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Propentofylline improves learning and memory deficits in rats induced by β-amyloid protein-(1-40)

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Abstract

We have reported that continuous infusion of β-amyloid protein-(1-40) into the cerebral ventricle produces learning and memory deficits in rats. Propentofylline has potent stimulatory effects on nerve growth factor (NGF) synthesis/secretion in mouse astrocytes in vitro and increases cerebral NGF content in aged rats. In the present study, we examined the effects of propentofylline on learning and memory deficits in β-amyloid protein-infused rats. The rats were given propentofylline orally once a day throughout the period of behavioral examination. In the β-amyloid protein-infused rats, spontaneous alternation behavior in a Y-maze, and performance in water maze and passive avoidance tasks were significantly impaired compared to sham-operated rats. Propentofylline prevented these behavioral deficits, but did not change the reduction of the activity of choline acetyltransferase in the hippocampus in the β-amyloid protein-infused rats. These results suggest that propentofylline is useful for the treatment of patients with Alzheimer's disease. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Alzheimer's disease; β-Amyloid protein; Choline acetyltransferase; Learning; Memory; Propentofylline; (Rat)

1. Introduction

In Alzheimer's disease, learning and memory deficits are caused, at least partly, by loss of neurons in the cholinergic system (Bartus et al., 1982; Sims et al., 1983). Several studies have shown that nerve growth factor (NGF) is a neurotrophic factor for the magnocellular cholinergic neurons of the basal forebrain (Hefti et al., 1984; Honegger and Lenoir, 1982). These neurons project from the medial septal area and nucleus basalis magnocellularis to the hippocampus and neocortex, respectively. It has been reported that NGF infusion into the cerebral ventricle of patients with Alzheimer's disease increases the nicotine receptor activity in the frontal and temporal cortices, and cortical blood flow (Olson et al., 1992). Thus, NGF may counteract the cholinergic dysfunction in Alzheimer's disease. However, in terms of the quality of life of the patient, the insertion of an i.c.v. delivery catheter is not an ideal therapeutic means. In addition, NGF does not cross the blood–brain barrier and is readily metabolized by peptidases when administered peripherally. NGF itself, therefore, may have a limited usefulness for medical treatment, unless an appropriate drug delivery system can be developed (Friden et al., 1993).

Propentofylline [3,7-dihydro-3-methyl-1-(5-oxohexyl)-7-propyl-1H-purine-2,6 dione], a xanthine derivative, exhibits several effects in the brain, e.g., prevention of cerebral metabolic disorder during anoxia (Stefanovich and Nagata, 1983), improvement of cerebral edema (Mrsulja et al., 1983) and rescuing of microglia from cytoxicity (Banati et al., 1993). It has been reported that propentofylline stimulates NGF synthesis and secretion in quiescent astroglial cells (Shinoda et al., 1990). Since propentofylline can cross the blood–brain barrier, it could be expected to stimulate NGF synthesis in the brain. Indeed, we found that this compound attenuated the reduced NGF content in the brains of aged rats (Nabeshima et al., 1993). Furthermore, the drug improved the memory impairment and cholinergic dysfunction observed in basal forebrain-le-

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sioned rats (Fuji et al., 1993a,b) and in rats which received continuous infusion of anti-NGF antibody into the septum (Nitta et al., 1996). Because of these findings, we believe NGF stimulators such as propentofylline may provide a new approach to the treatment of Alzheimer's disease (see, for review, Nabeshima and Itoh, 1997; Yamada et al., 1997).

Regarding clinical effects of propentofylline, a 12-month, randomized, placebo-controlled trial has shown that propentofylline has a favorable effect on cognitive function in patients with Alzheimer's disease as well as vascular dementia (Marcusson et al., 1997). To support the clinical data and clarify the mechanism of action, further studies of propentofylline should be carried out using an animal model of Alzheimer's disease.

We have previously demonstrated that continuous infusion of β-amyloid protein-(1-40) into the rat cerebral ventricle causes learning and memory deficits and a decrease in choline acetyltransferase activity in the frontal cortex and hippocampus (Nabeshima and Nitta, 1994; Nitta et al., 1994, 1997; Nabeshima and Itoh, 1996, 1997). In the present study, we investigated whether propentofylline exerts an effect on cognitive dysfunction in this animal model of Alzheimer's disease.

