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Research report

Effect of active fragments of arginine-vasopressin on the disturbance of spatial cognition in rats

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Abstract

The effect of arginine^8-vasopressin (AVP_{1-9}) and its metabolite C-terminal fragments on the scopolamine-induced disruption of spatial cognition were investigated using an 8-arm radial maze task in rats. AVP_{1-9} (10 μg/kg s.c.) markedly improved the disruption of spatial cognition by treatment with scopolamine (0.5 mg/kg i.p.), and 60% of the rats recovered to a normal level. The main metabolite of AVP_{1-9}, AVP_{4-9} (0.5 and 1 ng/kg s.c.) also significantly improved the scopolamine-induced deficit of spatial memory. The activity of AVP_{4-9} was determined to be about 10 000 fold greater than that of AVP_{1-9}. An intracerebroventricular (i.c.v.) injection of 10 fg of AVP_{5-8}, however, showed a lower activity. Both AVP_{6-8} and AVP_{5-7}, which are both metabolites of AVP_{5-8}, demonstrated no activity. The scopolamine-induced disruption of spatial memory was found to improve after a microinjection of AVP_{4-9} (1 fg) into the ventral hippocampus (VH) region, but not into the dorsal hippocampus (DH). In an in vivo microdialysis study, the scopolamine-induced acetylcholine (ACh) release from the VH was slightly potentiated by treatment with AVP_{4-9} (10 fg i.c.v.). In addition, an AVP_{4-9} analogue, No. 302, which is a synthetic hexapeptide and has a longer half-life, also demonstrated a markedly improved effect, which had a 10-fold higher activity than that with AVP_{4-9}. AVP_{4-9} is the most potent activity of all the endogenous metabolites of the AVP_{1-9} and the new synthetic AVP_{4-9} analogue, No. 302 (obtained from Nippon Chemiphar Co.), substituting Ser for Cys–Cys in hexapeptide, has higher activity than that of AVP_{4-9}. These results indicated [Ser^6] hexapeptide has an important role in behavioral activity. Based on these results, it is possible that AVP_{1-9} and its metabolite AVP_{4-9} could, thus, be useful in treating cholinergic dysfunction diseases, such as Alzheimer's disease. Hexapeptide may play an important role in improving the spatial memory by promoting the release of ACh in the VH region.

Keywords: 8-Arm radial maze task; Spatial memory; Scopolamine; Arginine^8-vasopressin (AVP_{1-9}) C-terminal fragment; Acetylcholine (ACh) release

1. Introduction

In the past few years, many peptides have been recognized as important factors in the functioning of the CNS [1]. Arginine^8-vasopressin (AVP_{1-9}), a neurohypophysial hormone traditionally associated with the regulation of water balance and blood pressure, has more recently been shown to act as a neurotransmitter or neuromodulator in the CNS [2] and to affect various aspects of cognitive functioning, including learning and memory retrieval [3–5]. The administration of AVP is known to affect both passive and active avoidance tasks in animals [6–8]. It has also been reported that treatment with vasopressin-like peptides can enhance memory retrieval in humans [9] and animals [10].

Vasopressin is widely distributed in the mesencephalon, pontine and medullary nuclei, with the largest amounts in the extrahypothalamus. AVP has been localized to the presynaptic vesicles, which thus makes it very likely that it is released as a neurotransmitter [11]. Therefore, a possible interaction of vasopressin with the neurotransmitter pathway in the CNS was indicated [12,13].

Memory deficits with aging in humans and animals are related to neurophysiological and neurochemical dysfunctions [14]. Such changes include a reduction in the extrahypothalamic vasopressin neurons in various brain regions in both senescent rats [15] and Alzheimer's disease patients with dementia. In Alzheimer's disease, numerous neurochemical changes have been reported.

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The most established finding is the degeneration of the cholinergic neurons in the basal forebrain, including Meynert's nuclei [16], which is closely related to the learning and memory process [17]. Numerous lesion studies have demonstrated the importance of the cholinergic septohippocampal system in performing memory tasks [18]. The hippocampus plays an important role in the spatial memory of animals [19,20]. Immunohistochemical and electrophysiological studies have provided clear evidence that AVP-specific neurons in the medial amygdaloid nucleus are transmitted to the ventral hippocampus [21]. In the CNS, AVP_{1-9} is endogenously metabolized to various fragments proteolytically in the extrahypothalamic brain regions [22], and its metabolites AVP_{4-9} and AVP_{5-9} improve the memory in both avoidance [23] and radial maze tasks [24].

