ELSEVIER

European Journal of Pharmacology 352 (1998) 135–142

ejp

NC-1900, an active fragment analog of arginine vasopressin, improves learning and memory deficits induced by β-amyloid protein in rats

Tomoko Tanaka [a,b], Kiyofumi Yamada [a], Kouji Senzaki [a], Hiroto Narimatsu [a], Kyoko Nishimura [a], Tsutomu Kameyama [b], Toshitaka Nabeshima [a,*]

[a] *Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University School of Medicine, Showa-ku, Nagoya 466-8560, Japan*
[b] *Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Tenpaku-ku, Nagoya 468-0077, Japan*

Received 15 January 1998; revised 22 April 1998; accepted 30 April 1998

Abstract

We have reported that the continuous infusion of β-amyloid protein-(1–40) into the rat cerebral ventricle produces learning and memory deficits accompanied by dysfunction in the cholinergic and dopaminergic systems. L-Pyroglutamyl-L-asparaginyl-L-seryl-L-prolyl-L-arginylglycinamide (NC-1900), an active fragment analog of arginine vasopressin in the rat brain, is a stable peptide with a five-fold longer half-life than that of arginine vasopressin-(4–9). In the present study, we examined the effects of NC-1900 on learning and memory deficits in β-amyloid protein-(1–40)-infused rats. The rats were injected subcutaneously with NC-1900 (0.1 and 1 ng kg^{-1}) once a day throughout the period of behavioral examination. In the β-amyloid protein-infused rats, learning and memory in water maze and passive avoidance tasks were impaired compared with these in the control rats. NC-1900 prevented the learning and memory deficits in β-amyloid protein-infused rats. Moreover, NC-1900 tended to increase the choline acetyltransferase activity in the frontal cortex of the β-amyloid protein-infused rats. These results suggested that NC-1900 could be useful for the treatment of patients with Alzheimer's disease.

Keywords: Arginine vasopressin; Alzheimer's disease; β-amyloid protein; Choline acetyltransferase; Learning; Memory; NC-1900

1. Introduction

Alzheimer's disease is characterized by the presence of senile plaques and neurofibrillary tangles accompanied by synaptic and neuronal loss (Coyle et al., 1983). The core of the plaques consists of β-amyloid protein (Masters et al., 1985). Although this protein has been well characterized biochemically, its primary biological function and role in the pathogenesis of Alzheimer's disease are not yet completely understood (Müller-Hill and Beyreuther, 1989). In patients with Alzheimer's disease, learning and memory are impaired with a concomitant loss of the cholinergic marker enzyme, choline acetyltransferase, in the cerebral cortex (Wilcock et al., 1982). However, there is yet no direct evidence that β-amyloid protein is related to the impairment of learning and memory.

We have previously demonstrated that the accumulation of β-amyloid protein-(1–40) in the brain results in the impairment of learning and memory after continuous infusion into the cerebral ventricles (i.c.v.) in adult rats (Nitta et al., 1994, 1997; Nabeshima and Nitta, 1994; Nabeshima and Itoh, 1996, 1997). Further, we observed dysfunction of cholinergic and dopaminergic neuronal systems as shown by the decrease in the nicotine- and KCl-induced stimulation of dopamine and acetylcholine release in vivo (Itoh et al., 1996), changes in ciliary neurotrophic factor levels in the brain (Yamada et al., 1995) and a deficiency of long-term potentiation formation in the hippocampal CA1 (Nabeshima and Itoh, 1996) in β-amyloid protein-infused rats. Therefore, we suggest that β-amyloid protein-infused rats are useful as a model of the dementia of Alzheimer's disease.

Arginine vasopressin-(1–9) is known to affect both passive and active avoidance tasks in animals (Bohus et al., 1972; De Wied and Versteeg, 1979; De Wied et al., 1984). It has also been reported that treatment with vasopressin-like peptides can enhance memory retrieval in humans and animals (Till and Beckwith, 1985; Alescio-Lautier et al., 1987). L-Pyroglutamyl-L-asparaginyl-L-seryl-

* Corresponding author. Tel.: +81-52-744-2670; fax: +81-52-744-2682; e-mail: tnabeshi@tsuru.med.nagoya-u.ac.jp

0014-2999/98/$19.00

L-prolyl-L-arginylglycinamide (NC-1900, No. 302), an active fragment [arginine vasopressin-(4–9)] analog of arginine vasopressin in the rat brain, is a new synthetic physiologically active peptide (Hirate et al., 1997). Unlike arginine vasopressin-(1–9), NC-1900 does not influence blood pressure. The administration of NC-1900 (1 fg, i.c.v.) has been shown to ameliorate scopolamine-induced spatial memory deficits (Fujiwara et al., 1997). Further, this arginine vasopressin analogue prevents the cycloheximide-induced disruption of passive avoidance behavior (Hirate et al., 1997).

In the present study, we investigated the effects of the repeated administration of NC-1900 on learning and memory impairment in rats which had received a continuous infusion of β-amyloid protein-(1–40) into the cerebral ventricle.

2. Materials and methods

2.1. Animals and surgery

Male Kbl Wistar rats (Charles River Japan, Yokohama, Japan), weighing 240–260 g at the beginning of the experiments, were used. They were housed in groups of two or three per cage in a temperature- and light-controlled room (23°C; 12-h light cycle starting at 0900 h) and had free access to food and water, except during the behavioral experiments.

