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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

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Abstract

In order to clarify the mechanism by which pGlu-Asn-Cys(Cys)-Pro-Arg-Gly-NH_2 (vasopressin-(4–9)), a major metabolite C-terminal fragment of [Arg^8]-vasopressin (vasopressin-(1–9)), improves learning and memory, we used several different drugs such as an acetylcholine receptor antagonist, a Ca^{2+}/calmodulin-dependent protein kinase II inhibitor, vasopressin receptor antagonists and L-type Ca^{2+} channel blocker to disrupt spatial memory in rats. Moreover, we examined the effect of vasopressin-(4–9) on acetylcholine release in the ventral hippocampus using microdialysis. Vasopressin-(4–9) (10 fg/brain, i.c.v.) improved the impairment of spatial memory in the eight-arm radial maze induced by scopolamine, pirenzepine and Ca^{2+}/calmodulin -dependent protein kinase II inhibitor. Pirenzepine, a vasopressin V_{1A} receptor antagonist, and L-type Ca^{2+} channel blocker, but not a vasopressin V_2 receptor antagonist, suppressed the effects of vasopressin-(4–9) on scopolamine-induced impairment of spatial memory. Moreover, vasopressin-(4–9) did not affect acetylcholine release in the ventral hippocampus of intact rats or of scopolamine-treated rats as assessed by microdialysis. These results suggest that vasopressin-(4–9) activates vasopressin V_{1A} receptors on the postsynaptic membrane of cholinergic neurons, and induces a transient influx of intracellular Ca^{2+} through L-type Ca^{2+} channels to interact with muscarinic M_1 receptors. The activation of these processes by vasopressin-(4–9) is critically involved in the positive effect of vasopressin-(4–9) on scopolamine-induced impairment of spatial memory.

Keywords: Vasopressin-(4–9); Ca^{2+}; Vasopressin V_{1A} receptor; Spatial memory; Eight-arm radial maze; Scopolamine

1. Introduction

[Arg^8]-vasopressin (vasopressin-(1–9)), a nine-amino-acid peptide secreted by the neurohypophysis traditionally associated with water balance and blood pressure modulation, has been shown to act as a neurotransmitter or a neuromodulator in the central nervous system (Brinton and McEwen, 1989; Brinton et al., 1994). Numerous studies have shown that vasopressin-(1–9) affects many kinds of behavior, especially learning and memory (Kovács and De Wied, 1994; De Wised, 1997; Landgraf et al., 1998). For example, it was reported that vasopressin-(1–9) administered systemically or centrally, facilitated the consolidation and retrieval processes of active (Engelmann et al., 1992a) or passive avoidance tasks (Burbach et al., 1983), and enhanced social recognition (Engelmann et al., 1996), the retention of a visual discrimination task (Paban et al., 1997) and working and reference memory in radial maze (Dietrich and Allen, 1997), and improved experimentally induced impairment of learning and memory (Fujiwara et al., 1997; Hirate et al., 1997). Vasopressin-(1–9) in the central nervous system was also reported to interact with classical neurotransmitter systems (Brinton and McEwen, 1989; Maegawa et al., 1992), to elicit electrophysiological effects (Joëls and Urban, 1984; Tanaka et al., 1994; Omura et al., 1999), to stimulate second messenger systems (Nakayama et al., 2000), to change Ca^{2+} channel activity (Brinton et al., 1994; Gouzenes et al., 1999) and to induce the expression of transcription factors (Giri et al., 1990;

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Zhou et al., 1995). Although these actions of vasopressin-(1–9) seem to be mediated via the vasopressin V_1 receptor subtype, the exact mechanisms of action of centrally released vasopressin-(1–9) in learning and memory are largely unclear. This is because vasopressin-(1–9) is rapidly degraded in the brain by membrane-bound aminopeptidases with a half-time of < 1 min (Burbach and Lebouille, 1983; Stark et al., 1989).

Burbach et al. (1983) explored the metabolites of vasopressin-(1–9) that enhance learning and memory, and showed that the main active metabolite of vasopressin-(1–9) produced by aminopeptidase was the carboxy-terminal 4–9 sequence [$pGlu^4$, Cyt^6] vasopressin-(4–9) (vasopressin-(4–9)), and that vasopressin-(4–9) and its derivative vasopressin-(4–8) were about 1000- to 10,000-fold more potent than vasopressin-(1–9) to affect consolidation and retrieval processes of passive avoidance. Vasopressin-(4–8), the cysteinyl methyl ester derivative of vasopressin-(4–9), has been studied behaviorally and biochemically in detail as a highly potent metabolite of vasopressin-(1–9) (Lin et al., 1990; Du et al., 1998). These peptides were found to be more selective on learning and memory without affecting the regulation of water balance and blood pressure (Burbach et al., 1983; Lin et al., 1990).

