9}. In addition, we investigated the effect of NC-1900 and V antagonists on the acquisition process in the radial maze and step-through PA tasks in nonamnesic mice (no CO_2 exposure).

2. Materials and methods

2.1. Animals

Male ddY mice (Kyudou, Kumamoto, Japan), 5–6 weeks old, were used in the step-through PA task, and 7- to 8-week-old mice were used in the eight-arm radial maze task. The animals were housed with free access to standard food (Clea Japan) and water in an air-conditioned room with a temperature of 24 ± 1 °C, humidity of $50 \pm 10\%$ and a constant 12-h light/dark cycle (lights on between 0700 and 1900 h). All behavioral experiments were carried out between 0900 and 1700 h. The procedures were approved by the Committee of Animal Experimentation, Dental School, Kagoshima University.

2.2. Drugs and treatment

The following drugs were used throughout the experiments: NC-1900, AVP_{4-9}, AVP_{1-9}, [Pmp^1-$Tyr(Me)^2$]-AVP ([1-(β-cyclopentamethylene propionic acid), 2-(*O*-methyl)-tyrosine]-Arg^8-vasopressin), [deamino-Pen^1, *O*-Me-Tyr^2]-AVP ([1-deamino-penicillamine 2-*O*-methyltyrosine-Arg^8-vasopressin]), and OPC-31260 (5-dimethylamino-1(4-(2-methylbenzoylamino)benzoyl-2,3,4,5-tetrahydro-1H-benzazepine) were obtained from Nippon Chemiphar (Saitama, Japan). These drugs were dissolved in 0.9% saline and injected subcutaneously (NC-1900: 0.1–100 ng/kg; AVP_{4-9}: 0.1–10 μg; AVP_{1-9}: 1–10 μg; Pmp,Tyr-AVP: 4 ng/kg–1 μg/kg; [deamino-Pen^1, *O*-Me-Tyr^2]-AVP (Pen,Me-AVP): 10 ng/kg–10 μg/kg; and OPC-31260: 10 ng/kg–10 μg/kg) 60 min before the acquisition trial (Acq.) of the step-through task or before each trial of the eight-arm radial maze task; the control groups were injected with saline in the same manner. Additionally, when two drugs were administrated simultaneously, the drugs were coinjected with a single syringe 60 min before the Acq. of the step-through task.

2.3. Eight-arm radial maze

The mice were tested in an eight-arm radial maze that was a modified version of the one originally developed by Olton and Samuelson (1976), as previously reported (Sato et al., 2003). In short, the maze used in the present study consisted of eight arms extending radially from a central area (22 cm diameter). Each arm was 50 cm long, 10 cm wide, and 5 cm high with gray vinyl chloride board walls. Food cups for the reinforcers were placed near the end of each arm. The maze was located in a room containing many extramaze visual cues.

Prior to pretraining, the mice were kept on a restricted diet and the body weight was reduced to 90% of normal weight over a 1-week period; water was freely available. Before the pretraining period, each mouse was handled for at least 5 min daily for 5 days. Before the radial maze trial began, mice underwent the pretraining session for 5 days, during which period, the mice were given 10 min daily to adapt to the apparatus. After the pretraining period, the learning and memory abilities of the mice were evaluated over 10 trials (one trial per day for 2 weeks). In each trial, a maximum of 10 min was allowed to visit all eight arms and eat the food reinforcements. To begin each trial, the mouse was placed on the central platform in random orientation and was then allowed to enter any of the arms. A visit to an arm was scored if all four limbs of the mouse were within an arm. Reentry into an already visited arm was regarded as an error. Accuracy of choice was scored by the number of correct choices until the first mistake. In addition, the total number of incorrect choices in a trial was also scored.

2.4. Step-through PA task

Learning and memory ability was also assessed by the step-through-type PA test, as previously reported (Sato et al., 2004), using methods modified from Matuoka et al. (1995). A two-compartment step-through-type PA apparatus was used in which the box was divided into bright ($9 \times 9 \times 36$ cm) and dark compartments ($26 \times 26 \times 36$ cm) by a guillotine door. In each trial, a mouse was placed in the

illuminated compartment for a 30-s habituation period, and then a guillotine door was raised to allow entry into the dark chamber. On the preexposure session (data not shown), the step-through latency (the length of time spent in the bright compartment after a habituation period) was measured, and mice that stepped through to the grids of the dark compartment were allowed to remain there for 30 s without electrical stimulation and were then returned to their home cage. Twenty-four hours after the measurement of the preexposure latency, the Acq. was conducted. When the hind legs of the mice entered into the dark chamber, the guillotine door was closed and electrical foot shock (20 V, duration 80 ms, repeat 500 ms, alternating current) was delivered through the grid floor for a total of 3 s. The time that elapsed prior to entry into the dark compartment (latency) was recorded. Retention trials (Ret.) were then performed 24 h after the Acq. and the latency was measured for up to 300 s.

2.5. CO_2 treatment

CO_2 amnesic mice were used in the experiments shown in Figs. 4 and 5. The method used to effect CO_2-induced amnesia was reported previously (Santo-Yamada et al., 2001). In short, some mice were given CO_2 treatment immediately after they had received the foot shock during the Acq. The animals were placed in a closed transparent box filled with CO_2 for 1 min. The mice were then revived by artificial respiration (the time necessary for respiratory arrest to occur was 35–45 s) Sham-treated mice (control groups in Figs. 4 and 5.) were placed in an identical but air-filled box.