To further investigate AVP_{4-9} and its metabolites (AVP_{5-8}, AVP_{5-7} and AVP_{6-8}), the effect of these peptides on deficits of spatial memory induced by scopolamine using an 8-arm radical maze task were examined. We compared the effect of the AVP_{4-9} with the AVP_{4-9} analogue, No. 302, which is a very stable peptide having a 5-fold longer half-life than that of AVP_{4-9} (Fig. 1). Furthermore, we also studied the effect of AVP_{4-9} on the acetylcholine (ACh) release in the hippocampus of freely moving rats using a microdialysis method.

2. Materials and methods

2.1. Eight-arm radial maze task

Male Wistar rats weighing 200–250 g, supplied by Kyu-Do Co. Ltd., were used for this experiment. They were caged under a controlled constant light–dark cycle (light 07.00–19.00 h) with a restricted diet (CE-2, Clea, Japan), and in an air-conditioned room (23±1°C, 60% humidity). Each rat was placed on a platform (25 cm in diameter) in the middle of an 8-arm radial maze, in which each arm had been baited with a food pellet. The performance of the animal in each session was assessed by 3 parameters; the number of correct choices, the number of errors, which is defined as choosing arms that they have already visited, and the elapsed time before the animal ate all 8 pellets. The behavioral observation was stopped after 10 min even when the animal did not finish the task.

In the drug test phase, we used only rats which had acquired spatial cognition, i.e., the rats which had made 7 or more correct choices and either one or no errors during the first 8 choices in each of 3 consecutive sessions. The significantly decreased number of correct choices in the presence of a significantly increased number of errors was considered to demonstrate a disruption of spatial cognition. Spatial cognition in the affected animals was considered to be improved when the treated rats made a significantly increased number of correct choices and significantly fewer errors compared with the untreated affected animals.

AVP_{1-9} and its metabolite C-terminal fragments (Nippon Chemiphar Co. Ltd., Japan dissolved in water) were subcutaneously (s.c.) injected after 30 or 60 min before each session. In addition, they were intracerebroventricularly (i.c.v.) and intradorsal (or ventral) hippocampally injected 10 min before each session. Scopolamine (Sigma, USA, dissolved in physiological saline) was intraperitoneally administered 30 min before the session.

The Wilcoxon's rank sum test was used for the data analysis of the experiments.

2.2. Brain microdialysis procedure

The animals anesthetized with 2% halothane were mounted onto a stereotaxic frame, and a guide cannula

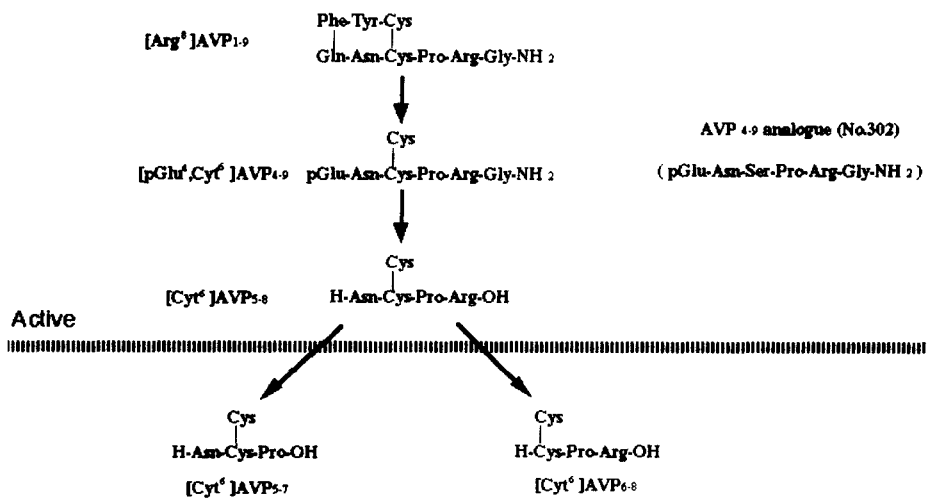

Fig. 1. $[Arg^8]AVP_{1-9}$ and its metabolites.