In a previous study (Fujiwara et al., 1997), we also found that vasopressin-(4–9) administered by i.c.v. injection and its newly synthesized analogue, L-Pyroglutamyl-L-asparaginyl-L-seryl-L-prolyl-L-prolyl-L-arginylglycinamide (NC-1900, Hirate et al., 1997), in which the cysteine residue of vasopressin-(4–9) was replaced by serine, at a dose in the fentogram range, improved the scopolamine-induced impairment of spatial memory in an eight-arm radial maze, and were about 1000–10,000 times as potent as vasopressin-(1–9), with its smaller fragments being much less active. In addition, the scopolamine-induced disruption of spatial memory was found to be improved by the microinjection of vasopressin-(4–9) into the ventral hippocampus but not into the dorsal hippocampus. Our results thus suggested that the hexapeptide containing the 4th to 9th amino acids (H-Asn-Cys(Cys)-Pro-Arg-OH) and the 6th amino acid (Cys–Cys bond) found in the structure of vasopressin-(1–9) were important for its effects and that the ventral hippocampus was the region where vasopressin-(4–9) exerted its effect. However, the mechanisms by which vasopressin-(4–9) improves learning and memory have not yet been clarified, because the vasopressin-(4–9) binding site remains questionable (Brinton et al., 1986; Jurzak et al., 1993; Nakayama et al., 2000), in spite of numbers of behavioral studies about learning and memory enhancement by vasopressin-(4–9) (Dietrich and Allen, 1997; Tanabe et al., 1999). The purpose of the present study was to clarify the mechanisms by which vasopressin-(4–9) improves learning and memory. We therefore used several different drugs such as an acetylcholine receptor antagonist, Ca^{2+}/calmodulin-dependent protein kinase II inhibitor, vasopressin receptor antagonists and an L-type Ca^{2+} channel blocker to disrupt spatial memory of rats and examined the interaction between vasopressin receptor and muscarinic receptor. We also examined the effect of vasopressin-(4–9) on acetylcholine release in the ventral hippocampus, using microdialysis.

2. Materials and methods

2.1. Animals and housing

Male Wistar, rats weighing 200–250 g, were obtained from Kyudo (Saga, Japan), and were housed in groups of four to five animals per cage, in a room with the temperature controlled at 23 ± 2 °C and a relative humidity of $60 \pm 10\%$ with the lights on from 7:00 am to 7:00 pm. The animals were allowed a restricted food intake (10–12 g/day, CE-2, Crea Japan, Tokyo, Japan). Their body weight was maintained at approximately 80% of their weight under free-feeding conditions during the experimental period. The animals had free access to their home cages. All procedures regarding animal care and use were carried out based on the regulations established by the Experimental Animal Care and Use Committee of Fukuoka University.

2.2. Apparatus

Behavior was tested in an eight-arm radial maze (neuroscience, Tokyo, Japan), which was a modification of the one originally developed by Olton and Samuelson (1976), as previously reported (Fujiwara et al., 1997). It was elevated 50 cm from the floor. The maze consisted of a central platform 24 cm in diameter, with eight arms extending radially. Each arm was 50 cm long, 10 cm wide, and 50 cm high with transparent plastic side walls. Food cups for the reinforcers were placed near the end of each arm. The maze was located in a room containing many extra-maze visual cues. For the behavioral analysis, an image motion analyzer, AXIS-30 (neuroscience), was used to quantify the task performance of rats in the eight-arm radial maze. The high-speed analyzer had an automatic tracking system which allowed us to track each rat's movement in the maze with a CCD camera equipped with a personal computer, which then analyzed the movement in real time and assessed the length of the route, frequency of arm visits, velocity of walking and time required to accomplish the task.

2.3. Preparation of animals for an eight-arm radial maze

A group of animals was trained so that they would become accustomed to the apparatus and food pellet for 3 days before each test. In this 3-day period, a 10-min period of habituation was repeated three times a day, at intervals

of more than 1 h. In each training session, the animal was placed within a circular plastic enclosure on a platform in the middle of the eight-arm radial maze. Then, after 1 min, the ring was lifted and the animal was allowed to move freely in the maze. The trial continued until the animal had entered all eight arms or until 10 min had elapsed. If the animals proceeded in the eight-arm radial maze according to sequential routines that consisted of repeating a given angular direction (e.g., 45° or 135°) on repeated training, these animals were excluded from the present experiment. The performance of a given animal in each trial was assessed on the basis of three parameters: the number of correct choices in the eight arms initially chosen, the number of errors, defined as choosing arms which had already been visited, and the time elapsed before the animal ate all eight pellets. If the animals made seven or eight correct choices and less than one error in three successive sessions, they were then used for the evaluation of vasopressin-(4–9) the next day.

2.4. Surgical operation for intraventricular injection

The animals that had acquired spatial memory according to the above criteria were first anesthetized with sodium pentobarbital (50 mg/kg, i.p., Tokyo Kasei, Tokyo, Japan) and placed in a stereotaxic apparatus (Type SR-6, Narishige Scientific Instrument Lab., Tokyo, Japan). The skull was exposed and drilled through to the duramater at the coordinate of the ventricle (0.8 mm anterior to bregma, 1.3 mm lateral to the midline and 3.8 mm ventral from the skull surface with the incisor bar set at −3.3 mm below the interaural line) using the brain atlas of Paxinos and Watson (1998). A 21-gauge stainless steel guide cannula was implanted at the outer edge of the dura mater through the drilled opening, lowered perpendicularly to the appropriate depth and then was permanently secured with dental cement and bone screws. A recovery period of approximately 3–7 days was allowed. Before the drugs were administered, it was confirmed that the animals did not show any abnormal behavior in the eight-arm radial maze.

2.5. Intraventricular injection

A 27-gauge stainless steel injection cannula was used to infuse the drug. The cannula was connected to a 1-ml Hamilton syringe (Hamilton Co., Reno, NV) via polyethylene tubing (type PE10, i.d. 0.28 mm, o.d. 0.61 mm, Becton Dickinson, Parsippany, NJ, USA). Five microliters of vasopressin-(4–9), pirenzepine or KN-62 was injected bilaterally into each lateral ventricle through the injection cannula that extended 0.5 mm beyond the tip of the guide cannula, using a Microinjection Pump (CMA/100 Carnegie Medicine, Stockholm, Sweden). The rate of injection was 1.0 μl/min and the injection cannula was left in place for 3 min, to allow the drug to diffuse away from the tip. Vasopressin-(4–9) and KN-62 were injected i.c.v. 10 min prior to the test, and pirenzepine was given i.c.v. 30 min prior to the test. The performance in the radial maze during non-injected trials was not affected by repeated intraventricular injections.