2.6. Statistical analysis

Results are expressed as the mean ± S.E.M. Data from the eight-arm radial maze experiments were analyzed using repeated-measures analysis of variance (ANOVA) with post hoc tests (Bonferroni/Dunn test). Data from step-through PA experiments were analyzed using one-way ANOVA with Bonferroni/Dunn test. Statistically significant differences between groups are indicated by $P<.05$. Data analyses were performed using Super ANOVA 1.11 software (Abacus Concepts, Berkeley, CA).

3. Results

3.1. Effect of NC-1900, AVP_{4-9}, and AVP_{1-9} on eight-arm radial maze performance

The number of correct choices until the first mistake during 10 consecutive trials is shown for each treatment group in Fig. 1A. A between-groups comparison of the four treatment groups showed that mice receiving 1 ng/kg NC-1900 or 10 μg/kg AVP_{4-9} made a greater number of correct

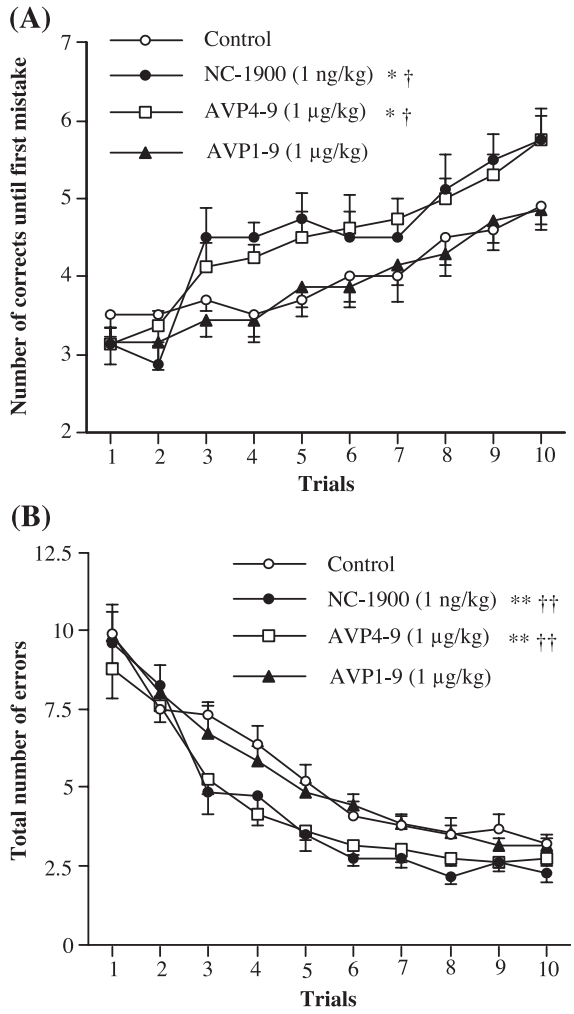

Fig. 1. Changes in the number of correct choices until the first mistake (A) and total number of incorrect choices (B) for radial maze performance of each treatment group of nonamnesic mice. All drugs were subcutaneously injected 60 min before each trial. Results were expressed as mean ± S.E.M. ($n=7-10$ per group). $^{*}P<.05$, $^{**}P<.01$ compared with control group. $^{\dagger}P<.05$, $^{\dagger\dagger}P<.01$ compared with AVP_{1-9} group (repeated-measures ANOVA followed by post hoc test; see Results section for details).

choices until the first mistake; repeated-measures ANOVA revealed a significant overall group effect [$F(3,29)=3.39$, $P<.05$] and relative to trial effect [$F(9,261)=30.5$, $P<.01$]. However, there was no significant Treatment × Trial interaction [$F(27,261)=1.28$, $P=$NS]. Post hoc tests revealed significant differences between control and NC-1900 ($P<.05$), control and AVP_{4-9} ($P<.05$), NC-1900 and AVP_{1-9} ($P<.05$), and AVP_{4-9} and AVP_{1-9} ($P<.05$) groups. In addition, there was no significant difference between the AVP_{1-9} and control, and the NC-1900 and AVP_{4-9} groups.

Fig. 1B shows the number of total errors in the 10 trials. The animals treated with the peptides were compared with each treatment group. Repeated-measures ANOVA showed a significant difference among the four treatment groups [$F(3,29)=8.03$, $P<.01$], and relative to the trial [$F(9,261)=103.9$, $P<.001$]. However, the Treatment × Trial interaction was not significant [$F(27,261)=$

1.00, P=NS]. Post hoc tests showed significant differences between control and NC-1900 ($P<.01$), control and AVP_{4-9} ($P<.01$), NC-1900 and AVP_{1-9} ($P<.01$), and AVP_{4-9} and AVP_{1-9} ($P<.01$) groups. However, there were no significant differences between the AVP_{1-9} and control, and NC-1900 and AVP_{4-9} groups.

