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Facilitation of Passive Avoidance Response by Newly Synthesized Cationized Arginine Vasopressin Fragment 4-9 in Rats

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TANABE, S., Y. SHISHIDO, M. FURUSHIRO, K. KADO, S. HASHIMOTO, T. YOKOKURA AND T. OHSAWA. *Facilitation of passive avoidance response by newly synthesized cationized arginine vasopressin fragment 4-9 in rats.* PHARMACOL BIOCHEM BEHAV **57**(1/2), 251–256, 1997.—The effects of a newly synthesized cationized arginine vasopressin fragment 4-9 analogue (C-AVP-(4-9)) on learning and memory in rats were studied by the passive avoidance test. C-AVP-(4-9) and its parent peptide, arginine vasopressin fragment 4-9 (AVP-(4-9)), a well known potent neuropeptide, were subcutaneously injected 1.5 hr prior to the retention test. The most effective doses of C-AVP-(4-9) and AVP-(4-9) were 8.6×10^{-2} and 1.3 nmol/ kg, respectively. To evaluate the distribution of C-AVP-(4-9) in the central nervous system (CNS), apparent tissue-plasma concentration ratios ($Kp_{,app}$) of intravenously administered radioiodinated C-AVP-(4-9) (^{125}I-C-AVP-(4-9)) in the CNS in mice were determined. At the apparent steady state of plasma concentration of ^{125}I-C-AVP-(4-9), the $Kp_{,app}$ values of the ^{125}I-C-AVP-(4-9) in the cerebrum, cerebellum and spinal cord were over 12 times higher than that of the vascular space marker which slightly penetrates the BBB. Moreover, the rat cerebral homogenate converted C-AVP-(4-9) into its parent peptide AVP-(4-9). These results suggest that the potent effects of C-AVP-(4-9) on learning and memory may be due to AVP-(4-9) generated as a result of distribution and metabolism of peripherally administered C-AVP-(4-9) in the CNS. © 1997 Elsevier Science Inc.

C-APV-(4-9) Passive avoidance response Central Nervous System Arginine vasopressin Rats

FACILITATION of learning and memory by several peptides (18) including arginine vasopressin (AVP) and its analogues (3,7,8) has been demonstrated previously. It was proposed that these neuropeptides pass through the blood-brain barrier (BBB) (24,28), reach the extracellular space and bind to membrane receptors in the central nervous system (CNS) (7,12,13). Therefore, it would be possible to regulate the effects of such peptides on learning and memory by controlling the penetration efficiency of these peptides through the BBB.

Recently, absorptive-mediated endocytosis (AME) has received much attention as a method for transporting cationic peptides to the extracellular space of the CNS via negatively charged sites on the BBB (16,21).

From these points of view, we modified AVP fragment 4-9 (AVP-(4-9)) a well studied potent neuropeptide which facilitates passive avoidance response (3,8), with cationic peptides and obtained cationized AVP-(4-9) (C-AVP-(4-9) to elevate its distribution efficiency to the extracellular space of the CNS. The present study was carried out to evaluate the effect of a novel AVP-(4-9) analogue, C-AVP-(4-9), on learning and memory. Moreover, our studies focused on distribution and metabolism of that peptide in the CNS.

METHODS

Reagents

[$pGlu_4$, Cyt_6, Arg_8]-vasopressin fragment 4-9 (AVP-(4-9), Fig. 1) was purchased from Sigma (St. Louis). The solid-phase synthesis of [$pGlu_4$, (Arg- His- Pro- Cyt)$_6$, Arg_8]-vasopressin fragment 4-9 (C-AVP-(4-9), Fig. 1) was performed by the American Peptide Company (Sunnyvale). AS22 (anti-staphylokinase immunoglobulin G) was prepared by Yakult Honsha

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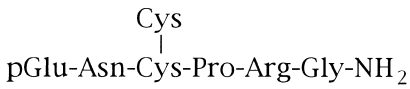
AVP-(4-9)



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C-AVP-(4-9)

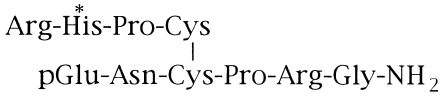

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FIG. 1. Amino acid sequences of AVP-(4-9) and C-AVP-(4-9). Abbreviations for residues; Arg, arginyl; Asn, asparaginyl; Cys, cysteinyl; Gly, glycyl; His, histidinyl; Pro, prolyl; pGlu, pyroglutamyl. *Represents ^{125}I-labeled position.