2.6. Brain microdialysis for acetylcholine release

Brain microdialysis was performed as previously described (Iwasaki et al., 1996). Briefly, only the animals that fulfilled the criterion of the eight-arm radial maze were stereotaxically implanted with a guide cannula (AG-8; Eicom, Kyoto, Japan) under pentobarbital anesthesia (50 mg/kg, i.p.) as well as given the above microinjection. The guide cannula was placed in the region selected according to the atlas of Paxinos and Watson (1998), and the coordinates were measured from the bregma: ventral hippocampus, A: −4.8, L: 5.0, V: 6.0. A microdialysis probe (A-I-8-03; 3 mm for ventral hippocampus, Eicom) was inserted into the guide cannula of rats housed in plastic cages (30 × 30 × 35 cm, 4–5 days following surgery), and was perfused with Ringer's solution containing 0.1 mM eserine sulfate (Sigma, St. Louis, MO, USA) at a flow rate of 2 μl/min by means of a syringe pump (CMA/100; Carnegie Medicine). The syringe was connected to the probe inlet with polyethylene tubing; the probe outlet was connected to the sample loop (300 μl) of the analytical system by polyethylene tubing. The sample (20 μl) was collected with a refrigerated collector (CMA/140; Carnegie Medicine) at 10-min intervals over a 120-min period. To achieve stable baseline readings, microdialysis was allowed to proceed for 30 min before the collection of fractions. The acetylcholine concentra-

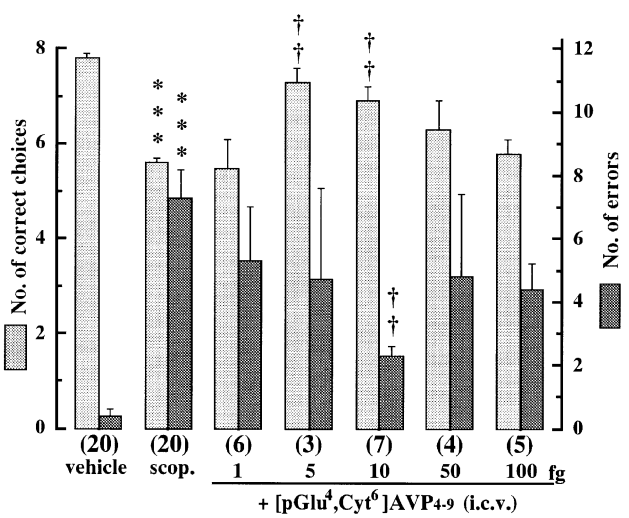

Fig. 1. Effects of i.c.v. injection of vasopressin-(4–9) on scopolamine-induced impairment of spatial memory in the eight-arm radial maze. Scopolamine and vasopressin-(4–9) were injected 30 min, i.p. and 10 min, i.c.v. prior to the test, respectively. $^{***}P < 0.001$ vs. vehicle, $\dagger\dagger P < 0.01$ vs. scop. (Kruskal–Wallis test followed by Wilcoxon's rank sum test). Number of rats is given at the bottom of each column. Scop.: scopolamine 0.5 mg/kg.

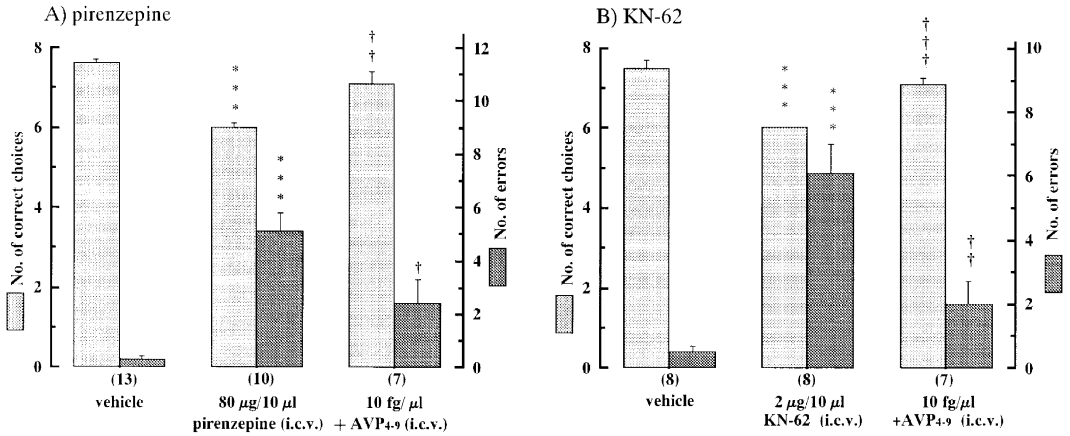

Fig. 2. Effects of i.c.v. injection of vasopressin-(4–9) on pirenzepine, KN-62-induced impairment of spatial memory in the eight-arm radial maze. Vasopressin-(4–9) was injected i.c.v. 10 min prior to the test, and pirenzepine and KN-62 were given i.c.v. 30 min prior to the test. $^{***}P < 0.001$ vs. vehicle, †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ vs. pirenzepine and KN-62 (Kruskal–Wallis test followed by Wilcoxon's rank sum test). Number of rats is given at the bottom of each column.

tions were then measured with a high-performance liquid chromatography-electrochemical detector (HPLC-ECD) system (Waters Assoc., Milford, MA), utilizing an Eicom-Pak AC column and enzyme column (EICOMPAK AC-GEL, Eicom) and were quantified by calculating the area under the curves using an integrator (Waters Model 730, Waters Assoc.). The acetylcholine concentration was then determined using an internal standard.