3.2. Effects of NC-1900 and AVP-related drugs on step-through PA performance in nonamnesic mice

Fig. 2 shows the effects of various doses of NC-1900, AVP_{4-9}, and AVP_{1-9}, and Pmp,Try-AVP, Pen,Me-AVP, and OPC-31260 on learning and memory in the step-through PA task. One-way ANOVA showed a significant difference in the Ret. [$F(13,129)=7.46$, $P<.01$]. Post hoc tests showed significant differences in comparison with the control group for the 1- ($P<.01$) and 10-ng/kg ($P<.01$) doses of NC-1900, and for the 1-μg/kg dose of AVP_{4-9} ($P<.01$). Latency tended to be increased in the 0.1-ng/kg NC-1900 ($P=.06$) and 0.1-μg/kg AVP_{4-9} ($P=0.06$) groups; however, the effects were not statistically significant. Treatment with AVP_{1-9} (0.1 and 1 μg/kg) did not have a significant effect on latency in the control group. Interestingly, the administration of Pen,Me-AVP (10 μg/kg), a V_1 receptor antagonist with low antidiuretic activity (Kaygisiz et al., 2001; Sato et al., 2002), produced a slight reduction of the latency, but the effect was not significant ($P=.09$). Pmp,Try-AVP (1 μg/kg), a more potent and selective vasopressin V_{1A} receptor antagonist (Mishima et al., 2001), significantly decreased latency in comparison with the control group ($P<.05$). However, high (10 μg/kg) and low (4 ng/kg) doses of OPC-31260, a potent $vasopressin_2$ (V_2) antagonist (Mishima et al., 2001), did not significantly affect latency.

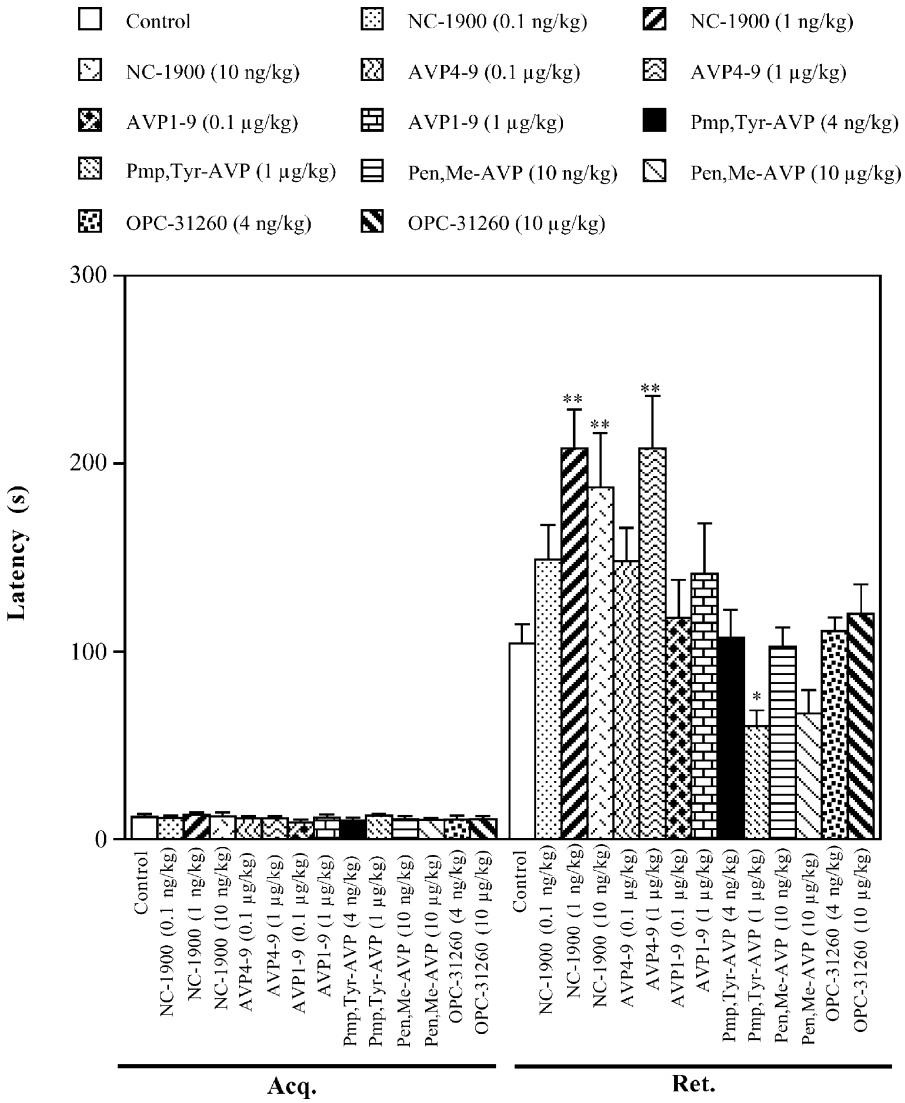

Fig. 2. Effects of NC-1900 and AVP_{1-9}-related drugs on step-through PA performance in nonamnesic mice. All drugs were subcutaneously injected 60 min before the Acq. Results were expressed as mean ± S.E.M. ($n=8-15$ per group). * $P<.05$, * * $P<.01$ vs. control group at the Ret. (one-way ANOVA followed by post hoc test).

3.3. Effects of coinjection with NC-1900 and V_1 or V_2 antagonist on step-through PA performance in nonamnesic mice

To examine the role of vasopressin receptors in the NC-1900-induced increase in step-through latency, mice were coinjected with NC-1900 (1 ng/kg) and a V_1 or V_2 receptor antagonist 60 min before the Acq. As shown in Fig. 3., the increase in latency on the Ret. in response to NC-1900 (1 ng/kg) occurred to a significantly lesser extent [$F(5,63) = 2.38$, $P < .05$] in mice that were coinjected with NC-1900 and 1 μg/kg of Pmp,Tyr-AVP. A similar trend was observed in the group coinjected with 10 μg/kg Pen,Me-AVP, although the effect was not significant ($P = .06$). However, step-through latency was clearly not reduced in the 1 μg/kg OPC-31260 coinjection group.