β-amyloid protein-(1–40) was obtained from Bachem California (Torrance, CA, USA). NC-1900 was kindly provided by Nippon Chemiphar (Saitama, Japan). The β-amyloid protein was dissolved in 35% acetonitrile/0.1% trifluoacetic acid. A continuous infusion of the β-amyloid protein (300 pmol 12 μl^{-1} day^{-1}) was maintained for 34 days, by attaching a cannula to a mini-osmotic pump (Alzet 2002; Alza, Palo Alto, CA, USA) (Nabeshima et al., 1991). The control rats were only infused with the vehicle (35% acetonitrile/0.1% trifluoacetic acid). We have confirmed that the vehicle itself failed to induce any behavioral or neurochemical changes at this flow rate (data not shown). The cannula was implanted into the right lateral ventricle (A −0.3, L 1.2, V 4.5, according to the atlas of Paxinos and Watson, 1986). On day 19 after the probe trial in the water maze task, each mini-osmotic pump was replaced with a new pump containing β-amyloid protein at the same concentration, because the capacity of the osmotic pump used was 230 μl.

2.2. Drug administration and experimental design

The behavioral study was started 10 days after the commencement of the β-amyloid protein infusion and the three tasks were carried out sequentially (Fig. 1). NC-1900 dissolved in saline was injected subcutaneously (s.c.) once a day for 38 consecutive days, at the doses of 0.1 and 1 ng kg^{-1}. The administration of NC-1900 started 3 days before the implantation of the mini-osmotic pump to detect a possible protective effect against the β-amyloid protein-induced behavioral impairments, and continued until the animals were killed (day 34). The behavioral test was performed in three batches, each consisted of four to six rats, and thus the number of animals in each group at the beginning of behavioral experiments was 14 to 16. If connecting tubing between the mini-osmotic pump and the infusion cannula was broken, the rats were excluded from the study. Since one batch of animals was not subjected to the working memory test in the water maze task, the number of animals in the experiment was eight to 10. Mnemonic capacity was assessed by performance in three different tasks: Y-maze, water maze (training, probe and working memory trials) and passive avoidance. On day 34 after the start of the β-amyloid protein infusion, the rats were killed, and the frontal cortex and hippocampus were dissected out. The tissue was stored at −80°C until used.

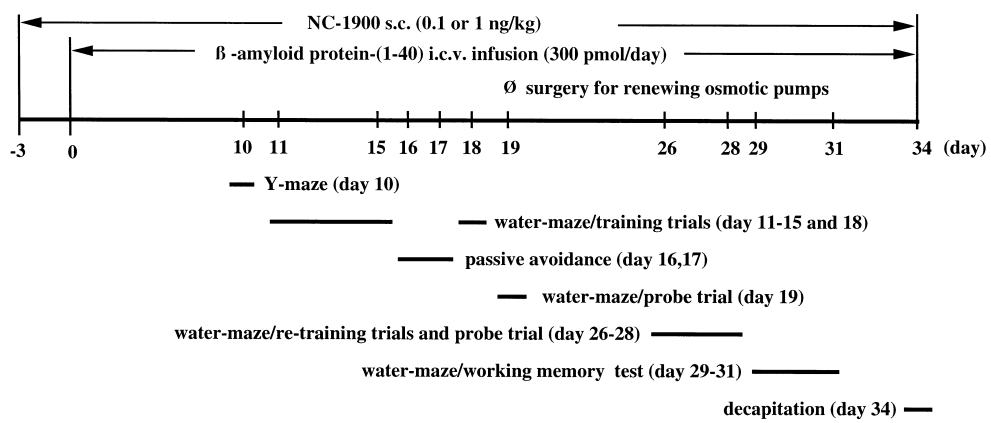

Fig. 1. Experimental schedule.

2.3. Y-maze task

The Y-maze task was carried out as described previously (Maurice et al., 1994) on day 10 after the start of the β-amyloid protein infusion. The experimental apparatus consisted of a black-painted Y-maze made of plywood. Each arm of the Y-maze was 35-cm long, 25-cm high and 10-cm wide and positioned at an equal angle. The rat was placed at the end of one arm and allowed to move freely through the maze for an 8-min session. The sequence of arm entries was recorded manually. Spontaneous alternation behavior was defined as successive entries into the three arms, on overlapping triplet sets (Maurice et al., 1994). The percent spontaneous alternation behavior was calculated as the ratio of actual to possible alternations, defined as [(total number of arm entries − 2) × 100].