2.7. Histology

After completion of the experiment, the animals were injected with 2 μl Cresyl violet dye to confirm cannula placement. Ten minutes after this microinjection, the animals were anesthetized with ether and decapitated. After the brain was removed, it was frozen and sliced to a thickness of 40 μm. The injected sites were confirmed by macroscopic examination. Only data from animals in which the injections were made into the desired sites were analyzed.

2.8. Drugs

Scopolamine-HBr (Research Biochemicals, Natick, MA), [Pmp^1, Tyr(Me)^2]-Arg^8-vasopressin ([1-(β-Mercapto-β, β-cyclopentamethylene propionic acid), 2-(*O*-methyl)tyrosine]-Arg^8-vasopressin, Peptide Institute, Osaka, Japan) and 5-dimethylamino-1(4-(2-methylbenzoylamino)benzoyl)-2,3,4,5-tetrahydro-1*H*-benzazepine (OPC-31260; Otsuka Pharmaceutical, Tokushima, Japan)

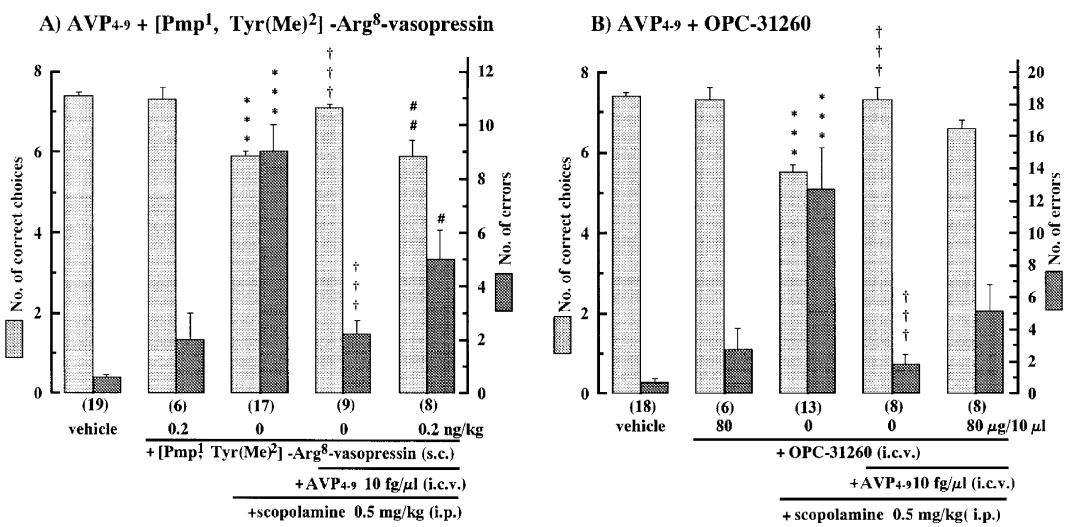

Fig. 3. Effects of [Pmp^1, Tyr(Me)^2], Arg^8-vasopressin and OPC-31260 on the ameliorative effect of vasopressin-(4–9) on the scopolamine-induced impairments of spatial memory. Vasopressin-(4–9) and OPC-31260 were injected i.c.v. 10 and 15 min prior to the test, respectively. Scopolamine and [Pmp^1, Tyr(Me)^2]-Arg^8-vasopressin were given 30 min i.p. and 60 min s.c., prior to the test, respectively. $^{***}P < 0.001$ vs. vehicle, †††$P < 0.001$ vs. scopolamine, #$P < 0.05$, ##$P < 0.01$ vs. scopolamine + vasopressin-(4–9) (Kruskal–Wallis test followed by Wilcoxon's rank sum test). Number of rats is given at the bottom of each column.