was stereotaxically implanted into the ventral hippocampus according to the brain coordinates of Paxinos and Watson. Following surgery, a microdialysis probe (Eicom; 2 mm dialysis membrane) was inserted into the guide cannula and was perfused with Ringer's solution at a flow rate of 2 μl/min by means of a microinfusion pump. The in vivo release of ACh in successive 10-min fractions was determined by HPLC–ECD. The HPLC–ECD system (Waters Assoc., Milford, MA), utilizing an EicomPak AC column and enzyme column (Eicom, Kyoto, Japan), was used for ACh determination. The brain ACh contents were quantified by calculating the area under the curves using an integrator (Waters Model 730, Waters Assoc.) and their contents were determined using an internal standard method.

The data on the ACh release were analyzed by a two-way (with repeated measures) analysis of variance (2-ANOVA) followed by Student's *t*-test for differences among the groups when overall significance was observed in the 2-ANOVA.

3. Results

3.1. Eight-arm radial maze task

3.1.1. Effect of systemic injection

When the rats were trained to perform the 8-arm radial maze task, about 90% of the rats reached the criterion of choice accuracy (number of correct choices, 7.8 ± 0.2; number of errors, 0.1 ± 0.05) after 14 training trials. Only those rats who had attained spatial memory underwent the experiment. The administration of scopolamine (0.5 mg/kg, i.p.) induced a significant deficit in choice accuracy compared to the vehicle control; that is, the number of correct choices significantly decreased and the errors significantly increased. This scopolamine-induced deficit of spatial memory then improved with 5 or 10 mg/kg s.c. AVP_{1-9} 60 min prior to the retention test (Fig. 2, left side), and approximately 60% of the rats thereafter recovered to normal levels (Fig. 2, right side). However, such improvement was attenuated when the injection was given 30 min before the retention test. These results suggested that the active component may thus be endogenous of AVP_{1-9} metabolites (data not shown). As shown in Fig. 2, AVP_{4-9} and the main endogenous metabolite of AVP_{1-9}, markedly ameliorated the scopolamine-induced deficit at 0.5 and 1.0 ng/kg s.c., and its efficiency index was about 60%. Thus, the activity of AVP_{4-9} is considered to be 10 000 fold higher than AVP_{1-9}.

3.1.2. Effect of i.c.v. administration

The effect of the intracerebroventricular (i.c.v.) administration of AVP metabolites on scopolamine-induced deficits was examined. After an i.c.v. injection, 10 fg/body of AVP_{1-9} significantly improved the disruption of spatial cognition at 10 min, and its efficiency index was 60% (Table 1). Ten fg/body i.c.v. of AVP_{5-8} or AVP_{4-9} metabolite, also demonstrated an improvement. In comparison to the efficacy of AVP_{4-9}, AVP_{5-8} had a lower activity with only 40% of the rats returning to normal levels. In addition, after a 10–100 fg/body i.c.v. administration of AVP_{6-8} and AVP_{5-7}, which are the main metabolites of AVP_{5-8}, a weaker activity with a low efficiency index of 10% was observed. Table 1 shows that the scopolamine-induced deficit of spatial memory improved after treatment with AVP_{1-9} and its metabolites with the degree of potency series being $AVP_{4-9} > > > AVP_{5-8} > AVP_{6-8} = AVP_{5-7}$.

3.1.3. Effect of intrahippocampal injection

The effect of a microinjection of AVP_{4-9} to the hippocampus on scopolamine-induced deficit was examined. When 1 fg of AVP_{4-9} was injected into the ventral hippocampus, but not into the dorsal hippocampus, the scopolamine-induced disruption of spatial memory markedly improved to normal levels (Fig. 3). Such evidence thus suggested that the ventral hippocampus may be an important site of action for the AVP fragment.