2.4. Water maze task

The training trials for the water maze task (Morris, 1984) were carried out on days 11 to 15 after the start of the β-amyloid protein infusion. On the day before the surgery for replacement with a new mini-osmotic pump containing β-amyloid protein (day 18), an additional two training trials were also carried out. The apparatus consisted of a circular water tank (140 cm in diameter and 45-cm high). A transparent platform (10 cm in diameter and 25-cm high) was set inside the tank, which was filled to a height of 27 cm with water at approximately 23°C; the surface of the platform was 2 cm below the surface of the water. The pool was located in a large test room, in which there were many cues external to the maze (e.g., pictures, lamps, etc.); these were visible from the pool and could be used by the rats for spatial orientation. The position of the cues remained unchanged throughout training. For each training trial, the rat was put into the water at one of five starting positions, the sequence of the positions being selected randomly. The platform was located in a constant position in the middle of one quadrant, equidistant from the center and edge of the pool. In each training trial, the latency to escape onto the hidden platform was recorded. If the rat found the platform, it was allowed to remain there for 15 s and was then returned to its home cage. If the rat was unable to find the platform within 90 s, the training trial was terminated and a maximum score of 90 s was assigned. Training trials were conducted for 6 days, twice a day, one session consisting of two trials (2 trials × 6 sessions).

On day 19, the platform was removed from the pool and the animals were tested on a 30-s spatial probe trial. The time spent in the quadrant where the platform had been located during training was measured.

The re-training trials were carried out on days 26 to 28 after the start of the β-amyloid protein infusion. The position of the platform was the same as during the training trials. Re-training trials were conducted on three consecutive days, and the rats underwent two trials on each day. Immediately after the 6th trial (day 28), the platform was removed from the pool and the animals were tested on a 30-s spatial probe trial.

The working memory test was carried out on days 29 to 31 after the start of the β-amyloid protein infusion. In these trials, the position of the platform was changed daily. Since the position in space to which the animal had to swim was changed daily, this task could evaluate the working memory component (Miyagawa et al., 1998). First, the rat was placed on the platform in a new position for 15 s for orientation. Then, the rat was placed into the four quadrants of the pool in a random sequence. The time taken to find the hidden platform was measured. Four trials were conducted on each day. Spatial memory was assessed as the mean latency of 12 trials (four trials per day for 3 days).

2.5. Multiple-trial passive avoidance task

The multiple-trial passive avoidance task was carried out on days 16 and 17 after the start of the β-amyloid protein infusion, as described previously (Yamada et al., 1996). The experimental apparatus consisted of two compartments (25 × 15 × 15-cm high), one illuminated and one dark, both equipped with a grid floor. The two compartments were separated by a guillotine door. In the acquisition trial on day 16, each rat was placed in the illuminated compartment; as soon as the animal entered the dark compartment, the door was closed and an inescapable footshock (0.3 mA, 5 s) was delivered through the grid floor. The rat was removed after receiving the footshock and was placed back into the light compartment by the experimenter. The door was again opened 30 s later to start the next trial. Training continued in this manner until the rat stayed in the light compartment for 120 s. In the retention trial on day 17, given 24 h after the acquisition test, the rat was again placed in the illuminated compartment, and the time until it entered the dark compartment was measured as step-through latency. When the rat did not enter for at least 300 s, a score of 300 s was assigned.

2.6. Measurement of choline acetyltransferase activity

The measurement of choline acetyltransferase activity was carried out as reported previously (Kaneda and Nagatsu, 1985), with a minor modification (Nitta et al., 1993).

2.7. Statistical analysis

Statistical significance was determined by one-way analysis of variance (ANOVA) or the Kruskal–Wallis test, followed by Bonferroni's test for multi-group comparison.

For two-group comparisons, the Welch test or Wilcoxon test was utilized. Two-way ANOVA was also utilized for the water maze task.

3. Results

3.1. Effects of NC-1900 on the spontaneous alternation behavior of the β-amyloid protein-infused rats in the Y-maze task

The incidence of spontaneous alternation behavior in the β-amyloid protein-infused group ($61.5 \pm 3.6\%$) was lower than that in control group ($66.5 \pm 2.1\%$), although this change did not reach statistical significance. NC-1900 did not change the spontaneous alternation behavior induced by the β-amyloid protein infusion ($64.6 \pm 1.9\%$ at 0.1 ng kg^{-1}, $67.3 \pm 1.8\%$ at 1 ng kg^{-1}). The number of arm entries did not differ significantly among the control (18.9 ± 1.6), β-amyloid protein-infused (21.3 ± 1.4) and NC-1900-treated groups (19.3 ± 1.2 at 0.1 ng kg^{-1}, 21.0 ± 1.8 at 1 ng kg^{-1}).

3.2. Effects of NC-1900 on the performance of the β-amyloid protein-infused rats in the training trials and the probe trial in the water maze task

As shown in Fig. 2A, the latency to escape onto the hidden platform in the β-amyloid protein-infused group in the first training trial was not different from that in the control group. The repeated training trials rapidly shortened the latencies in the control group, while in the β-amyloid protein-infused group, they shortened the latencies more slowly ($P < 0.01$, two-way ANOVA). In the 7th training trial, the latency in the β-amyloid protein-infused group was significantly longer than those in the control group ($P < 0.05$). The two-way ANOVA revealed that the daily administration of NC-1900 at a dose of 1 ng kg^{-1} significantly attenuated the β-amyloid protein-induced impairment of spatial learning in the water maze task ($P <$