(Tokyo) (14). The specific prolyl endopeptidase inhibitor Z-Pro-prolinal (ZPP) (25) was synthesized at Yakult Honsha.

Animals

Eight- to nine-week-old male Wistar rats and ICR mice (CLEA Japan Inc., Tokyo) were used. All animals had free access to food, drinking water and were kept under a controlled 12 h light-dark cycle with lights on between 8 a.m. and 8 p.m.

Passive Avoidance Test

The effects of peptides on learning and memory in rats were determined by a slightly modified method of Ader et al. (1), using a step-through apparatus equipped with a shock generator (PAS-SSR0lA and SGS-001; Muromachi Kikai, Tokyo). For training, the rats were placed in an illuminated box attached to a large dark compartment and allowed to enter the dark compartment. After habituation to the dark compartment for 2 min, the rats were placed in an illuminated compartment and allowed to enter the dark compartment again. As soon as rats entered the dark compartment, the door separating the two compartments was shut and an unavoidable scrambled foot shock (0.5 mA, 3 s, 50 Hz) was delivered through the grid floor (learning trial). Twenty-four hours after the learning trial, the rats were placed in an illuminated compartment and the passive avoidance latencies (max.; 300 s) were recorded (retention test).

Treatment for the Passive Avoidance Test

Peptides were dissolved in saline before use and were subcutaneously (s.c.) administered at 1 ml/kg immediately after the learning trial and/or 1.5 h prior to the retention test. As a placebo, a group of rats received the same volume of saline.

Radioiodination of C-AVP-(1-9) and AS22

Radioiodinated C-AVP-(4-9) and AS22 (^{125}I-C-AVP-(4-9) and ^{125}I-AS22) were prepared by the chloramine T method (11) as follows. C-AVP-(4-9) or AS22 (10 μl, 1 μg/ml) was mixed with 0.5 mCi of Na ^{125}I and 50 μl of 0.5 M phosphate buffer (pH 7.4), and 10 μl of 0.25% chloramine T were added and allowed to react at room temperature for 20 s. The reactions were stopped by addition of 25 μl of 0.25% sodium metabisulfate and 10 μl of 10% KI. ^{125}I-C-AVP-(4-9) was purified by HPLC with an LC-module 1 system (Waters, Milford) equipped with a 2 ml sample loop. The column (J'sphere ODS-H80; YMC Inc., Wilmington) was eluted with a linear gradient of 0.05% trifluoroacetic acid (TFA) (solvent A) and acetonitrile (solvent B). The gradient ran from 0 to 40% B in A during 40 min at a flow rate of 1.5 ml/min. The eluate was collected automatically and the radioactivity in each fraction (1.5 ml) was counted using a γ-counter (Auto-gamma 5550; Packard Instrument Co., Downers Grove). ^{125}I-AS22 was purified by gel-filtration with PD-10 (Pharmacia, Uppsala). ^{125}I-C-AVP-(4-9) and ^{125}I-AS22 had specific activities of 380 and 16 Ci/mmol, respectively, and their chemical purity were $>$ 96%.

Distribution of Radiolabeled C-AVP-(4-9) After a Single Intravenous Administration in Mice