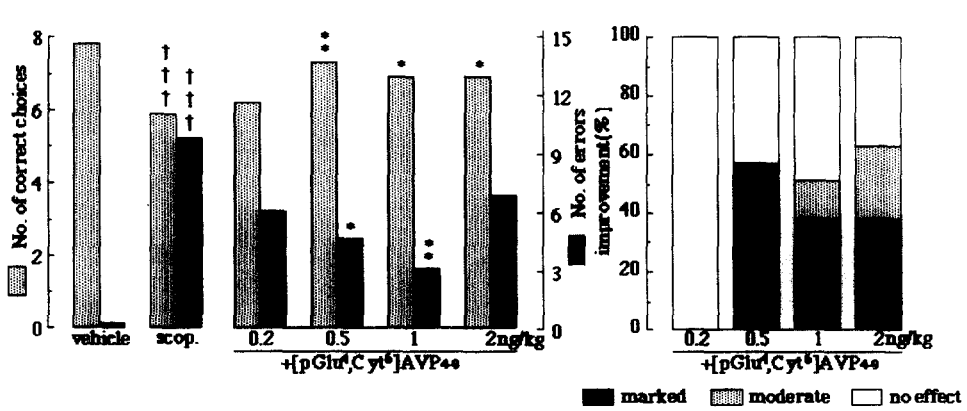

Fig. 2. The effect of a subcutaneous injection of AVP_{4-9} on the scopolamine-induced disruption of spatial cognition in an 8-arm radial maze task in rats. scop., scopolamine 0.5 mg/kg i.p. †††$P < 0.001$ vs. vehicle; *$P < 0.05$, **$P < 0.01$ vs. scopolamine.

Table 1
Summary of [Arg^8]AVP$_{1-9}$ and the activities of its metabolites on the scopolamine-induced disruption of spatial cognition in an 8-arm radial maze task in rats

		Effective doses	Efficacy(%)
[Arg^8]AVP$_{1-9}$	Phe-Tyr-Cys Gln-Asn-Cys-Pro-Arg-Gly-NH$_2$	5 ~ 10 μg (s.c.)	60 %
[pGlu^1,Cyt^6]AVP$_{4-9}$	Cys pGlu-Asn-Cys-Pro-Arg-Gly-NH$_2$	0.5 ~ 1 mg (s.c.)	60 %
		10 fg (i.c.v.)	60 %
[Cyt^6]AVP$_{5-9}$	Cys H-Asn-Cys-Pro-Arg-OH	10 fg (i.c.v.)	40 %
[Cyt^6]AVP$_{6-9}$	Cys H-Cys-Pro-Arg-OH	10 ~ 100 fg (i.c.v.)	10 %
[Cyt^6]AVP$_{5-7}$	Cys H-Asn-Cys-Pro-OH	10 ~ 100 fg (i.c.v.)	10 %
No.302	pGlu-Asn-Ser-Pro-Arg-Gly-NH$_2$	1 fg (i.c.v.)	50 %

3.1.4. Effect of AVP_{4-9} on the ACh release in the ventral hippocampus

The effect of an i.c.v. administration of AVP_{4-9} on ACh release in the ventral hippocampus of the vehicle-treated rats was next examined. The administration of AVP_{4-9} (10 fg/body i.c.v.), itself demonstrated no change on the ACh basal level in the control rats (Fig. 4a). On the other hand, the ACh level from the ventral hippocampus increased by approximately 240% after the administration of scopolamine 0.5 mg/kg i.p. As shown in Fig. 4b, the increase in the ACh level by scopolamine was facilitated, but not statistically significant, by a behaviorally effective dose of AVP_{4-9} (10 fg/body i.c.v.).