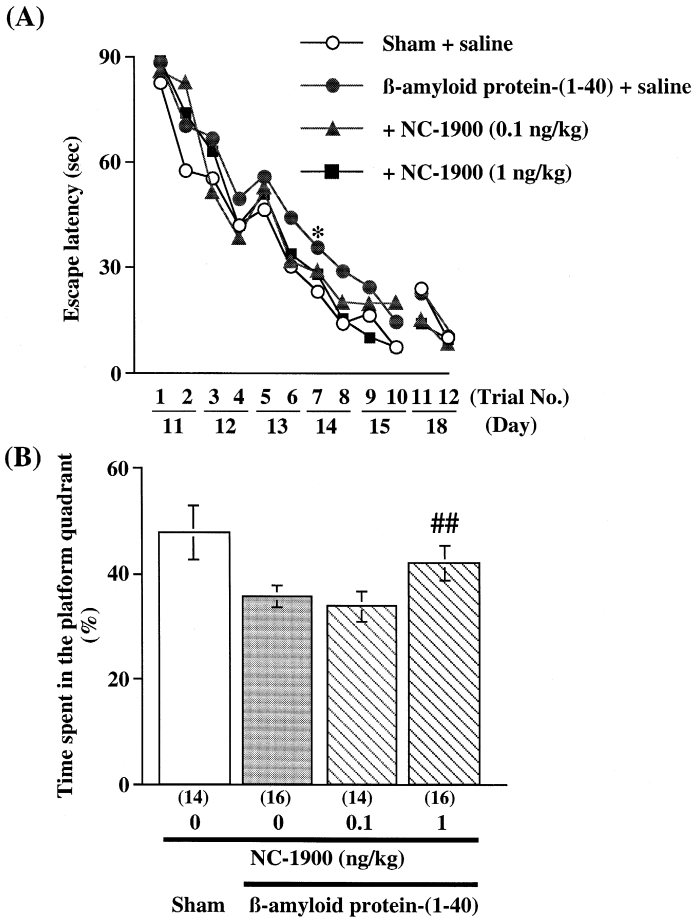

Fig. 2. Effect of NC-1900 on the performance of the β-amyloid protein-infused rats in the training trials (A) and the probe trial (B) in the water maze task. The training trials (two trials per day) were carried out on days 11–15 and 18 after the start of the β-amyloid protein infusion. The probe trial was carried out on day 19. Columns indicate means ± S.E. The number of rats used in each group is shown in parentheses. $* P < 0.05$ vs. control group (Wilcoxon test). $\#\# P < 0.01$ vs. β-amyloid protein-infused group (Bonferroni's test). Repeated training trials rapidly shortened the latencies in the control group, while in the β-amyloid protein-infused group they shortened the latencies more slowly ($P < 0.01$, two-way ANOVA). NC-1900 at a dose of 1 ng kg^{-1} significantly attenuated the β-amyloid protein-induced impairment of spatial learning in the water maze task ($P < 0.05$, two-way ANOVA).

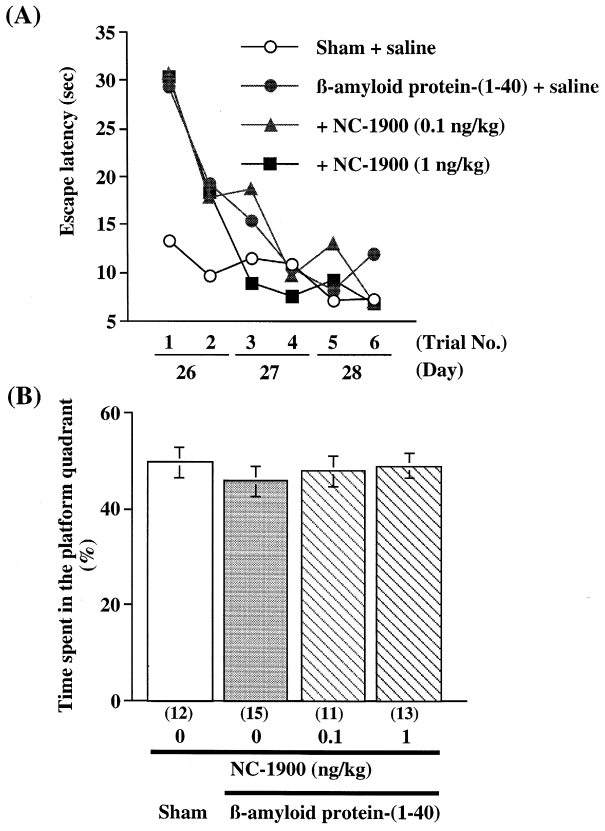

Fig. 3. Effect of NC-1900 on the performance of the β-amyloid protein-infused rats in the re-training trials (A) and the 2nd probe trial (B) in the water maze task. The re-training trials (two trials per day) were carried out on days 26–28 after the start of β-amyloid protein infusion. The 2nd probe trial was carried out on day 28 after the 6th re-training trial. Columns indicate means ± S.E. The number of rats used in each group is shown in parentheses. The performance of β-amyloid protein-infused group in the re-training trials was significantly impaired compared to that of the control group ($P < 0.05$, two-way ANOVA).

0.05), while the effect of NC-1900 at a dose of 0.1 ng kg^{-1} was not significant ($P = 0.06$).