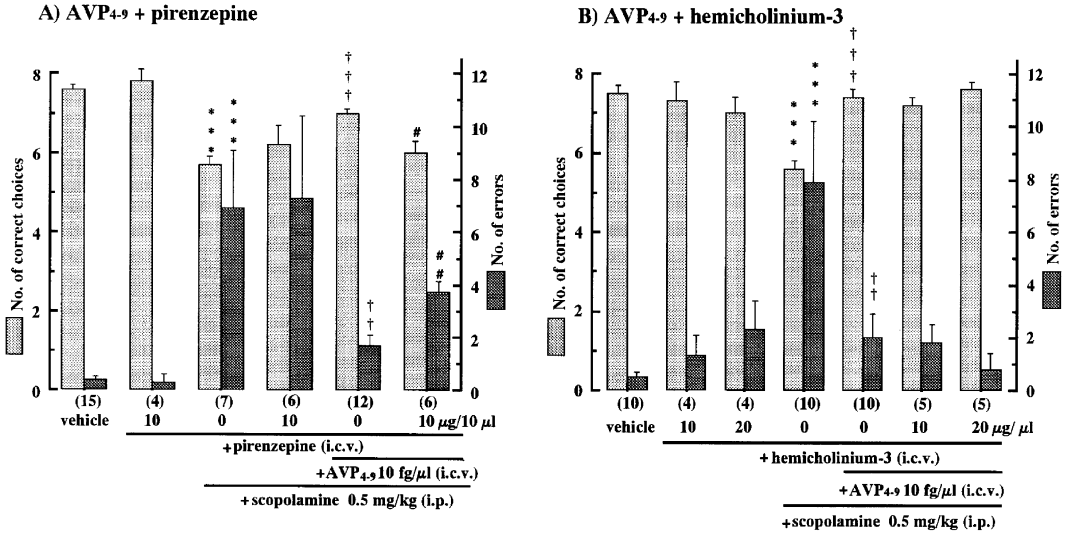

Fig. 4. Effects of pirenzepine and hemicholinium-3 on the ameliorative effect of vasopressin-(4–9) on the scopolamine-induced impairments of spatial memory. Vasopressin-(4–9) and hemicholinium-3 were injected i.c.v. 10 min prior to the test. Scopolamine and pirenzepine were given i.p. and i.c.v., 30 min prior to the test, respectively. $^{***}P < 0.001$ vs. vehicle, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$ vs. scopolamine, $\#P < 0.05$, $\#\#P < 0.01$ vs. scopolamine + vasopressin-(4–9) (Kruskal–Wallis test followed by Wilcoxon's rank sum test). Number of rats is given at the bottom of each column.

were dissolved in saline. Pirenzepine dihydrochloride (Research Biochemicals), vasopressin-(4–9) (Peptide Institute) and hemicholinium-3 (Sigma) were dissolved in artificial CSF (D-glucose 3.4 mM, NaCl 119 mM, $NaHCO_3$ 21 mM, KCl 3.3 mM, Na_2HPO_4 0.5 mM, $MgCl_2$ 1.2 mM, $CaCl_2$ 1.3 mM). 1-[*N*,*O*-bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenyl-piperazine (KN-62, Research Biochemicals) and nicardipine (Sigma) were suspended in 1% dimethylsulfoxide in saline and 1% Tween in saline, respectively.

2.9. Statistical analysis

Data from the eight-arm radial maze were evaluated for significant differences using the Kruskal–Wallis test followed by the Wilcoxon's rank sum test. Acetylcholine release was analyzed with a two-way (with repeated measures) analysis of variance (ANOVA). Values were considered as statistically significant at $P < 0.05$.

3. Results

3.1. Effects of vasopressin-(4–9) on scopolamine-induced impairments of spatial memory

All animals performed successfully in the maze within a maximum of 20 days. When the rats underwent cannula implantation and drug injection, they were re-trained and it was confirmed that they maintained their previous performance level.

As shown in Fig. 1, after scopolamine (0.5 mg/kg, i.p.), the number of correct choices decreased significantly while that of errors increased. Vasopressin-(4–9) (10 fg/brain, i.c.v.) reversed the decrease in the number of correct choices and the increase in errors induced by scopolamine (correct choices and errors; $P < 0.01$). The data showed a bell-shaped dose–response curve.

3.2. Effects of vasopressin-(4–9) on pirenzepine and KN-62-induced impairments of spatial memory

As shown in Fig. 2, after pirenzepine (80 μg/brain, i.c.v.), a selective muscarinic M_1 receptor antagonist, and

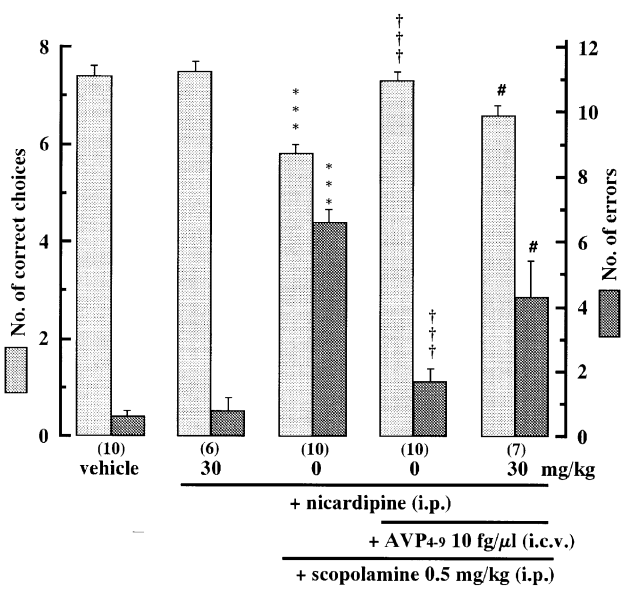

Fig. 5. Effect of nicardipine on the ameliorative effect of vasopressin-(4–9) on the scopolamine-induced impairments of spatial memory. Vasopressin-(4–9) was injected i.c.v. 10 min prior to the test. Scopolamine and nicardipine were given i.p. 30 min prior to the test. $^{***}P < 0.001$ vs. vehicle, $\dagger\dagger\dagger P < 0.001$ vs. scopolamine, $\#P < 0.05$ vs. scopolamine + vasopressin-(4–9) (Kruskal–Wallis test followed by Wilcoxon's rank sum test). Number of rats is given at the bottom of each column.

KN-62 (2 μg/brain, i.c.v.), a Ca^{2+}/calmodulin-dependent protein kinase II inhibitor, the number of correct choices decreased significantly while that of errors increased at 30 and 10 min after the injection, respectively. Vasopressin-(4–9) (10 fg/brain, i.c.v.) significantly reversed the decrease in correct choices and the increase in errors induced by pirenzepine (correct choices; $P < 0.01$ and errors; $P < 0.05$) and KN-62 (correct choices; $P < 0.001$ and errors; $P < 0.01$).