Twenty μCi of ^{125}I-C-AVP-(4-9) (equivalent to 53 pmol) or 1 μCi of ^{125}I-AS22 (equivalent to 64 pmol) was intravenously (i.v.) administered rapidly into tail vein of mice. At 1, 5, 15, 30, 60 or 300 min after the administration, mice were sacrificed and plasma, cerebrum, cerebellum and spinal cord were removed and weighed. All were frozen with liquid nitrogen immediately after weighing. ^{125}I-AS22 and ^{125}I-C-AVP-(4-9) were determined as follows. In the case of ^{125}I-AS22, 5 volumes (V/W) of 15% ice-cold trichloroacetic acid were added to frozen samples and homogenized. After centrifugation at 10,000 × *g* for 10 min (4°C), the radioactivity in the precipitates was counted. ^{125}I-C-AVP-(4-9) was quantified by HPLC. The frozen samples for HPLC were homogenized with 5 volumes (V/W) of ice-cold methanol and the homogenates were centrifuged twice for 1 h each time at 3800 × *g* (4°C). The supernatants were evaporated to dryness at 25°C and redissolved in 500 μl of 0.05 % TFA. These solutions were filtered through 0.1 μm filters and 450 μl aliquots of the filtered solutions were analyzed by HPLC under the same conditions as those used for radioiodination.

Conversion of C-AVP-(4-9) by the Rat Cerebral Homogenate

C-AVP-(4-9) (0.5 μmol/ml) was incubated with 100 μg/ml protein of the rat cerebral homogenate in 250 μl of 20 mM Tris-HCl (pH 7.4) for 6 h at 37°C in the presence or absence of ZPP (20 μmol/ml). The reactions were stopped by addition of 1.25 ml of ice-cold methanol. Samples were centrifuged for 10 min at 10,000 × *g* (4°C). The supernatants were evaporated to dryness at 25°C and redissolved in 250 μl of 0.05% TFA. These solutions were filtered through 0.1 μm filters and 200 μl aliquots of filtered solutions were analyzed by HPLC under the same conditions as those used for radioiodination and absorbance at 215 nm was recorded. In this study, the quantity of metabolites was expressed as the percentage of peak area from peak area of C-AVP-(4-9), that was obtained after incubation of C-AVP-(4-9) with the homogenate in the absence of ZPP for 0 h (control experiment).

Data Analysis

Differences in passive avoidance latencies were analyzed by Kruskal-Wallis test and subsequently with Steel-test or

TABLE 1

EFFECT OF C-AVP-(4-9) AFTER TWICE ADMINISTRATION ON THE RETENTION OF THE PASSIVE AVOIDANCE RESPONSE

Treatment[a]	(nmol/kg)[b]	*n*	Latency of the Retention Test (Seconds, Mean ± SEM)
Placebo		20	30.5 ± 8.5
C-AVP-(4-9)	8.6×10^{-3}	8	61.6 ± 35.8
	8.6×10^{-2}	8	240.5 ± 32.1†
	0.43	9	179.2 ± 45.4*
	1.7	8	94.1 ± 44.8
	17	8	19.6 ± 6.3

[a]Treatment was performed s.c. immediately after the learning trial and 1.5 hr prior to the retention test.

[b]Number of animals per group.

Different from placebo treatment ($*p < 0.05$, $\dagger p < 0.001$) Kruskal-Wallis test and subsequent Steel-test.

Wilcoxon's test. Metabolites converted from C-AVP-(4-9) were analyzed by Dunnet's test. The plasma concentration-time curve of ^{125}I-C-AVP-(4-9) was fitted to a bioexponential equation (Equation A) by MULTI weighted nonlinear regression analysis (26).

$$C(t) = A \exp(-\alpha t) + B \exp(-\beta t) \qquad (A)$$

$C(t)$ represents the plasma concentration of the peptide at time "t", A and B are the intercepts, and α and β are the rate constants of the peptide at distribution and elimination phase (apparent steady state), respectively. The inverse square of the observed plasma concentration served as a weighting factor. The half-lives of C-AVP-(4-9) at distribution phase and apparent steady state ($t_{1/2\alpha}$ and $t_{1/2\beta}$) were calculated by Equation (B).

$$t_{1/2\alpha} = 0.693/\alpha$$

$$t_{1/2\beta} = 0.693/\beta \qquad (B)$$

The tissue distributions of ^{125}I-C-AVP-(4-9) and ^{125}I-AS22 were expressed as the apparent tissue-plasma concentration ratio ($Kp_{,app}$) in Equation (C) (2,20).