A spatial probe trial was carried out on day 19 after the start of β-amyloid protein infusion to examine whether the rats had learned the position of the platform (Fig. 2B). The β-amyloid protein-infused group, compared with the control group, showed a decrease in the time spent in the quadrant (platform quadrant) in which the platform had been located during training trials (control group: 47.7 ± 5.2%, β-amyloid protein-infused group: 35.6 ± 2.1%) ($P = 0.051$). NC-1900 reversed the decrease in time spent in the platform quadrant in the β-amyloid protein-infused group in a dose-dependent manner (33.8 ± 2.9% at 0.1 ng kg^{-1}, 41.9 ± 3.3% at 1 ng kg^{-1}), and the effect of NC-1900 at a dose of 1 ng kg^{-1} was significant ($P < 0.01$).

3.3. Effects of NC-1900 on the performance of the β-amyloid protein-infused rats in the re-training trials and the 2nd probe trial of the water maze task

The performance of β-amyloid protein-infused group in the re-training trials of the water maze task (day 26–28) was significantly impaired ($P < 0.05$), compared to that of the control group. NC-1900 did not improve the impairment of spatial learning caused by β-amyloid protein. After repeated training for 3 days, there were no apparent differences in escape latency among the four different treatment groups (Fig. 3A). The results in the re-training trials were also analyzed separately in the 1st re-training trial and the 2nd to 6th trials as retrieval and re-learning phases, respectively. Although it appears that in the 1st re-training trial, escape latencies in the β-amyloid protein-infused groups with or without NC-1900 treatment were longer than that in control group, the change did not reach statistical significance.

A spatial 2nd probe trial was carried out after the 6th re-training trial to test whether the rats had learned the position of the platform. The time spent in the platform quadrant did not differ among the control, β-amyloid protein-infused and NC-1900 (0.1 and 1 ng kg^{-1})-treated groups (Fig. 3B).

3.4. Effects of NC-1900 on the performance of the β-amyloid protein-infused rats in the working memory trials in the water maze task

The mean escape latency of the β-amyloid protein-infused rats in the working memory test for 3 days, was significantly longer than that of the control rats (Fig. 4; $P < 0.05$). NC-1900 at a dose of 1 ng kg^{-1} apparently improved the working memory deficits induced by β-

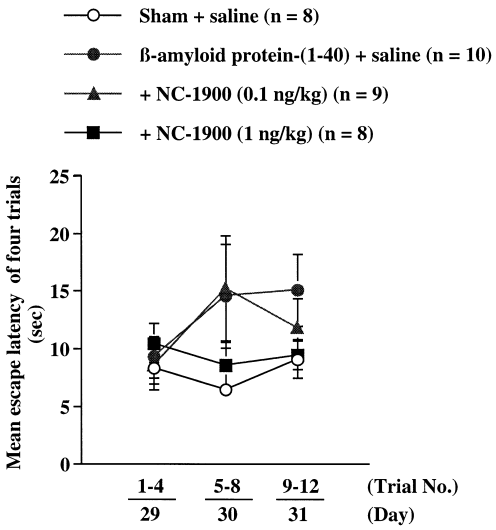

Fig. 4. Effect of NC-1900 on the performance of the β-amyloid protein-infused rats in the working memory trials in the water maze task. The working memory trials (four trials per day) were carried out for 3 days on day 29–31 after the start of β-amyloid protein infusion. Bars indicate means ± S.E. The mean escape latency in the working memory test for 3 days in β-amyloid protein-infused rats was significantly longer than that in control rats ($P < 0.05$, two-way ANOVA).

amyloid protein, but the effect was not statistically significant.

3.5. Effects of NC-1900 on the performance of the β-amyloid protein-infused rats in the multiple-trial passive avoidance task

The step-through latencies and numbers of trials in the acquisition trial were not different among the four groups (Fig. 5A,B). In the retention trial, the step-through latency of the β-amyloid protein-infused group was shorter than that the control group ($P = 0.05$) (Fig. 5A). NC-1900 at a dose of 1 ng kg^{-1} significantly ameliorated the decrease in the step-through latency in the retention trial of the β-amyloid protein-infused group ($P < 0.05$).

3.6. Effects of NC-1900 on choline acetyltransferase activity in the β-amyloid protein-infused rats

As shown in Fig. 6, the choline acetyltransferase activity in the hippocampus and frontal cortex in the control rats was 940.8 ± 105.4 and 608.0 ± 75.6 pmol min^{-1} mg^{-1} protein, respectively. The choline acetyltransferase activity in the β-amyloid protein-infused rats did not differ from that in the control rats in the hippocampus and frontal cortex. NC-1900 (0.1 and 1 ng kg^{-1}) tended to increase the cortical choline acetyltransferase activity in the β-amyloid protein-infused rats ($P = 0.07$), although it had no effect on activity in the hippocampus.