3.3. Effects of [Pmp[1], Tyr(Me)[2], Arg[8]]-vasopressin and OPC-31260 on the effect of vasopressin-(4–9) on scopolamine-induced impairment of spatial memory

Neither [Pmp^1, $Tyr(Me)^2$, Arg^8]-vasopressin (0.2–10 ng/kg, s.c. and 0.1–100 μg/brain, i.c.v.), a vasopressin V_{1A} receptor antagonist, nor OPC-31260 (10–80 μg/brain, i.c.v.), a vasopressin V_2 receptor antagonist, affected spatial memory in the eight-arm radial maze (data not shown). Co-administration of scopolamine (0.5 mg/kg) and [Pmp^1, $Tyr(Me)^2$, Arg^8]-vasopressin (0.2 ng/kg, s.c.) or OPC-31260 (80 μg/brain, i.c.v.) did not change scopolamine-induced impairment of spatial memory (data not shown). As shown in Fig. 3, [Pmp^1, $Tyr(Me)^2$, Arg^8]-vasopressin, but not OPC-31260, significantly suppressed the effects of vasopressin-(4–9) (10 fg/brain) on scopolamine-induced impairment of spatial memory (correct choices; $P < 0.01$ and errors; $P < 0.05$).

3.4. Effects of pirenzepine and hemicholinium-3 on the effect of vasopressin-(4–9) on scopolamine-induced impairment of spatial memory

Neither pirenzepine (10 μg/brain, i.c.v.) nor hemicholinium-3 (10–20 μg/brain, i.c.v.), a choline uptake inhibitor, affected spatial memory in the eight-arm radial maze (data not shown). Co-administration of scopolamine (0.5 mg/kg) and pirenzepine (10 μg/brain, i.c.v.) or hemicholinium-3 (10–20 μg/brain, i.c.v.) did not change the scopolamine-induced impairment of spatial memory (data not shown). As shown in Fig. 4, pirenzepine significantly suppressed the effects of vasopressin-(4–9) (10

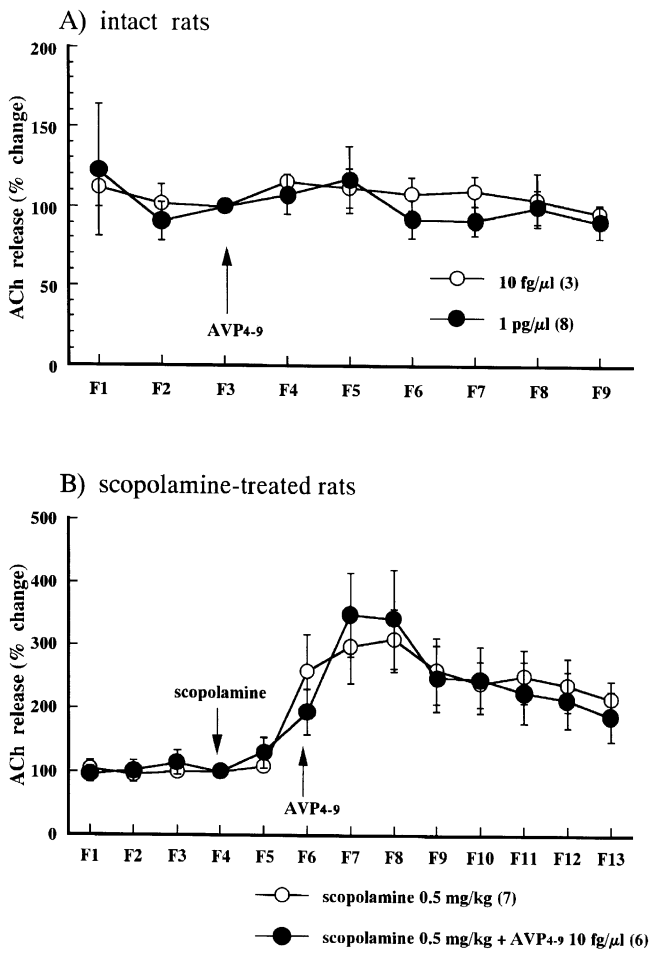

Fig. 6. Effects of vasopressin-(4–9) on the acetylcholine release in the ventral hippocampus in intact and scopolamine-treated rats. (A) Intact rats; vasopressin-(4–9) was injected i.c.v. to the highly trained rats immediately after the 3rd fraction (F3). (B) Scopolamine-treated rats; scopolamine (0.5 mg/kg, i.p.) and vasopressin-(4–9) (10 fg/μl, i.c.v.) were injected to the highly trained rats immediately after the 4th fraction (F4) and 6th fraction (F6). The acetylcholine concentrations are expressed as the % of the pre-sample average (F1–F3).

fg/brain) on scopolamine-induced impairment of spatial memory, but hemicholinium-3 did not suppress the effects of vasopressin-(4–9) (correct choices; $P < 0.05$ and errors; $P < 0.01$).

3.5. Effects of nicardipine on the effects of vasopressin-(4–9) on scopolamine-induced impairment of spatial memory

Nicardipine (3–30 mg/kg, i.p.), an L-type Ca^{2+} channel blocker, did not affect spatial memory in the eight-arm radial maze (data not shown). Co-administration of scopolamine (0.5 mg/kg) and nicardipine (30 mg/kg, i.p.) did not change the scopolamine-induced impairment of spatial memory (data not shown). As shown in Fig. 5, nicardipine significantly suppressed the effects of vasopressin-(4–9) (10 fg/brain) on scopolamine-induced impairment of spatial memory (correct choices; $P < 0.05$ and errors; $P < 0.05$).