$$Kp_{,app}\ (ml/g) = (fmol/g\ of\ tissue)/(fmol/ml\ of\ plasma) \qquad (C)$$

RESULTS

Effects of C-AVP-(4-9) on Passive Avoidance Response

The effects of newly synthesized C-AVP-(4-9) on the learning and memory were observed with a passive avoidance test. As shown in Table 1, C-AVP-(4-9) was administered twice. Animals treated with C-AVP-(4-9) at 8.6×10^{-2} and 4.3×10^{-1} nmol/kg showed significantly facilitated passive avoidance latencies compared with placebo treatment. The administration of C-AVP-(4-9) (8.6×10^{-2} nmol/kg) immediately after the learning trial (consolidation study) or 1.5 h prior to the retention test (retrieval study) elongated passive avoidance latencies significantly compared with placebo treatment (Table 2). In the retrieval study, the most effective doses of C-AVP-(4-9) and AVP-(4-9) were 8.6×10^{-2} and 1.3 nmol/kg, respectively (Table 2).

Distribution of Radiolabeled C-AVP-(4-9) After a Single Intravenous Administration in Mice

The plasma concentration and the distribution of C-AVP-(4-9) to the CNS were studied following i.v. administration of ^{125}I-C-AVP-(4-9) in mice. The plasma concentration of ^{125}I-C-AVP-(4-9) disappeared rapidly within 5 min after administration, then reached the apparently steady state. The clearance of ^{125}I-C-AVP-(4-9) from plasma was characterized by a bi-exponential equation. The half-lives of ^{125}I-C-AVP-(4-9) at the distribution phase and apparent steady state were 1.5 and 18.1 min, respectively (Fig. 2). To obtain the $Kp_{,app}$ values of ^{125}I-C-AVP-(4-9) in the CNS at apparent steady state, concentrations of ^{125}I-C-AVP-(4-9) in the cerebrum, cerebellum and spinal cord at 15 min after administration were quantified; values of 12.6, 15.6 and 15.8 fmol/g tissue were obtained, respectively. These values were divided by ^{125}I-C-AVP-(4-9) plasma concentration in each mouse at 15 min (mean value; 90.7 fmol/ml plasma). The $Kp_{,app}$ values of ^{125}I-C-AVP-(4-9) in the cerebrum, cerebellum and spinal cord at apparent steady state were 1.4×10^{-1}, 1.8×10^{-1} and 1.7×10^{-1} ml/ g tissue, respectively (Table 3). The $Kp_{,app}$ values of AS22 at apparent steady state in the CNS were obtained from the data at 6 h. The $Kp_{,app}$ values of ^{125}I-AS22 in the cerebrum, cerebellum and spinal cord were 8.4×10^{-3}, 7.6×10^{-3} and 1.4×10^{-2} ml/g tissues, respectively (Table 3).

Conversion of C-AVP-(4-9) by the Rat Cerebral Homogenate

The enzymatic conversion of C-AVP-(4-9) in the CNS was observed in vitro by incubation of C-AVP-(4-9) with the rat

TABLE 2

EFFECTS OF C-AVP-(4-9) AND AVP-(4-) ON THE RETENTION OF CONSOLIDATION AND RETRIEVAL STUDIES

Treatment	(nmol/kg)	*n*[c]	Latency of the Retention Test (Seconds, Mean ± SEM)
Placebo (consolidation)[a]		8	84.9 ± 35.3
C-AVP-(4-9)[a]	8.6×10^{-2}	8	264.1 ± 25.6*
Placebo (retrieval)[b]		14	92.2 ± 40.7
AVP-(4-9)[b]	1.3×10^{-2}	8	82.6 ± 42.7
	0.13	8	102.9 ± 23.7
	1.3	8	223.3 ± 38.0‖
	13	8	82.5 ± 36.5
C-AVP-(4-9)[b]	8.6×10^{-3}	8	114.0 ± 48.5
	8.6×10^{-2}	10	240.5 ± 34.9‡
	0.86	8	229.6 ± 38.1†
	8.6	8	101.5 ± 36.8

[a]Treatment was performed s.c. immediately after the learning trial.

[b]Treatment was performed s.c. 1.5 h prior to the retention test.

[c]Number of animals per group.

Different from placebo treatment (consolidation) ($*p < 0.01$) Wilcoxon's test.