3.4. Comparison of the effects of NC-1900 and AVP-related drugs on CO_2-induced amnesia in the step-through PA task

The effects of NC-1900 (1 ng/kg), AVP_{4-9} (1 μg/kg), AVP_{1-9} (10 μg/kg), Pmp,Tyr-AVP (10 ng/kg), Pen,Me-AVP (10 μg/kg), and OPC-31260 (10 μg/kg) on CO_2-induced amnesia in the step-through PA task are presented in Fig. 4. One-way ANOVA showed significant differences in the Ret. [$F(7,67) = 4.84$, $P < .01$]. CO_2 exposure significantly decreased latency compared to the control group ($P < .05$), and the administration of NC-1900 (1 ng/kg) ameliorated the CO_2-induced amnesia ($P < .01$). A similar effect was observed in AVP_{4-9} ($P < .01$), although the effective dose (1 μg/kg) was 1000-fold more than that of NC-1900. Additionally, the administration of AVP_{1-9} (10 μg/kg), Pmp,Tyr-AVP (1 μg/kg), Pem,Me-AVP (10 μg/kg), or

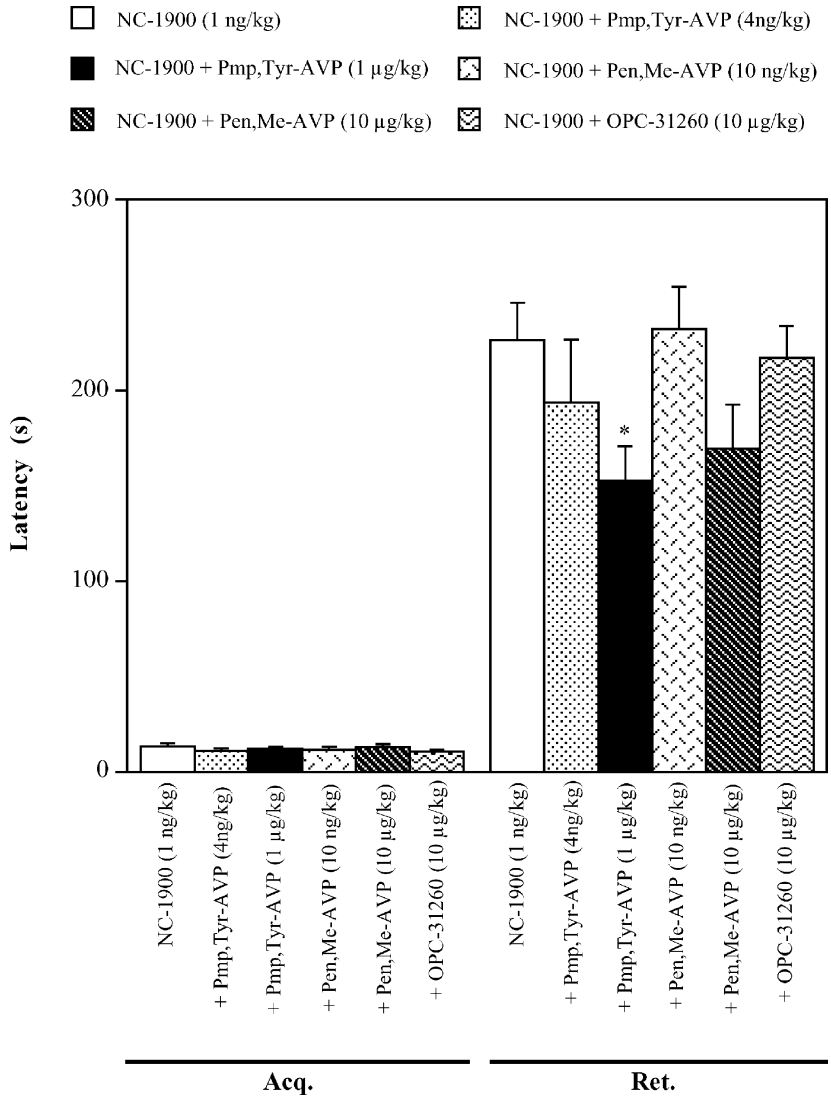

Fig. 3. Effect of coinjection with NC-1900 and V antagonists on step-through PA performance in nonamnesic mice. All drugs were subcutaneously injected or coinjected 60 min before the Acq. Results were expressed as mean ± S.E.M. ($n = 8–14$ per group). * $P < .05$ vs. NC-1900 (1 ng/kg) group at the Ret. (one-way ANOVA followed by post hoc test).