3.6. Effects of vasopressin-(4–9) on acetylcholine release in the ventral hippocampus

As shown in Fig. 6, vasopressin-(4–9), at the effective dose of 10 fg/brain, induced no change in the basal level of acetylcholine in the ventral hippocampus of intact rats. Furthermore, vasopressin-(4–9) at the higher dose of 1 pg/brain produced no change in acetylcholine release in the ventral hippocampus. On the other hand, scopolamine (0.5 mg/kg, i.p.) induced marked increases in acetylcholine release in the ventral hippocampus with 297.0 ± 56.0% of the pre-fraction (4th fraction) value at 30 min (7th fraction) after the injection. However, vasopressin-(4–9) did not affect the increase in acetylcholine release in the ventral hippocampus of scopolamine-treated rats.

4. Discussion

In the present study, we found that vasopressin-(4–9) administered by i.c.v. injection, even at the very small dose of 10 fg, improved the scopolamine-induced impairment of spatial memory of rats in the eight-arm radial maze in agreement with our previous report (Fujiwara et al., 1997). Moreover, we examined whether vasopressin-(4–9) could improve the impairment of spatial memory induced by pirenzepine, a muscarinic M_1 receptor antagonist, in rats. The results demonstrated that vasopressin-(4–9) significantly improved the pirenzepine-induced impairment of spatial memory. Furthermore, pirenzepine at a dose which did not induce impairment of spatial memory, suppressed the effects of vasopressin-(4–9) on the scopolamine-induced impairment of spatial memory. It is known that the muscarinic M_1 receptor plays an important role in learning and memory function, and that activation of this receptor is associated with a transient intracellular influx of calcium ions, as well as activation of various protein kinases. Among various protein kinases, the Ca^{2+}/calmodulin-dependent protein kinase II is known to constitute 30–50% of the total protein in the postsynaptic density (Kennedy et al., 1983). The likely hood of a Ca^{2+}/calmodulin-dependent protein kinase II in learning and memory is strengthened by the findings that mutant mice with the Ca^{2+}/calmodulin-dependent protein kinase II gene knocked out, not only showed an impaired long-term potentiation, a prevailing neurobiological model for learning and memory, in their hippocampal slices but also was an impaired acquisition process for the water maze (Silva et al., 1992a,b). Recently it was shown that spatial learning performance is impaired after intra-hippocampal injection of KN-62, a Ca^{2+}/calmodulin-dependent protein kinase II inhibitor, and that inhibitory avoidance learning is impaired after intra-amygdala injection of KN-62 in rats (Wolfman et al., 1994; Tan and Liang, 1997). Therefore, we examined the effect of vasopressin-(4–9) on the KN-62-induced impairment of spatial memory. The results showed that vasopressin-(4–9) significantly improved KN-62-induced impairment of spatial memory, suggesting that vasopressin-(4–9) may improve the experimentally induced impairment of spatial memory by an intracellular Ca^{2+} influx through its binding site.

Vasopressin-(4–9) receptors have been classified into two major subtypes, vasopressin V_{1A} (Morel et al., 1992), V_{1B} (Lolait et al., 1995) and V_2 receptors (Lolait et al., 1992), based on their intracellular transduction mechanisms. The vasopressin V_{1A} and V_{1B} receptors are associated with phosphoinositol turnover, while the vasopressin V_2 receptor activates adenylate cyclase (Gouzenes et al., 1999; Omura et al., 1999). To further clarify the subtype of the vasopressin-(4–9) binding site in the behavioral study, we examined whether a vasopressin V_{1A} or a vasopressin V_2 receptor antagonists could suppress the effect of vasopressin-(4–9) on scopolamine-induced impairment of spatial memory. The results demonstrated that [Pmp^1, $Tyr(Me)^2$, Arg^8]-vasopressin, a vasopressin V_{1A} receptor antagonist (Manning and Sawyer, 1989), but not OPC-31260, a vasopressin V_2 receptor antagonist, suppressed the effects of vasopressin-(4–9) on scopolamine-induced impairment of spatial memory. Several reports have, however, suggested that vasopressin-(4–9) and vasopressin-(4–8) bind to receptors different from those of vasopressin-(1–9) (Brinton et al., 1986; Du et al., 1994; Nakayama et al., 2000). In the present study, the vasopressin V_{1A} receptor antagonist inhibited the effect of vasopressin-(4–9) on scopolamine-induced impairment of spatial memory, suggesting at least that vasopressin-(4–9) binds to the vasopressin V_{1A} receptor and that the vasopressin V_{1A} receptor interacts with the muscarinic M_1 receptor by intracellular Ca^{2+} influx. In addition, it was recently demonstrated that vasopressin-(1–9) and vasopressin-(4–9) enhanced intracellular Ca^{2+} in several types of cells via the vasopressin V_1 receptor (Brinton et al., 1994; Gouzenes et al., 1999). Therefore, we assumed that

Ca^{2+} influx was involved in the effect of vasopressin-(4–9). If so, a Ca^{2+} channel blocker should inhibit the effect of vasopressin-(4–9) on the scopolamine-induced impairment of spatial memory. Therefore, we examined whether nicardipine, an L-type Ca^{2+} channel blocker, could suppress the effect of vasopressin-(4–9). Nicardipine significantly inhibited the effect of vasopressin-(4–9) on the scopolamine-induced impairment of spatial memory. These results indicated that vasopressin-(4–9) might bind to the vasopressin V_{1A} receptor and interact with the muscarinic M_1 receptors by inducing intracellular Ca^{2+} influx through L-type Ca^{2+} channels.