Different from placebo treatment (retrieval) ($\|p < 0.15$, $\dagger p < 0.1$, $\ddagger p < 0.05$). Kruskal-Wallis test and subsequent Steel-test.

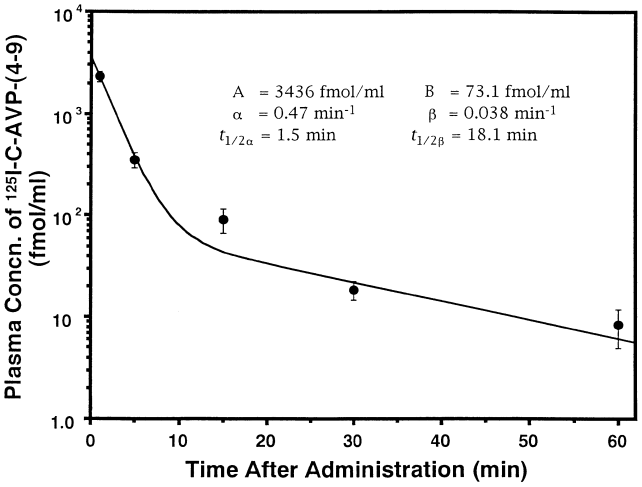

FIG. 2. The plasma concentration of ^{125}I-C-AVP-(4-9) after i.v. administration in mice. Mice were injected with 20 μCi/mouse (53 pmol/mouse) of ^{125}I-C-AVP-(4-9). The plasma concentration of ^{125}I-C-AVP-(4-9) was determined with HPLC as described in ''Methods.'' The data were fitted to a biexponential function (see under ''Methods''). Data at each time point are means ± S.D. of 3 mice. The line indicates fitted value.

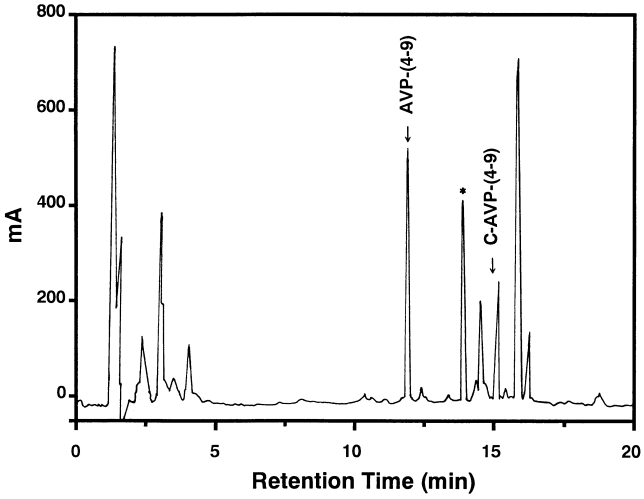

FIG. 3. HPLC profile of C-AVP-(4-9) metabolites produced by the rat cerebral homogenate. C-AVP-(4-9) was incubated with the rat cerebral homogenate for 6 h at 37°C. The HPLC conditions were described in ''Methods.'' The metabolite eluted at 14.02 min (marked with an asterisk) is an unknown metabolite which was derived from C-AVP-(4-9) as judged by parallel incubation in the absence of C-AVP-(4-9). The arrows at 12.05 and 15.02 min indicate retention times of authentic AVP-(4-9) and C-AVP-(4-9), respectively.

cerebral homogenate. The authentic C-AVP-(4-9) and AVP-(4-9) were eluted at 15.02 and 12.05 min, respectively. Addition of ZPP in the control experiment had slight effects on the level of C-AVP-(4-9), a metabolite eluted at 12.05 min and a metabolite eluted at 14.02 min (Table 4). Following incubation of C-AVP-(4-9) for 6 h at 37°C with the rat cerebral homogenate in the absence of ZPP, the metabolites eluted at 12.05 and 14.02 min were significantly increased to 25.14 and 18.43% compared with the control experiment. In this experiment, C-AVP-(4-9) was decreased to 0.05% (Table 4 and Fig. 3). In the presence of ZPP, a metabolite eluted at 12.05 min was slightly increased, but C-AVP-(4-9) was significantly decreased to 49.10% and the level of a metabolite eluted at 14.02 min was significantly increased to 17.60% relative to the control experiment (Table 4).