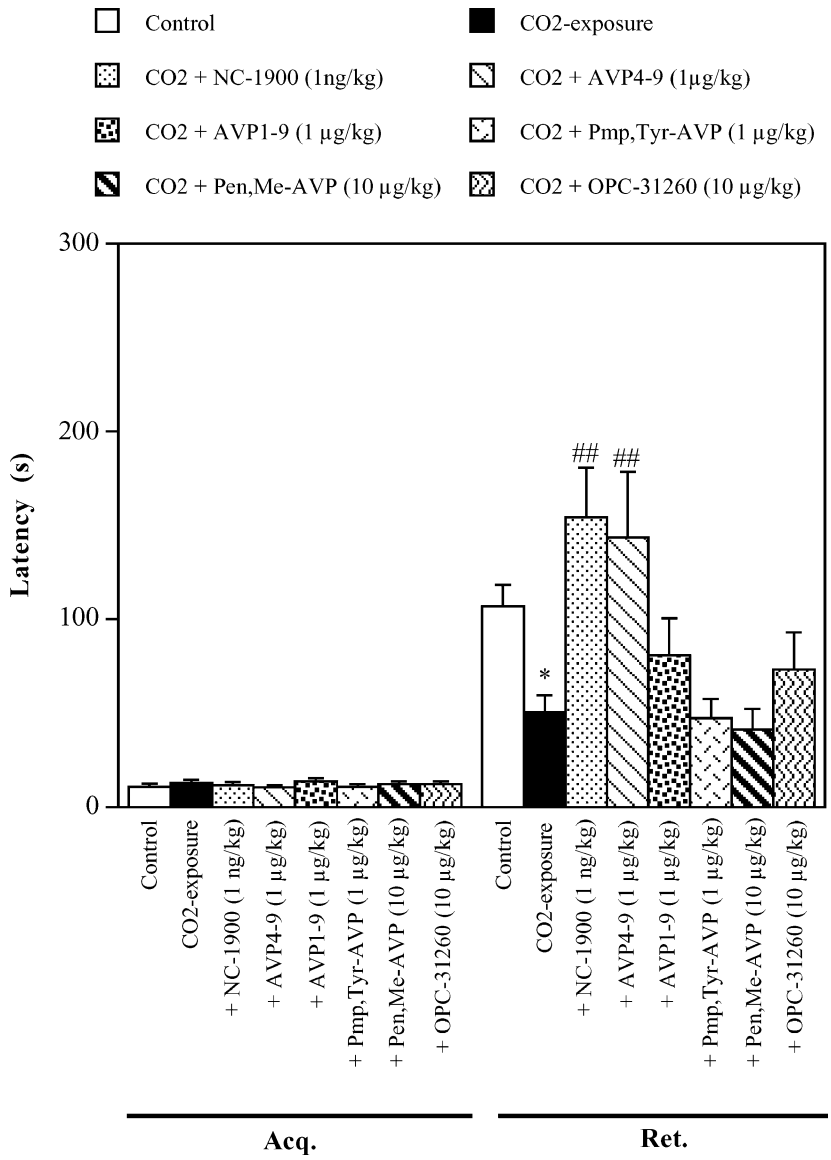

Fig. 4. Effects of NC-1900 and AVP_{1-9}-related drugs on PA performance in CO_2-induced amnesic mice. All drugs were subcutaneously injected 60 min before the Acq., and the CO_2 exposure was conduced immediately after the Acq. Results were expressed as mean ± S.E.M. ($n = 8-12$ per group). * $P < .05$ vs. control (sham-treated) group; $^{\#\#}P < .01$ vs. CO_2 exposure + NC-1900 group at the Ret. (one-way ANOVA followed by post hoc test).

OPC-31260 (10 µg/kg) had no effect on CO_2-induced amnesia.

3.5. Effects of coinjection with NC-1900 and V_1 or V_2 antagonist on CO_2-induced amnesia in the step-through PA task

As shown in Fig. 5, coinjection with V_1 antagonist and NC-1900 inhibited the ameliorative effect of NC-1900 (1 ng/kg) on CO_2-induced amnesia in the step-through PA task [$F(3,42) = 2.88$, $P < .05$]. Among CO_2-exposed mice, post hoc tests revealed significant differences on the Ret. between the NC-1900 group and the groups coadministered with 1 µg/kg Pmp,Tyr-AVP and NC-1900 ($P < .05$) or 10 µg/kg Pen,Me-AVP and NC-1900 ($P < .05$). However, coadministration of OPC-31260 did not influence the ameliorative effect of NC-1900 on CO_2-induced reduction of latency.

4. Discussion

One of the key findings in the present experiment was that NC-1900 enhanced performance in the eight-arm radial maze and step-through PA task, and the effective dose was 1000-fold lower than that of AVP_{4-9}. A learning effect over the course of radial maze experiments by AVP_{4-9} has been already reported (Dietrich and Allen, 1997a,b), and our study showed similar results. Burbach et al. (1983) reported that AVP_{4-9} is about 1000-fold more potent than AVP_{1-9}. In the present study, AVP_{1-9} did not have a facilitative

