Effects of neuroactive peptides, vasopressin, vasopressin fragments, and its analog (NC-1900) on learning and memory

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Abstract

Here we describe effects of neuroactive peptides, vasopressin, vasopressin fragment, and its analog, NC-1900, on cognitive functions and CO_2-induced amnesia. Vasopressin (AVP_{1-9}) and its fragments are synthesized in the central nervous, and it is well known to regulate diuretic action. However, vasopressin receptors are distributed not only in the supraoptic nucleus and the paraventricular nucleus but also throughout the central nervous system. This fact shows that an action of AVP_{1-9} is not restricted to the anti-diuretic action, and it is suggested that AVP_{1-9} and its metabolites regulate cognitive function such as learning and memory.

In order to determine the mechanism of action of a new AVP_{4-9} analog, NC-1900, on memory process, we investigated the facilitative effect of NC-1900 on memory performance in eight-arm radial maze and in passive avoidance (PA) tasks in nonamnesic and amnesic mice. The improved effect of NC-1900 on the CO_2-induced amnesia was caused by V1A receptor but not V2, and the effect of NC-1900 on memory retention test performance appeared to be due to activation of the protein kinase C (PKC) signaling pathway via V1A receptors.

Key words: AVP_{1-9}; AVP_{4-9}; NC-1900; memory; passive avoidance; radial maze; vasopressin receptor; PKC

Introduction

An increasing population of patients with geriatric disease would cause an important social problem for Japan. In particular, dementia would decrease the quality of life (QOL) not only for the patients themselves but also near relatives who nurse them. Naturally, an anti-dementia drug is need for these patients and the near relatives to inhibit the progress of dementia and to maintain their QOL. In recent years, it is reported that Donepezil, which is a cholinesterase inhibitor and only authorized as an anti-dementia drug in Japan, is not cost effective, and it is suggested that more effective treatments than cholinesterase inhibitors are needed for Alzheimer's disease which is one of a form of dementia[1]. Therefore, we believe that it is necessary to study drugs, except cholinesterase inhibitors, which have been suggested as having an anti-dementia effect.

AVP_{1-9} has been suggested to play an important role in memory formation (Fig. 1-A). For example, it has been reported that in Alzheimer's disease or Down's syndrome,

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which are accompanied by the hypofunction of memory, AVP_{1-9}-mRNA is overexpressed in the temporal lobe[2]. In addition, it has been confirmed that AVP_{1-9} facilitates learning and memory processes in several animal models[3,4]. However, these studies have 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 some peripheral factor, such as attention, motivation, or arousal[5]. Thereafter, a carboxy-terminal 4-9 sequence that was generated by aminopeptidase was discovered (Fig.1-B). AVP_{4-9} reportedly has a more potent facilitative effect on performance in PA[6] and other memory tasks[7,8,9] and does not have peripheral effects, such as antidiuretic or pressor effects[6,7,9].

NC-1900 (Fig.1-C) is a newly synthesized AVP_{4-9} analog in which the cysteine residue of AVP_{4-9} is replaced with a serine residue[10,11]. This peptide was found to be more selective and more potent than AVP_{4-9} on scopolamine-induced impairment of spatial memory[12]. In addition, Hirate et al.[13] reported that NC-1900 ameliorate cyclohexamide-induced memory impairment of PA behavior, and Hori et al.[10] 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, memory impairment and cell damage to cultured cerebro-cortical neurocytes induced by glutamic acid[14]. In addition, it has been reported that NC-1900 inhibits glycine-induced Cl^- currents in the CA1 region of an isolated rat hippocampus and that the inhibition is due to activation of protein kinase A[15]. Most recently, Mishima et al.[11] 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 V1A receptors at postsynaptic cholinergic nerves and by interaction with postsynaptic M_1 receptors.

In this mini-review, we summarize several lines of videnc e implicating the mnemonic effect of AVP_{1-9}, AVP_{4-9}, and NC-1900, and attempt to conjecture the action mechanism of NC-1900 on cognitive functions.