DISCUSSION

Recently, it has been reported that cationized peptides effectively penetrate the BBB to the extracellular space of the CNS by the mechanism known as AME (17,22). In the present study, we combined the cationic peptide ''Argnyl-Histidinyl-Proline'' with the free cysteine residue of AVP-(4-9) and obtained C-AVP-(4-9) (Fig. 1). Moreover, C-AVP-(4-9) was designed to be converted into AVP-(4-9) by prolyl endopeptidase (PEP) in the CNS (5,27).

Administration of C-AVP-(4-9) twice significantly elongated the passive avoidance latencies compared with placebo treatment. This result indicates that C-AVP-(4-9) has effects on passive avoidance response (Table 1). To clear whether C-AVP-(4-9) affects consolidation or retrieval of learning and memory, 8.6×10^{-2} nmol/kg of C-AVP-(4-9), the most effective dose in the double administration schedule used here, was examined in consolidation or retrieval studies. Gaffori et al. (10) demonstrated that AVP analogues give the most effective facilitation of the passive avoidance response, when s.c. administered 1 h prior to the retention test. However, administration schedule for studying retrieval of memory used here was 1.5 h prior to the retention test, because of the time

TABLE 3

DISTRIBUTION OF ^{125}I-C-AVP-(4-9) TO THE CNS AFTER I.V. ADMINISTRATION

Tissues	Concn. of ^{125}I-C-AVP-(4-9) (fmol/g tissue)[a,c]	$Kp_{,app}$ value (ml/g tissue)[b]	
		^{125}I-C-AVP-(4-9)[c]	^{125}I-AS22[c]
Cerebrum	12.6 ± 3.7	$1.4 \times 10^{-1} \pm 4.5 \times 10^{-3}$	$8.4 \times 10^{-3} \pm 2.8 \times 10^{-3}$
Cerebellum	15.6 ± 1.1	$1.8 \times 10^{-1} \pm 4.5 \times 10^{-2}$	$7.6 \times 10^{-3} \pm 5.2 \times 10^{-3}$
Spinal cord	15.8 ± 7.3	$1.7 \times 10^{-1} \pm 5.2 \times 10^{-2}$	$1.4 \times 10^{-2} \pm 1.3 \times 10^{-3}$

Values represent means ± S.D. of 3 to 5 experiments.
[a]The concentration of ^{125}I-CAVP-(4-9) was quantified by HPLC as described in "Methods."
[b]$Kp_{,app}$ values were determined from the ratio of the fmol/g tissue divided by the fmol/ml plasma.
[c]Values at apparent steady state of plasma concentration.

TABLE 4

CONVERSION OF C-AVP-(4-9) BY THE RAT CEREBRAL HOMOGENATE

Incubation (h)	ZPP[a]	Peak area (% of control)[b] C-AVP-(4-9) (15.02 min)[c]	AVP-(4-9) (12.05 min)[c]	Unknown (14.02 min)[c]
0	−[d]	100.00 ± 5.79	1.85 ± 0.13	0.48 ± 0.00
	+	114.35 ± 15.79	2.86 ± 4.40	0.54 ± 0.10
6	−	0.05 ± 0.02†	25.14 ± 0.34*	18.43 ± 0.13*
	+	49.10 ± 0.40†	3.79 ± 0.09	17.60 ± 0.14*

[a]C-AVP-(4-9) was incubated with the rat cerebral homogenate in the presence (+) or absence (−) of ZPP at 37°C for 0 or 6 h.

[b]Values represent means ± S.D. ($n = 3$) of the peak area percentage vs. C-AVP-(4-9) area obtained in the control experiment.

Different from same substance obtained in the control experiment (*$p < 0.01$, †$p < 0.001$) Dunnet's test.

[c]Retention time.