Finally, we attempted to determine whether the vasopressin V_{1A} receptor stimulated by vasopressin-(4–9) existed at the presynaptic or the postsynaptic membrane of cholinergic neurons. If vasopressin-(4–9) stimulated the vasopressin V_{1A} receptor of the presynaptic membrane of cholinergic neurons, vasopressin-(4–9) would improve the scopolamine-induced impairment of spatial memory by enhancing acetylcholine release. Therefore, we examined the effect of vasopressin-(4–9) on acetylcholine release, using microdialysis, and we selected the ventral hippocampus to measure it, because our previous study had shown that the ventral hippocampus was more sensitive than the dorsal hippocampus to the effects of vasopressin-(4–9) (Fujiwara et al., 1997) and was found to be the important regions of the scopolamine-induced impairment of spatial memory in the eight-arm radial maze (Mishima et al., 2000). Moreover, it is of interest that the ventral hippocampus contains abundant terminals of extrahypothalamic vasopressinergic pathways (Buijs et al., 1983; Buijs and Kalsbeek, 1995) and vasopressin receptors (Elands et al., 1992). In the present study, vasopressin-(4–9), at a behaviorally effective dose of 10 fg/brain and the more than 100-fold higher dose of 1 pg/brain, did not affect acetylcholine release in the ventral hippocampus of intact rats. Furthermore, vasopressin-(4–9) did not affect the increase in acetylcholine release in the ventral hippocampus of scopolamine-treated rats. However, in contrast with our results, it was reported that vasopressin-(4–9) stimulated acetylcholine release in the rat hippocampus in vivo (Maegawa et al., 1992) and in vitro (Tanabe et al., 1999). The authors proposed these findings as possible mechanism by which vasopressin-(4–9) facilitated learning and memory. For example, it is reported that, despite direct infusion of vasopressin-(4–9) (10 μg/ml) into the ventral hippocampus for 30 min, an increase in acetylcholine release was observed for 1.5 to 3 h after the injection (Maegawa et al., 1992). It is considered that the increase in acetylcholine release induced by vasopressin-(4–9) may not be a direct effect of vasopressin-(4–9) on nerve endings, but an indirect effect. Another in vitro study (Tanabe et al., 1999) demonstrated that vasopressin-(4–9) at far higher concentrations slightly increased acetylcholine release in hippocampal slices and that the increase was suppressed by [Pmp^1, $Tyr(Me)^2$, Arg^8]-vasopressin, a vasopressin V_{1A} receptor antagonist, but that the vasopressin V_{1A} receptor antagonist did not affect basal acetylcholine release. If the vasopressin V_{1A} receptor was activated by vasopressin-(4–9) at cholinergic terminals, the vasopressin V_{1A} receptor antagonist must have decreased acetylcholine release. These findings led us to ask whether vasopressin-(4–9) had directly enhanced acetylcholine release in the hippocampus, and was it possible that, at a high concentration, vasopressin-(4–9) indirectly enhances acetylcholine release without mediating the presynaptic membrane of cholinergic neurons in the ventral hippocampus, in agreement with the present study that hemicholinium-3, a choline uptake inhibitor, which act the presynaptic membrane of cholinergic neurons, did not suppress the effect of vasopressin-(4–9).

A possible explanation for the bell-shaped dose–response of vasopressin-(4–9) is the following: first, we already reported that scopolamine impaired spatial memory in the eight-arm radial maze by blocking not only the postsynaptic muscarinic M_1 receptor but also the presynaptic muscarinic M_2 receptor, thus resulting in the increase in acetylcholine release by inhibiting its feedback (Mishima et al., 2000). These results suggest that the scopolamine-induced disruption of spatial memory may be influenced by the balance between the postsynaptic muscarinic M_1 receptor blocking action and the presynaptic muscarinic M_2 receptor blocking action. Vasopressin-(4–9) may affect this balance and may yield the "bell-shaped" dose–response observed in the present study. Second, since vasopressin-(4–9) was reported to influence motivational, arousal and attentional processes rather than memory (Sahgal, 1984; Bunsey et al., 1990), a "bell-shaped" dose-response can be interpreted psychologically as changes in these processes influenced by vasopressin-(4–9).

In the present study, vasopressin V_{1A} and V_2 receptor antagonists did not affect spatial memory in the eight-arm radial maze. A vasopressin V_{1A} receptor antagonist was reported to impair passive avoidance (Elands et al., 1992) and pole-jumping avoidance behavior (Engelmann et al., 1992a) requiring strong reinforcement such as electric shock. On the other hand, it is reported that a V_{1A} receptor antagonist did not affect spatial learning and memory in the water maze (Engelmann et al., 1992b) and the radial maze (Dietrich and Allen, 1997). These facts suggest that endogenous vasopressin may regulate non-spatial but not spatial learning and memory in rats.

Recently, Nakayama et al. (2000) proposed that vasopressin-(4–9) binds to a novel type of receptor in rat hippocampus because vasopressin-(4–9) at very low doses has a more potent effect on various functions than does vasopressin-(1–9), although they did not clone a novel type of receptor for vasopressin-(4–9). We also showed that both vasopressin-(4–9) and NC-1900 at a very low fentogram dose improved the scopolamine-induced impairment of spatial memory in the eight-arm radial maze, and

was about 1000–10000 times as potent as vasopressin-(1–9) (Fujiwara et al., 1997). Thus, these facts suggest that vasopressin-(4–9) and NC-1900 may bind to a novel type of vasopressin receptor.

In conclusion, we suggest that vasopressin-(4–9) activates a novel vasopressin receptor in the postsynaptic membrane of cholinergic neurons, and produces a transient intracellular influx of Ca^{2+} via L-type Ca^{2+} channels, resulting in an interaction with the muscarinic M_1 receptor. Activation of these processes induced by vasopressin-(4–9) is critically involved in the effects of vasopressin-(4–9) on scopolamine-induced impairment of spatial memory in the eight-arm radial maze.

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