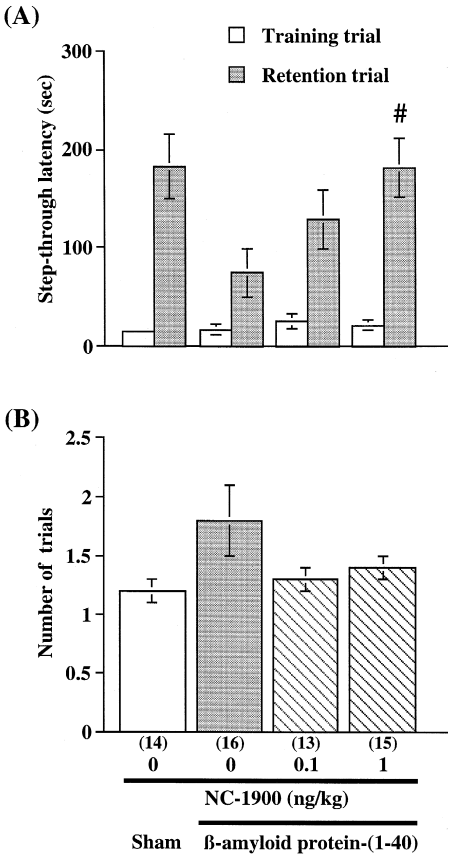

Fig. 5. Effect of NC-1900 on step-through latency (A) and the number of training trials (B) of the β-amyloid protein-infused rats in the multiple-trial passive avoidance task. The task was carried out on days 16 and 17 after the start of the β-amyloid protein infusion. Columns indicate means ± S.E. The number of rats used in each group is shown in parentheses. #$P < 0.05$ vs. β-amyloid protein-infused group (Bonferroni's test).

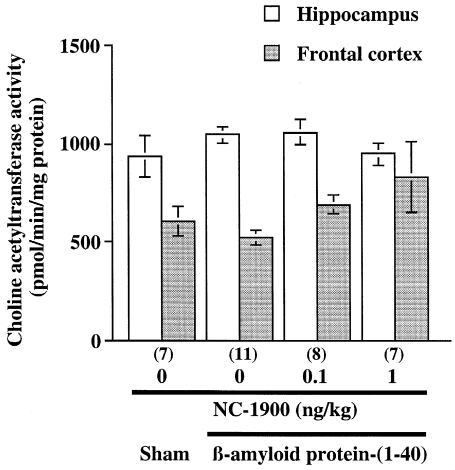

Fig. 6. Effect of NC-1900 on choline acetyltransferase activity in the β-amyloid protein-infused rats. Rats were killed on day 34 after the start of the β-amyloid protein infusion. Columns indicate mean ± S.E. The number of rats used in each group is shown in parentheses.

4. Discussion

In the present study, we observed that the continuous infusion of β-amyloid protein-(1–40) into the cerebral ventricle caused impairments of spatial reference memory in a water maze task and memory retention in passive avoidance task, in agreement with results of previous studies (Nitta et al., 1994, 1997; Nabeshima and Nitta, 1994). Moreover, we found that working memory in the water maze task was impaired by the infusion of β-amyloid protein. Working memory refers to memory in which the information to be remembered changes in repeated trials (Olton et al., 1979). Thus, working memory is trial-dependent and can be assessed in versions of the water maze task which require learning rapidly changing information (Olton et al., 1979). Since performance in the 6th retraining trial and 2nd probe trial after the 6th retraining trial in the β-amyloid protein-infused group did not differ from that of the control group, it is unlikely that deficits in the working memory task in the β-amyloid protein-infused rats were due to the secondary effects of reference memory formation. Instead, the continuous infusion of β-amyloid protein into the brain results in an impairment of spatial working memory. Furthermore, we have confirmed that the reversed β-amyloid protein-(40–1) has no effect on performance in these behavioral tasks (unpublished obser-

vation). We therefore conclude that learning and memory function in rats are impaired by the continuous infusion of β-amyloid protein into the cerebral ventricle.

We previously reported that choline acetyltransferase activity in the hippocampus was minimally (but significantly) decreased by the continuous infusion of β-amyloid protein into the cerebral ventricle (Nitta et al., 1994, 1997; Nabeshima and Nitta, 1994), while no significant changes in choline acetyltransferase activity were observed in the present study. We have previously demonstrated, however, that the continuous infusion of β-amyloid protein causes a marked impairment of the nicotine- and KCl-induced stimulation of dopamine and acetylcholine release in vivo without apparent changes in their basal levels (Itoh et al., 1996).

The structure of NC-1900 is with Ser substituted for Cys–Cys in the hexapeptide of arginine vasopressin-(4–9) which is mainly metabolized from arginine vasopressin-(1–9). NC-1900 demonstrated a markedly improved effect, with a 10-fold higher behavioral activity than that obtained with arginine vasopressin-(4–9). This result indicates that [Ser^6] hexapeptide has an important role in behavioral activity (Fujiwara et al., 1997). In the present study, we selected the doses of NC-1900 on the basis of a earlier results (Hirate et al., 1997). NC-1900 improved β-amyloid protein-induced deficits in spatial reference memory formation in the water maze task and the memory retention in the passive avoidance task.