3.1.5. Comparison of the effects of the AVP_{4-9} and its analogue No. 302

AVP_{4-9} analogue, No. 302, at 1 fg/body i.c.v. significantly improved the scopolamine-induced deficit and 50% of rats showed an improved memory in the inverted-U-shaped dose–response function (data not shown). This

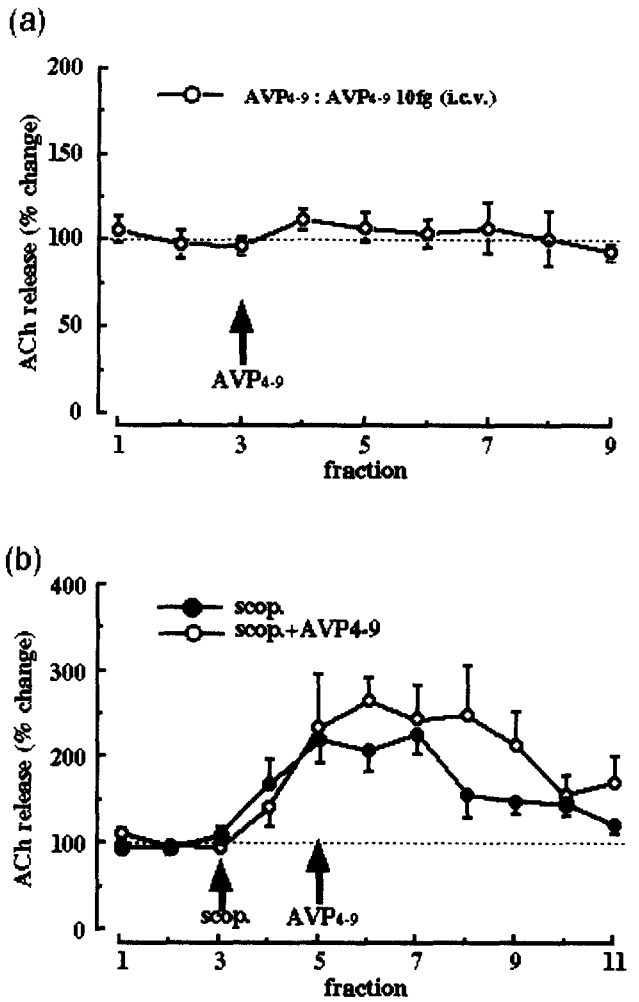

Fig. 4. The effect of AVP_{4-9} on ACh release from the ventral hippocampus in scopolamine-treated rats. a: intact rat. b: scopolamine-treated rat. scop., scopolamine 0.5 mg/kg i.p.; AVP_{4-9}, AVP_{4-9} 10 fg i.c.v.

improvement was attenuated after administration of higher doses of 10 and 100 fg/body. In addition, the AVP_{4-9} analogue, No. 302, had a 10-fold higher activity in comparison to the effect of AVP_{4-9}.

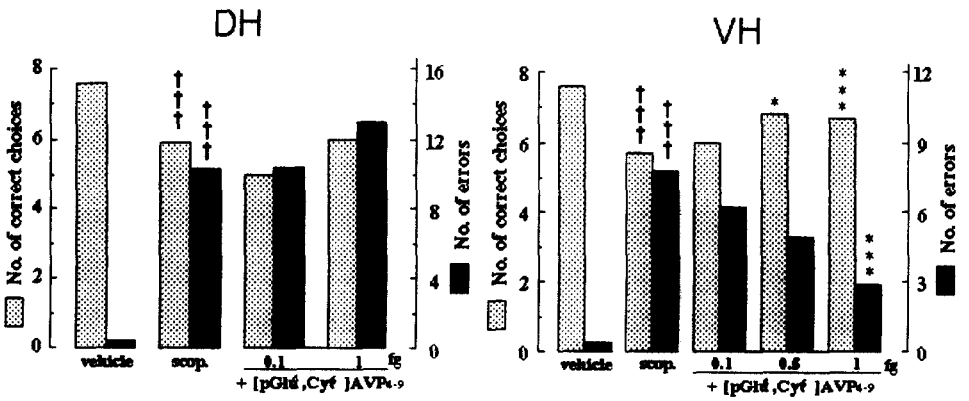

Fig. 3. The effect of an AVP_{4-9} intrahippocampal injection on the scopolamine-induced disruption of spatial cognition in an 8-arm radial maze task in rats. scop., scopolamine 0.5 mg/kg i.p. $^{\dagger\dagger\dagger}P<0.001$ vs. vehicle; $^{*}P<0.05$, $^{***}P<0.001$ vs. scopolamine.

4. Discussion

In the present study, we examined the effect of AVP_{1-9} and its endogenous metabolites on the disruption of spatial memory induced by scopolamine.