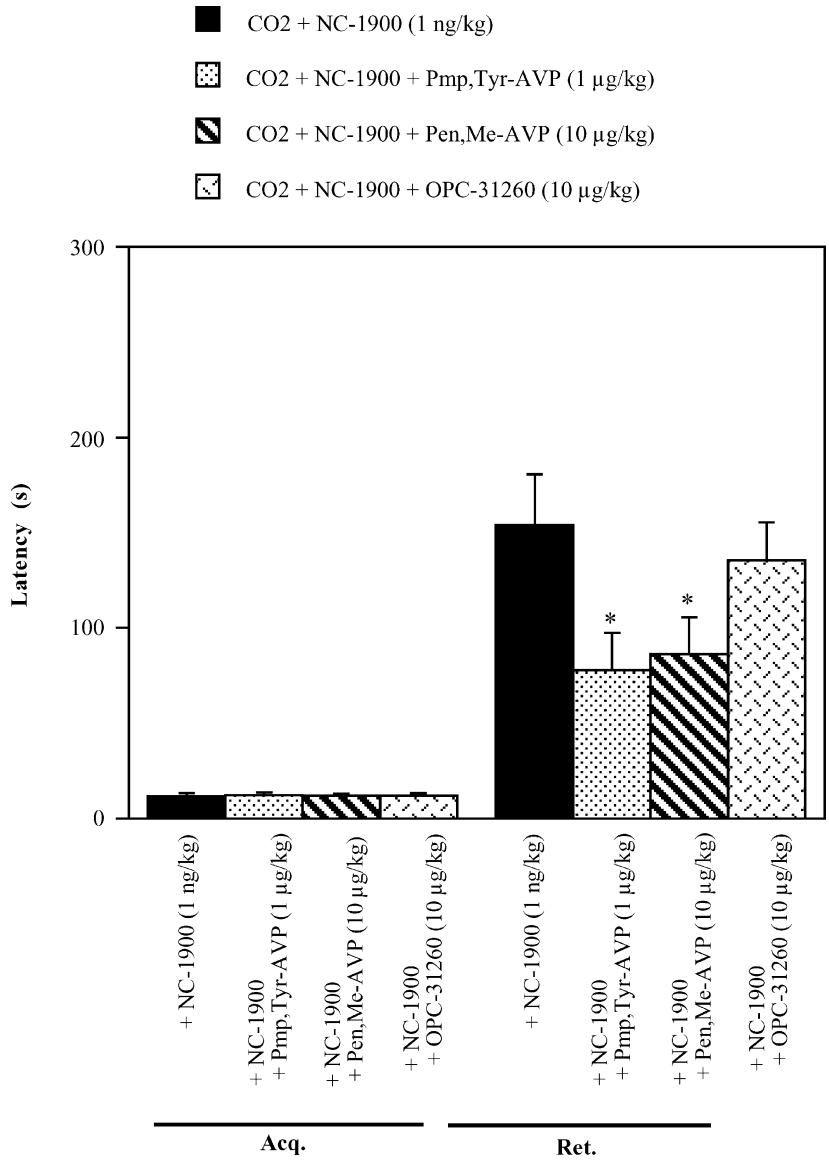

Fig. 5. Effects of coinjection with NC-1900 and V antagonists on step-through PA performance in CO_2-induced amnesic mice. All drugs were subcutaneously injected or coinjected 60 min before the Acq., and the CO_2 exposure was conducted immediately after the Acq. Results were expressed as mean ± S.E.M. ($n = 10-15$ per group). * $P < .05$ vs. CO_2 exposure + NC-1900 group at the Ret. (one-way ANOVA followed by post hoc test).

effect at the same doses at which AVP_{4-9} was effective: thus, confirming that AVP_{4-9} is more potent than AVP_{1-9}. Based on our previous study (Sato et al., 2002) and the present results, it appears that AVP_{1-9} had a poor effect on learning and memory task, and we think that a cause for the dull effect of AVP_{1-9} may be the cysteine residues in themselves or a stereoconfiguration formed by the dislufide bond between first and sixth cysteine residues. Likewise, the antidiuretic effect (Fog et al., 1964), the tertiary structure shaped by the disulfide bond, may be unessential for effect on learning and memory. In fact, AVP_{4-9} does not have the structure of the parent peptide, although AVP_{4-9} presents a more potent effect on learning and memory than AVP_{1-9}. Similarly, NC-1900 has no cysteine residue, thus, it seems that the new peptide has stably revealed a facilitative effect on learning and memory performance (Sato et al., 1999, 2002). Accordingly, it is noteworthy that NC-1900 enhanced radial maze and step-through PA performance at lower doses than AVP_{4-9}.

The enhancement of eight-arm radial maze performance may be due to improved working memory because NC-1900-treated mice had a higher number of correct responses until the first mistake and a lower number of total errors than the control animals. Moreover, an increase in latency by NC-1900 would explain the low movement of animals treated with the drug, although the enhancement of PA performance was not caused by inhibitory activation of the mice because the animals administered with 1 ng/kg of NC-1900 were able to acquire radial maze task rewards within a limited time and were able to move freely in the

apparatus during the tasks. In addition, it is also possible that enhancement of latency by NC-1900 in the step-through task was due to a change in the electrical sensitivity of the animals. However, Hirate et al. (1997) reported that the administration of NC-1900 (0.1–10 ng/kg) did not change the electrical sensitivity of animals in a PA test. Thus, it is unlikely that the enhancement effect of NC-1900 in the PA task was due to inhibition of activation or a change in the electrical sensitivity of the animals. A possible explanation for the enhancement of performance by NC-1900 is that the peptide acts on peripheral sites, such as the heart or the endocrine system. However, this possibility can be excluded, because like AVP_{4-9} (Dietrich and Allen, 1997a,b; Mishima et al., 2001), there are several reports (Hirano et al., 1998; Hirate et al., 1997) indicating that NC-1900 is void of any pressor or antidiuretic effects. In fact, our results showed that coinjection with Pmp,Tyr-AVP (1 μg/kg), a V_{1A} antagonist, inhibits the facilitating effect of NC-1900 on PA performance, but that OPC-31260, a V_2 antagonist, does not. Thus, it appears that NC-1900-induced memory facilitation was not due to the abovementioned peripheral effects or classical endocrine effect via V_{1B} receptors, such as adrenocorticotropic hormone secretion.