We observed that NC-1900 increased the choline acetyltransferase activity in the frontal cortex in rats that had received a continuous infusion of β-amyloid protein. This NC-1900-induced increase in choline acetyltransferase activity may be, at least in part, related to its behavioral effects. Arginine vasopressin-(4–9) also improves learning and memory deficits (Burbach et al., 1983; De Wied et al., 1987; Strupp, 1989; Dietrich and Allen, 1997; Fujiwara et al., 1997; Hirate et al., 1997). The facilitation of memory by arginine vasopressin-(4–9) is perhaps induced by the stimulation of acetylcholine release in the hippocampus (Maegawa et al., 1992; Fujiwara et al., 1997) and the stimulation of inositol phospholipid metabolism in the hippocampus (Stephens and Logan, 1986). Therefore, it is also possible that the effects of NC-1900 in the β-amyloid protein-infused rats are mediated through these mechanisms. However, in the present study, we injected NC-1900 after the behavioral test, and 3 days before the infusion of β-amyloid protein. The improvement effect of NC-1900 on β-amyloid protein-induced deficits may thus be due to protection against the β-amyloid protein-induced toxicity. Zhou et al. (1995) demonstrated that exogenous arginine vasopressin-(4–9) enhances nerve growth factor (*NGF*) gene expression in the hippocampus and cerebral cortex. We have observed that propentofylline, which increases *NGF* synthesis in the brain (Shinoda et al., 1990; Nabeshima et al., 1993), ameliorates behavioral deficits in β-amyloid protein-infused rats (Yamada et al., 1998). Accordingly, NC-1900 may improve β-amyloid protein-induced learning and memory deficits by inducing *NGF* in the brain. Further experiments, including investigation of the effects of NC-1900 on mnemonic ability in intact animals, should be carried out to clarify the mechanism of action of this novel derivative of arginine vasopressin.

5. Conclusion

We have demonstrated that the repeated administration of NC-1900 ameliorated learning and memory deficits in rats which were continuously infused with β-amyloid protein into the cerebral ventricle. We therefore believe that clinical trials of NC-1900 for the treatment of Alzheimer's disease are warranted.

Acknowledgements

We are grateful to Nippon Chemiphar for the supply of NC-1900. This study was supported in part by a Grant-in-Aid for Science Research from the Ministry of Education, Science, and Culture of Japan (No. 07557009 and 07557303), a grant from the Ministry of Health and Welfare's Foundation for Gerontological Science Research (91A-2406 and 94A-2405), and an SRF Grant from Biomedical Research.

References

Alescio-Lautier, B., Devigne, C., Soumireu-Mourat, B., 1987. Hippocampal lesions block behavioral effects of central but not of peripheral pre-test injection of arginine vasopressin in an appetitive learning task. Behav. Brain Res. 26, 159–169.

Bohus, B., Ader, R., De Wied, D., 1972. Effects of vasopressin on active and passive avoidance behavior. Horm. Behav. 3, 191–197.

Burbach, J.P., Kovacs, G.L., De Wied, D., Van Nispen, J.W., Greven, H.M., 1983. A major metabolite of arginine vasopressin in the brain is a highly potent neuropeptide. Science 221, 1310–1312.

Coyle, J.T., Price, D.L., Delong, M.R., 1983. Alzheimer's disease: a disorder of cortical cholinergic innervation. Science 219, 1184–1190.

De Wied, D., Versteeg, D.H., 1979. Neurohypophyseal principles and memory. Fed. Proc. 38, 2348–2354.

De Wied, D., Gaffori, O., Van Ree, J.M., De Jong, W., 1984. Central target for the behavioral effects of vasopressin neuropeptides. Nature 308, 276–278.

De Wied, D., Gaffori, O., Burbach, J.P., Kovacs, G.L., Van Ree, J.M., 1987. Structure activity relationship studies with C-terminal fragments of vasopressin and oxytocin on avoidance behaviors of rats. J. Pharmacol. Exp. Ther. 241, 268–274.

Dietrich, A., Allen, J.D., 1997. Vasopressin and memory: II. Lesion to the hippocampus block the memory enhancing effects of AVP(4–9) in the radial maze. Behav. Brain Res. 87, 201–208.

Fujiwara, M., Ohgaki, Y., Inada, K., Iwasaki, K., 1997. Effect of active fragments of arginine vasopressin on the disturbance of spatial cognition in rats. Behav. Brain Res. 83, 91–96.

Hirate, K., Hirano, M., Nakajima, Y., Hiyama, A., Maeda, O., Asakura, W., 1997. No. 302, a newly synthesized [pGlu[4], Cyt[6]] AVP(4–9) analogue, prevents the disruption of avoidance behavior. Behav. Brain Res. 83, 205–208.

Itoh, A., Nitta, A., Nadai, M., Nishimura, K., Hirose, M., Hasegawa, T., Nabeshima, T., 1996. Dysfunction of cholinergic and dopaminergic neuronal systems in β-amyloid protein-infused rats. J. Neurochem. 66, 1113–1117.

Kaneda, N., Nagatsu, T., 1985. Highly sensitive assay for choline acetyltransferase activity by high-performance liquid chromatography with electrochemical detection. J. Chromatogr. 341, 23–30.

Maegawa, H., Katsube, N., Okegawa, T., Aishita, H., Kawasaki, A., 1992. Arginine vasopressin fragment 4–9 stimulates the acetylcholine release in hippocampus of freely moving rats. Life Sci. 51, 285–293.

Masters, C.L., Simms, G., Weinman, N.A., Multhaup, G., McDonald, B.L., Beyreuther, K., 1985. Amyloid plaque core protein in Alzheimer's disease and Down syndrome. Proc. Natl. Acad. Sci. USA 82, 4245–4249.