[d]Control experiment.

lag for conversion of C-AVP-(4-9) into AVP-(4-9) in the CNS. In both consolidation and retrieval studies, C-AVP-(4-9) facilitated the passive avoidance latencies significantly compared with placebo treatment. In the retrieval study, the most effective dose of C-AVP-(4-9) was 8.6×10^{-2} nmol/kg, one-15th of that of AVP-(4-9) (1.3 nmol/kg) (Table 2). These results imply that C-AVP-(4-9) has the potent effects on consolidation and retrieval of learning and memory processes similarly to previously reported AVP-(4-9) (10).

It is suitable for the characterization of tissue distribution of drugs to compare $Kp_{,app}$ values at apparent steady state of plasma concentration with that of the vascular space marker (15,16). In this study, ^{125}I-AS22 was used as a vascular space marker. Except for the case when the specific binding sites, transporter or antigen for the macromolecules exist at the luminal side of the brain capillaries constructing the BBB, the tight-junction structure of the BBB limits the penetration of macromolecules like immunoglobulin G (IgG) (6,22). Therefore, it was supposed that AS22 would not penetrate the BBB, because of its molecular size (M.W.; 150,000) and its antigen specificity (anti-staphylokinase; enzyme from *Staphylococcus aureus*) (14). Moreover, it was indicated in many cases, that the plasma concentration of IgG which was used as the vascular space marker reaches an apparently steady state within 6 h after i.v. administration (15,16). Consequently, the $Kp_{,app}$ values of ^{125}I-AS22 at 6 h were taken as control parameters indicating the distribution of peptides only at the luminal side of the BBB. The $Kp_{,app}$ values of ^{125}I-C-AVP-(4-9) in the CNS at 15 min, when the plasma concentration of ^{125}I-C-AVP-(4-9) reached an apparently steady state (Fig. 2), were compared with those of ^{125}I-AS22. The $Kp_{,app}$ values of ^{125}I-C-AVP-(4-9) in the CNS were not only over 12 times higher than the $Kp_{,app}$ values of ^{125}I-AS22 (Table 3), but were comparable to the previously reported $Kp_{,app}$ values of other cationized or cationic proteins (15, 16). Accordingly, we could suppose that C-AVP-(4-9) has very high efficiency on distribution to the BBB or the extracellular space of the CNS. In this point, more precise characterization remains to be determined by the capillary depletion method which distinguishes the peptide entering the extracellular space of the CNS from the peptide existing in the BBB (19,23).

Incubation of C-AVP-(4-9) with the rat cerebral homogenate for 6 h increased the metabolites eluted at 12.05 and 14.02 min significantly compared with control experiment (Table 4 and Fig. 3). Since the authentic AVP-(4-9) was eluted at 12.05 min and the simultaneous application of authentic AVP-(4-9) with sample obtained after 6 h incubation on HPLC showed increasing of a metabolite eluted at 12.05 min as a single peak numerical rationally (not shown), the increased metabolite eluted at 12.05 min was supposed to be AVP-(4-9). In this study, conversion of C-AVP-(4-9) into AVP-(4-9) was inhibited completely by the PEP inhibitor ZPP (Table 4). Thus, these results may suggest that C-AVP-(4-9) is converted by cleavage of the Pro-Cys bond in C-AVP-(4-9) by PEP in the cerebrum. On the other hand, previously it was proposed that AVP-(4-9) is relatively stable against aminopeptidases degradation by the existence of NH_2-terminal pGlu residue (3,4). Moreover, the conversion of C-AVP-(4-9) into the unknown metabolite eluted at 14.02 min was not inhibited by ZPP (Table 4). Therefore, the unknown metabolite eluted at 14.02 min seems to be an intermediary metabolite from C-AVP-(4-9) into AVP-(4-9) by the aminopeptidases other than PEP.

In conclusion, the present study demonstrates the potent effects of a novel AVP-(4-9) analogue, C-AVP-(4-9), on the passive avoidance response in rats. It is possible that peripherally administered AVP-(4-9) may be effectively distributed to the extracellular space of the CNS by AME. Moreover, AVP-(4-9) converted from C-AVP-(4-9) by the PEP in the CNS would contribute to learning and memory as one of active substances.

To clarify the mechanism of the potent behavioral effects of C-AVP-(4-9), the binding affinities of C-AVP-(4-9) itself and the obtained metabolites to the membrane receptors in the CNS (7,9,12) were presently under investigation.

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