Neurohypopysis hormones and vasopressin

Neurohypopysis hormones are classified into 9 kinds of hormones, and various kinds of mammalian have vasopressin and oxytocin (Table 1), and vasopressin is known as a multifunctional peptide[16]. Most of vasopressin has arginine residue in position eight, and it is so called, an arginine vasopressin (AVP_{1-9}). Lysine vasopressin (LVP) are recognized in limited mammalians such as porcine or hippopotami[17]. As a hormone that shows an anti-diuretic effect, there is a no difference between the two vasopressins mentioned above. However, vasopressin re-

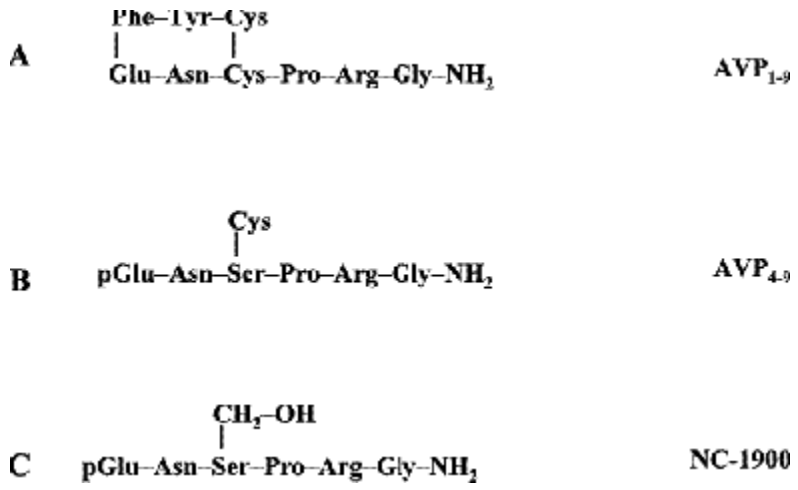

Fig. 1. Amino acid sequences of AVP_{1-9}, AVP_{4-9}, and NC-1900

ceptors are known to exist in not only supraoptic nucleus and paraventricular nucleus but also in subfornical organa, suprachiasmatic nucleus, hippocampus[19] and cerebral cortex[16]. These reports suggest that AVP_{1-9} has some effect, which is due to a centrally-acting but not an anti-diuretic, for a living body.

Vasopressin fragments

Burbach et al.[6] first reported that the metabolites of argine[8]-vasopressin (AVP_{1-9}) can enhance learning and memory, and that the main active metabolite of AVP_{1-9} produced by aminopeptidase was a carboxy-terminal 4-9 sequence [$pGlu^4$, Cyt^6] vasopressin (Fig. 2). Since then, AVP_{4-9} has been studied behaviorally[7, 8, 9, 13] and biochemically[15, 18], and it was shown that the metabolite fragment of AVP_{1-9} has a more potent effect than AVP_{1-9} itself on learning and memory performance[13, 20]. It has also been reported that AVP_{4-9} causes a more marked reduction of the ischemic decrease of CA1 presynaptic fiber spikes than treatment with AVP_{1-9} itself[21]. As mentioned above, NC-1900 is a novel synthetic AVP_{4-9} analog, and this peptide was found to be more selective and more potent than AVP_{4-9} or AVP_{1-9}.

Assessment of learning and memory

In assessment of learning and memory, we have used step-through type passive avoidance (PA) test using methods modified from Matuoka et al.[22] or an eight-arms radial maze task[23].

1) Step-through type PA task

A two-compartment step-through type PA apparatus was used. The box was divided into bright (9 cm × 9 cm × 36 cm) and dark compartments (26 cm × 26 cm × 36 cm) by a guillotine door. In each trial, a mouse was placed in the illuminated compartment for a 30s habituation period, and then the guillotine door was raised to allow entry into the dark chamber. On the pre-exposure session, the step-through latency (the length of time spent in the bright

Table 1. Structure of neurohypophysis hormones in vertebrates

Mammal	Cys Tyr Phe Gln Asn Cys Pro Arg Gly NH_2 Arginine-Vasopressin Cys–Tyr–Phe–Gln–Asn–Cys–Pro–Lys–Gly–NH_2 Lisine-Vasopressin	Cys Tyr Ile Gln Asn Cys Pro Leu Gly NH_2 Oxcytocin
Aves, Reptilia, Amphibia, Lungfish	Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Arg–Gly–NH_2 Vasotocin	Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Ile–Gly–NH_2 Mesotocin
Bony fishes	Vasotocin	Cys–Tyr–Ile–Ser–Asn–Cys–Pro–Ile–Gly–NH_2 Isotocin
Cartilaginous fishes	Vasotocin	Cys Tyr Ile Ser Asn Cys Pro Gln Gly NH_2 Glumitocin
		Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Val–Gly–NH_2 Valitocin
		Cys–Tyr–Ile–Asn–Asn–Cys–Pro–Leu–Gly–NH_2 Aspartocin

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. The acquisition trial (Acq.) was conducted over 24 h for the measurement of pre-exposure latency. When the hind legs of the mice entered into the dark chamber, the guillotine door was closed and electrical foot shock 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. The latency was measured for up to 300 s.