Maurice, T., Hiramatsu, M., Itoh, J., Kameyama, T., Hasegawa, T., Nabeshima, T., 1994. Behavioral evidence for a modulating role of σ ligands in memory processes: I. Attenuation of dizocilpine (MK-801)-induced amnesia. Brain Res. 647, 44–56.

Miyagawa, H., Hasegawa, M., Fukuta, T., Amano, M., Yamada, K., Nabeshima, T., 1998. Dissociation of impairment between spatial memory and motor function and emotional behavior in aged rats. Behav. Brain Res., in press.

Morris, R.G.M., 1984. Developments of water maze procedure for studying spatial learning in the rat. J. Neurosci. Methods 11, 47–60.

Müller-Hill, B., Beyreuther, K., 1989. Molecular biology of Alzheimer's disease. Annu. Rev. Biochem. 58, 287–307.

Nabeshima, T., Itoh, A., 1996. Toxicity of β-amyloid protein: neurochemical, histological and behavioral changes. In: Iqbal, K. et al. (Eds.), Alzheimer's Disease: Biology, Diagnosis and Therapeutics. Chichester Press, England, pp. 623–630.

Nabeshima, T., Itoh, A., 1997. Alzheimer's disease animal models induced by continuous infusion of β-amyloid protein and anti-nerve growth factor antibody. Rev. Heteroatom Chem. 16, 229–255.

Nabeshima, T., Nitta, A., 1994. Memory impairment and neuronal dysfunction induced by β-amyloid protein in rats. Tohoku J. Exp. Med. 174, 241–249.

Nabeshima, T., Ogawa, S., Ishimaru, H., Kameyama, T., Fukuta, T., Takeuchi, R., Hayashi, K., 1991. Memory impairment and morphological changes in rats induced by active fragment of anti-nerve growth factor-antibody. Biochem. Biophys. Res. Commun. 175, 215–219.

Nabeshima, T., Nitta, A., Hasegawa, T., 1993. Impairment of learning and memory and the accessory symptom in aged rats as senile dementia model (3): oral administration of propentofylline produces recovery of reduced *NGF* content in the brain of aged rats. Jpn. J. Psychopharmacol. 13, 89–95.

Nitta, A., Hayashi, K., Hasegawa, T., Nabeshima, T., 1993. Development of plasticity of brain function with repeated trainings and passage of time after basal forebrain lesions in rats. J. Neural Transm. 93, 37–46.

Nitta, A., Itoh, A., Hasegawa, T., Nabeshima, T., 1994. β-Amyloid protein-induced Alzheimer's disease animal model. Neurosci. Lett. 170, 63–66.

Nitta, A., Fukuta, T., Hasegawa, T., Nabeshima, T., 1997. Continuous infusion of β-amyloid protein into cerebral ventricle induces learning impairment and neuronal and morphological degeneration. Jpn. J. Pharmacol. 73, 51–57.

Olton, D.S., Becker, J.T., Handeimann, G.E., 1979. Hippocampus, space, and memory. Behav. Brain Sci. 2, 313–365.

Paxinos, G., Watson, C., 1986. The Rat Brain in the Stereotaxic Coordinates. Academic Press, New York.

Shinoda, I., Furukawa, Y., Furukawa, S., 1990. Stimulation of nerve growth factor synthesis/secretion by propentofylline in cultured mouse astroglial cells. Biochem. Pharmacol. 49, 1813–1816.

Stephens, L.R., Logan, S.D., 1986. Arginine vasopressin stimulates inositol phospholipid metabolism in rat hippocampus. J. Neurochem. 46, 649–651.

Strupp, B.J., 1989. Improvement of memory by a vasopressin fragment: importance of individual differences in mnemonic function. Behav. Neurosci. 103, 743–754.

Till, R.E., Beckwith, B.E., 1985. Sentence memory affected by vasopressin analog (DDAVP) in cross-over experiment. Peptides 6, 397–402.

Wilcock, G.K., Esiri, M.M., Bowen, D.M., Smith, C.C., 1982. Alzheimer's disease. Correlation of cortical choline acetyltransferase activity with the severity of dementia and histological abnormalities. J. Neurol. Sci. 57, 407–417.

Yamada, K., Nitta, A., Saito, T., Hu, J., Nabeshima, T., 1995. Changes in ciliary neurotrophic factor content in the rat brain after continuous intracerebroventricular infusion of β-amyloid (1–40) protein. Neurosci. Lett. 201, 155–158.

Yamada, K., Noda, Y., Hasegawa, T., Komori, Y., Nikai, T., Sugihara, H., Nabeshima, T., 1996. The role of nitric oxide in dizocilpine-induced impairment of spontaneous alternation behavior in mice. J. Pharmacol. Exp. Ther. 276, 460–466.

Yamada, K., Tanaka, T., Senzaki, K., Kameyama, T., Nabeshima, T., 1998. Propentofylline improves learning and memory deficits in rats induced by β-amyloid protein-(1–40). Eur. J. Pharmacol. 349, 15–22.

Zhou, A.W., Guo, J., Wang, H.Y., Gu, B.X., Du, Y.C., 1995. Enhance of *NGF* gene expression in rat brain by the memory-enhancing peptide AVP(4–8). Peptides 16, 581–586.