The scopolamine-induced deficit returned to normal levels with an injection of AVP_{1-9} at 60 min, but not 30 min prior to the retention test. Among the AVP_{1-9} metabolites studies, AVP_{4-9} showed the most potent ameliorating effect on memory deficit. In addition, this ameliorating effect was observed when AVP_{4-9} was injected into the ventral hippocampus, but not into the dorsal hippocampus. In an in vivo microdialysis study, AVP_{4-9} facilitated the scopolamine (0.5 mg/kg i.p.) stimulated-ACh release from the ventral hippocampus. The present results indicate that the ventral hippocampus is more sensitive to the behavioral effects of vasopressin than the dorsal hippocampus. An immunohistochemical analysis proved the transmission of AVP-specific neurons from the medial amygdaloid nucleus to the ventral hippocampus [21] and electrophysiological observations using intracellular recording techniques has shown that AVP-induced excitation was found in 81% of ventral and 29% of dorsal hippocampal pyramidal cell [25]. AVP-synthesizing neurons exist within the hypothalamic nuclei, including the supraoptic and paraventricular, bed nucleus of the stria terminals, and amygdala [26]. It has also been shown that the vasopressin-converting aminopeptidase exists in the hippocampus [27].

In behavioral studies, de Wied et al. have reported that AVP_{1-9}, following microinjection into the ventral hippocampus, but not the dorsal hippocampus, facilitated the passive avoidance [8]. These facts strongly support our present result. Furthermore, AVP has been localized in presynaptic vesicles [11]. This means that AVP is closely associated with neurotransmitter release in the hippocampus. One of the cholinergic neurons is transmitted from the medial septum to the hippocampus. We therefore examined the effect of AVP_{4-9} on the ACh release from the dorsal and ventral hippocampus of freely moving rats using a brain microdialysis technique. AVP_{4-9} was found to have no effect on the ACh release of the normal brain, but had a marked effect on such abnormal states as the dysfunction of cholinergic neurons induced by scopolamine treatment.

The ACh level in the dialysates from hippocampus was increased by treatment of scopolamine-blocking muscarinic receptor, and its antagonist induced the disruption of spatial memory. On the other hand, co-administration of AVP_{4-9} slightly facilitate the ACh release through the stimulating effect of ACh nerve terminal; however, this result showed the marked improvement of spatial memory loss induced by scopolamine. The facilitation on learning and memory and the stimulation of ACh release in the hippocampus by AVP_{4-9} involving the V_1-like vasopressin receptors were reported [28], and vasopressin potentiation of norepinephrine-induced c-AMP accumulation was found to be regulated by extracellular calcium and dependent upon calcium/calmodulin. [29]. Stephens and Logan reported that AVP stimulates inositol phospholipid metabolism in the hippocampus [30]. Our present studies of AVP_{4-9} left the problem unresolved, however. The following possibilities for this are implicated: (1) intrahippocampally injected AVP_{4-9} diffused to other brain sites, such as lateral septum, amygdala and thalamus etc.; (2) AVP_{4-9} bound to V_1-vasopressin receptors in the hippocampus; (3) AVP_{4-9} may act to regulate Ca^{2+} pump in addition to induction of PI hydrolysis, that is common to all mechanism, such as release of neurotransmitter, expression and maintenance of long-term potentiation. To clarify this hypothesis, further experiments are necessary for understanding the role of AVP_{4-9} both in scopolamine-treated and normal brain function.

In addition, the AVP_{4-9} analogue, No. 302, is a stable peptide with a long half-life and also has a very long activity. One fg i.c.v. of No. 302 markedly improved the scopolamine-induced deficit in the inverted-U-shape dose–response function. Such mechanisms may also include the rapid desensitization of cholinergic receptor, the balance between neurotransmitters, such as the noradrenergic and cholinergic system, and the activation of inhibitory regulatory system, etc. Based on the above findings, it is possible that AVP_{1-9} and its metabolites AVP_{4-9}, could therefore potentially be useful in the treatment of Alzheimer's disease. These findings may also lead to an improved understanding of the role of AVP_{4-9} in both disease as well as in the normal brain.

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