2) Eight-arm radial maze task

Method of Olton and Samuelson[24] modified version was used[23]. In short, the maze used in 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 pre-training, the mice were kept on a restricted diet and their body weight was reduced to 90% of normal weight over a 1-week period; water was freely available. Before the pre-training period, each mouse was handled for at least 5 min daily for 5 days. Before the radial maze trial began, mice underwent the pre-training session for 5 days, during which the mice were given 10 min daily to adapt to the apparatus. After the pre-training period, the learning and memory abilities of the mice were evaluated over ten 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 a random orientation and 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. Re-entry 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.

Experimental results and their out-line on learning and memory

(1) Effect of NC-1900, AVP_{4-9}, AVP_{1-9}, and vasopressin receptor antagonists on memory retention in the passive avoidance (PA) task[25]

Previous studies showed that NC-1900 and AVP_{4-9} facilitated memory retention in the PA task after an interval of 21 days. The latency in the test was significantly increased by NC-1900 (100 ng/kg) or AVP_{4-9} (0.1 and 1 ng/kg) as compared with the control group. In addition, low doses of AVP_{4-9} (0.1 and 1 ng/kg) did not show a facilitative

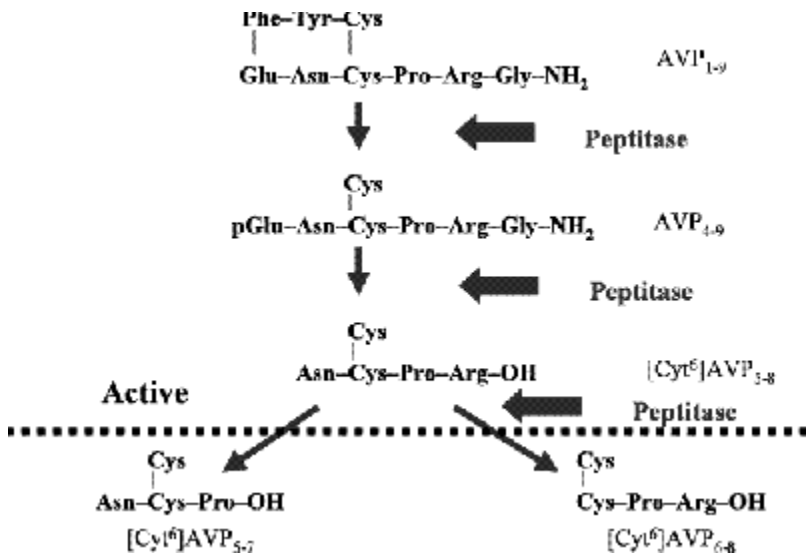

Fig. 2. AVP_{1-9} and its metabolites

effect on the task. Latency was shorter in mice injected with 1 μg/kg Pmp, Tyr-AVP, a V1A antagonist[11] as compared with mice in the control group. Administration of 100 ng/kg or 1 μg/kg AVP_{1-9} did not affect the memory retention of mice. In addition, the application of the V1A (10 ng/kg) or V2 (10 ng/kg and 10 μg/kg) antagonist did not influence the latency in the test. Furthermore we examined the effect of co-injection with NC-1900 and V1A or V2 antagonist on the latency. The co-injection with V1A antagonist blocked the facilitative effect of 1 ng/kg NC-1900 on the test latency (the latency was lower in mice that were co-administered 1 ng/kg NC-1900 with 1 μg/kg of Pmp, Tyr-AVP than in those that received NC-1900 alone. However, the co-administration with OPC-31260, a V2 antagonist, did not prevent the prolongation of latency by NC-1900.

(2) Effect of NC-1900, AVP_{4-9}, and AVP_{1-9} on eight-arm radial maze performance[21]

The number of correct choices until the first mistake during 10 consecutive trials is compared among control, NC-1900, AVP_{4-9}, and AVP_{1-9} groups. 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 choices until their first mistake. However, there was no significant difference between the AVP_{1-9} treated and control, and the NC-1900 and AVP_{4-9} groups.

(3) Effect of PMA and 4αPDD and NPC-15437 on memory retention in the PA task

Since V1A receptors are linked to the phospholipase C (PLC)-PKC signaling pathway, we studied whether the application of PMA, an activator PKC, facilitates memory retention. The group of mice that received intracisternal administration (i.cist.) of PMA (200 ng) had a higher latency than the control group. However, the same dose of 4αPDD, an inactive phorbol ester, did not facilitate memory retention at the test.

NPC-15437 is a one of PKC inhibitors, and we examined the effect of NPC-15437 on the facilitation of memory retention in the PA task by NC-1900. The administration of 0.1mg/kg of NPC-15437 did not affect the latency on the test after 21 days; however it did prevent the increase in the latency by 1 ng/kg NC-1900. These results suggest that the effect of NC-1900 on memory retention is due to the activation of the PKC signaling pathway.