Dantzer et al. (1987) have reported that intraseptal injection of Pen,Me-AVP has a amnesic-like effect on social memory in rats. Although Pen,Me-AVP did not show a clear amnesic effect in our PA task, we did find that the administration of Pmp,Tyr-AVP, a more selective and potent antagonist, inhibited PA task performance. In addition, both V_1 antagonists inhibited the protective effect of NC-1900 on CO_2-induced amnesia. As mentioned in the Introduction, Mishima et al. (2003) suggested that NC-1900 ameliorates scopolamine-induced impairment of spatial memory via activation of V_{1A} receptors. Together, these results suggest that V_{1A} receptors are involved in NC-1900-induced facilitation of the retention process in PA under non- or CO_2-exposed conditions.

To evaluate the effects of new compounds on learning and memory, many experimental amnesic models have been used, and one of the most useful is CO_2-induced amnesia PA model (Eguchi et al., 1994; Santo-Yamada et al., 2001; Sato et al., 2002). Unlike scopolamine-induced amnesia, CO_2 produces amnesia via the alternation of not only cholinergic, but also noradrenergic and serotonergic neuronal function in the CNS (Chleide and Ishikawa, 1990; Rigter et al., 1975). The present experiment showed that NC-1900 ameliorates CO_2-induced amnesia in the step-through PA task. Fujiwara et al. (1997) and Mishima et al. (2003) reported that NC-1900 administration improves scopolamine-induced impairment of spatial memory in the eight-arm radial maze. Therefore, NC-1900 may ameliorate the learning deficits induced by CO_2 at least partly via the activation of cholinergic neurons. However, as mentioned above, NC-1900 may also attenuate dysmnesia via non-cholinergic neurotransmitter systems, including noradrenaline, serotonin, and glutamate. In fact, it has been reported that AVP_{1-9} causes an increase in catecholamine utilization in the limbic structures of rats (Tanaka et al., 1977), that V_1 receptors in the lateral septum are partly located on catecholaminergic nerve terminals (Ishizawa et al., 1990), and that intracerebroventricular injection of AVP_{1-9} improves 6-hydroxy-dopamine-induced impairment (which results from the disappearance of norepinephrine and dopamine) of memory in the step-through test (Winnicka and Wisniewski, 1998). These facts reveal that a relationship exists between AVP_{1-9}, catecholamines, and improved memory; thus, the modulation of catecholaminergic neurotransmission would explain why NC-1900 and AVP_{4-9} had an ameliorating effect on learning and memory. In the present study, the administration of NC-1900 was conducted 60 min before the Acq., and just after the trial, CO_2 exposures were carried out. Therefore, there may be other possible mechanisms underlying the protective effect of NC-1900 against CO_2-induced memory impairment. For example, NC-1900 may have protected against neuronal damage induced by CO_2 exposure, because CO_2 inhalation reportedly causes an increase of glutamate in the hippocampus (Gos et al., 2002). Thus, it is possible that NC-1900 protects against lesions caused by excitatory amino acids, such as glutamate. In fact, we previously reported that NC-1900 ameliorates damage to cultured rat cerebrocortical neurocytes induced by 100 and 1000 μM glutamic acid (Sato et al., 1999).

In conclusion, the present results indicate that NC-1900 ameliorates learning and memory impairment induced by CO_2 through the V_{1A} receptor, and that this novel peptide was more potent than either AVP_{1-9} or AVP_{4-9} in normal and amnesic mice.

Acknowledgements

This work was supported partly by the Japanese Ministry of Education, Science and Culture Grants-in Aid for Scientific Research (13771099).

References

Burbach JPH, Kovacs GL, De Wied D, Van Nispen JW, Greven HM. A major metabolite of arginine vasopressin in the brain is a highly potent neuropeptide. Science 1983;221:1310–2.

Chleide E, Ishikawa K. Hypoxia-induced decrease of brain acetylcholine release detected by microdialysis. NeuroReport 1990;1:197–9.

Dantzer R, Bluthe RM, Koob GF, Le Moal M. Modulation of social memory in the male rat by neurohypophyseal peptides. Pychopharmacology 1987;91:363–8.

De Wied D. Long term effect of vasopressin on the maintenance of conditioned avoidance response in rats. Nature 1971;232:58–60.

De Wied D, Gaffori O, van Ree JM, de Jong W. Central target for the behavioural effects of vasopressin neuropeptides. Nature 1984;308:276–8.

Dietrich A, Allen JD. Vasopressin and memory: I. The vasopressin analogue AVP_{4-9} enhances working memory as well as reference memory in the radial maze. Behav Brain Res 1997a;87:195–200.

Dietrich A, Allen JD. Vasopressin and memory: II. Lesion to the hippo-

campus block the memory enhancing effects of AVP4–9 in the radial maze. Behav Brain Res 1997b;87:201–8.
Eguchi J, Yuasa T, Egawa M, Tobe A. Effects of novel compound MCI-225 on impaired learning and memory in rats. Pharmacol Biochem Behav 1994;48:345–9.
Ettenberg A, van der Kooy D, Le Moal M, Koob GF, Bloom FE. Can aversive properties of (peripherally-injected) vasopressin account for its putative role in memory? Behav Brain Res 1983;7:310–50.
Fog CT, Silver L, Louie DD. Necessity of the disulfide bond of vasopressin for antidiuretic activity. Biochem Biophys Res Commun 1964;14: 302–6.
Fujiwara M, Ohgami Y, Inada K, Iwasaki K. Effect of active fragments of arginine–vasopressin on the disturbance of spatial cognition in rats. Behav Brain Res 1997;83:91–6.
Gos T, Hauser R, Krzyzanowski M. Regional distribution of glutamate in the central nervous system of rat terminated by carbon dioxide euthanasia. Lab Anim 2002;36:127–33.
Hirano M, Hirate K, Miyake N. NC-1900, a [pGlu^4,Cyt^6]AVP(4–9) analogue, facilitates the learning performance of rats on delayed nonmatching to sample task in Y-water maze. Methods Find Exp Clin Pharmacol 1998;20:567–73.
Hirate K, Hirano M, Nakajima Y, Hiyama A, Maeda O, Asakura W. No. 302, a newly synthesized [pGlu^4,Cyt^6]AVP(4–9) analogue, prevents the disruption of avoidance behavior. Behav Brain Res 1997; 83:205–8.
Hori E, Uwano T, Tamura R, Miyake N, Nishijo H, Ono T. Effects of a novel arginine–vasopressin derivative, NC-1900, on the spatial memory impairment of rats with transient forebrain ischemia. Cogn Brain Res 2002;13:1–15.
Ishizawa H, Tabakoff B, Mefford IN, Hoffman PL. Reduction of arginine vasopressin binding sites in mouse lateral septum by treatment with 6-hydroxy-dopamine. Brain Res 1990;507:189–94.
Kaygisiz Z, Kabadere TE, Dernek S, Erden SH. The effects of vasopressin in isolated rat hearts. Indian J Physiol Pharmacol 2001;45:54–62.
Lande S, Witter A, De Wied D. Pituitary peptides: an octapeptide that stimulates conditioned avoidance acquisition in hypophysectomized rats. J Biol Chem 1971;246:2058–62.
Matuoka Y, Hasegawa H, Okuda S, Muraki T, Uruno T, Kubota K. Ameliorative effects of tea catechins on active oxygen-related nerve cell injuries. J Pharmacol Exp Ther 1995;274:602–8.
Mishima K, Tsukikawa H, Inada K, Fujii M, Iwasaki K, Matsumoto Y, et al. Ameliorative effect of vasopressin-(4–9) through vasopressin V_{1A} receptor on scopolamine-induced impairments of rat spatial memory in the eight-arm radial maze. Eur J Pharmacol 2001;427: 43–52.
Mishima K, Tsukikawa H, Miura I, Inada K, Abe K, Matumoto Y, et al. Ameliorative effect of NC-1900, a new AVP_{4-9} analog, through vasopressin V_{1A} receptor on scopolamine-induced impairments of spatial memory in the eight-arm radial maze. Neuropharmacology 2003;44: 541–52.
Olton DS, Samuelson RJ. Remembrance of places passed: spatial memory in rats. J Exp Psychol Anim Behav Process 1976;2:97–116.
Omura T, Nabekura J, Akaike N. Intracellular pathways of V_1 and V_2 receptors activated by arginine vasopressin in rat hippocampal neurons. J Biol Chem 1999;274:32762–70.
Paban V, Soumireu-Mourat B, Alescio-Lautier B. Behavioral effects of arginine^8–vasopressin in the Hebb–Williams maze. Behav Brain Res 2003;141:1–9.
Rigter H, Van Eys G, Leonard BE. Hippocampal monoamine metabolism and the CO_2 induced retrograde amnesia gradient in rats. Pharmacol Biochem Behav 1975;3:781–5.
Santo-Yamada Y, Yamada K, Wada K. Posttraining administration of gastrin-releasing peptide improves memory loss in scopolamine- and hypoxia-induced amnesic mice. Physiol Behav 2001;74:139–43.
Sato T, Tanaka K, Ohnishi Y, Teramoto T, Hirate K, Nishikawa T. The ameliorating effects of a novel NC-1900 on impairments of learning/memory caused by glutamic acid. Folia Pharmacol Jpn 1999;114(Suppl. 1):198P–203P.
Sato T, Tanaka K, Ohnishi Y, Teramoto T, Hirate K, Nishikawa T. The improvement of memory retention and retrieval of a novel vasopressin fragment analog NC-1900. Folia Pharmacol Jpn 2002;120(Suppl. 1): 57P–60P.
Sato T, Teramoto T, Tanaka K, Ohnishi Y, Irifune M, Nishikawa T. Effects of ovariectomy and calcium deficiency on learning and memory of eight-arm radial maze in middle-aged female rats. Behav Brain Res 2003;142:207–16.
Sato T, Tanaka K, Ohnishi Y, Teramoto T, Irifune M, Nishikawa T. Effects of steroid hormones on (Na^+,K^+)-ATPase activity inhibition-induced amnesia on the step-through passive avoidance task in gonadoectomized mice. Pharm Res 2004;49:151–9.
Tanaka M, Versteeg DHG, De Wied D. Regional effects of vasopressin on rat brain catecholamine metabolism. Neurosci Lett 1977;4: 321–5.
Winnicka MM, Wisniewski K. 6-OHDA bilateral lesions to the nucleus septi lateralis attenuate vasopressin improvement of recall in rats. Pharmacol Res 1998;37:145–50.