(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), AVP1-9 (10 μg/kg), Pmp,Tyr-AVP (10 ng/kg), and OPC-31260 (10 μg/kg) on CO_2-induced amnesia in the step-through PA task are compared. CO_2 exposure significantly decreased latency compared to the control group, and the administration of NC-1900 (1 ng/kg) ameliorated the CO_2-induced amnesia. A similar effect was observed in AVP_{4-9}, although the effective dose (1 μg/kg) was 1000-fold more than that of NC-1900. The administration of AVP_{1-9} (10 μg/kg), Pmp,Tyr-AVP (1 μg/kg), or OPC-31260 (10 μg/kg) had no effect on CO_2-induced amnesia. In addition, coinjection with V1 antagonist (Pmp,Tyr-AVP: 1 μg/kg) and NC-1900 (1 ng/kg) inhibited the ameliorative effect of NC-1900 (1 ng/kg) on CO_2-induced amnesia in the step-through PA task. However, coadministration of OPC-31260 did not influence the ameliorative effect of NC-1900 on CO_2-induced reduction latency. These results suggested that NC-1900 has a more potent effect on facilitation of memory via the V1A receptor than AVP_{4-9} in CO_2-amnesic conditions.

Conclusive remarks

The effect of NC-1900 on memory retention and CO_2-induced amnesia appeared to be mediated by activation of V1A but not V2 receptors (Fig. 3). One of the key findings in the present experiment was that the effective dose of NC-1900 was 1000-fold lower than that of AVP_{4-9}.

It is claimed that the V1A receptor is coupled with $G_{q/11}$ protein[26], which modulates the PLC (phospholipase C) /PKC/ calcium-calmodulin (CaM)-dependent protein kinase II signaling pathway[25]. It suggests a possibility that the facilitation of memory retention by NC-1900 via activation of V1A receptors may be due to the modulation of PKC signaling pathway activation. Mishima et al.[11] revealed that NC-1900 improves KN-62 (a CaM-dependent protein kinase II inhibitor) -induced impairment of spatial memory. Bourtchuladze et al.[28] reported that PKC levels are increased in chicks after inhibitory avoidance training. Therefore, we speculate that the following sequence of events take place: the activation of V1 receptors by NC-1900 would induce the production of IP3, thereby causing

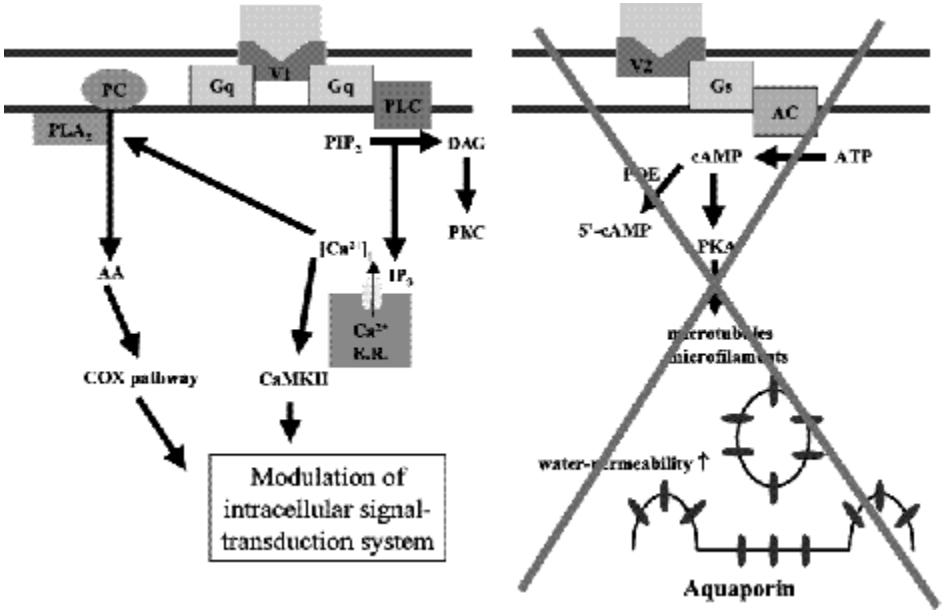

Fig. 3. Schematic drawing of the signaling pathway induced by activation of vasopressin receptors

the release of Ca^{2+} from IP3-sensitive Ca^{2+} storage sites, and the Ca^{2+} would subsequently bind to CaM, resulting in the activation of Ca^{2+}/CaM-sensitive adenylate cyclase and the production of cAMP. Together, these facts suggest that facilitation of memory retention by NC-1900 may be primarily caused by an increase in the activity of PLC/PKC/CaM-dependent protein kinase II signaling pathway.

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