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 in a temperature- and light-controlled room (23°C; 12-h light cycle starting at 9:00 AM) and had free access to food and water, except during the behavioral experiments.

β-Amyloid protein-(1-40) was obtained from Bachem (Torrance, CA, USA). Propentofylline was kindly provided by Nippon Hoechst Marion Roussel (Tokyo, Japan). The β-amyloid protein was dissolved in 35% acetonitrile/0.1% trifluoacetic acid. Continuous infusion of the β-amyloid protein (300 pmol/day) was maintained for at least two weeks by attaching a cannula to a mini-osmotic pump (Alzet 2002; Alza, Palo Alto, CA, USA) (Nabeshima et al., 1991). The control rats were infused with the vehicle only (35% acetonitrile/0.1% trifluoacetic acid). We have confirmed that the vehicle itself failed to induce any behavioral and neurochemical changes at this flow rate (data not shown). The cannula was implanted into the right ventricle (A −0.3, L 1.2, V 4.5) according to the atlas of Paxinos and Watson (1986).

2.2. Drug administration and experimental design

Propentofylline dissolved in distilled water, was administered orally for 21 consecutive days at doses of 10 and 25 mg/kg per day (Fuji et al., 1993a,b; Nabeshima et al., 1993; Nitta et al., 1996). One group consisted of 10–12 rats. Learning and memory capacity was measured by performance in four tasks: habituation, Y-maze, water maze and passive avoidance. The behavioral study was started 8 days after the surgery and the four tasks were carried out sequentially. The oral administration of propentofylline started 3 days before the implantation of the mini-osmotic pump, and continued throughout the period during which the behavioral studies were conducted.

2.3. Habituation task

The habituation task (Platel and Porsolf, 1982) was carried out on days 8 and 9 after the start of β-amyloid protein-infusion. The apparatus consisted of a locomotor cage ($25 \times 42 \times 20$ cm), with photobeams placed 2 cm above the floor at 1-in. intervals along two sides of the cage (Colombus Instruments, USA). Locomotor activity was measured for 10 min on the first and the second days. Locomotor activity was measured at the same time each day to avoid the influence of the circadian rhythm.

2.4. Y-maze task

The Y-maze task was carried out on day 10 after the start of β-amyloid protein infusion, as described previously (Yamada et al., 1996). The 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. Each 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 the entry into all three arms on consecutive choices in overlapping triplet sets. The percent spontaneous alternation behavior was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries $- 2) \times 100$.

2.5. Water maze task

The water maze was carried out on day 11 to 15 after the start of β-amyloid protein infusion (Morris, 1984). 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 several 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 orienta-

tion. The position of the cues remained unchanged throughout training. For each training session, the rat was put into 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 session, 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 session was terminated and a maximum score of 90 s was assigned. Training was conducted for 5 consecutive days, twice a day, one session consisting of 2 trials. (2 trials × 5 sessions).

Immediately after the 10th trial, the platform was removed from the pool and animals were tested on a 60-s spatial probe trial. The time spent in the quadrant where the platform had been located during training was measured.

2.6. Multiple-trial passive avoidance task

Multiple-trial passive avoidance task was carried out on day 16 and 17 after the start of 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, each rat was placed in the illuminated compartment; when 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 on a single trial. In the retention trial, 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.

2.7. Measurement of choline acetyltransferase activity

Choline acetyltransferase activity was measured as reported previously (Kaneda and Nagatsu, 1985).

2.8. 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 comparison, the Welch test or Wilcoxon test was utilized. Paired *t*-test was also utilized for the habituation task.

3. Results

3.1. Effects of propentofylline on performance in the habituation task of the β-amyloid protein-infused rats

Locomotor activity in a novel environment was recorded for a 10-min period on 2 successive days (Fig. 1). On the first day, the locomotor activity did not differ among sham-operated, β-amyloid protein-infused and propentofylline-treated groups. On the second day, locomotor activity was significantly decreased, compared with that on the first day, in all treatment groups (Fig. 1A). As shown in Fig. 2B, the mean values for the percent reduction in locomotor activity on the second day were 29.6, 22.0, 27.6 and 33.9% in the sham-operated, β-amyloid protein-infused and propentofylline (10 and 25 mg/kg)-treated groups, respectively. The percent reduction of motor activ-

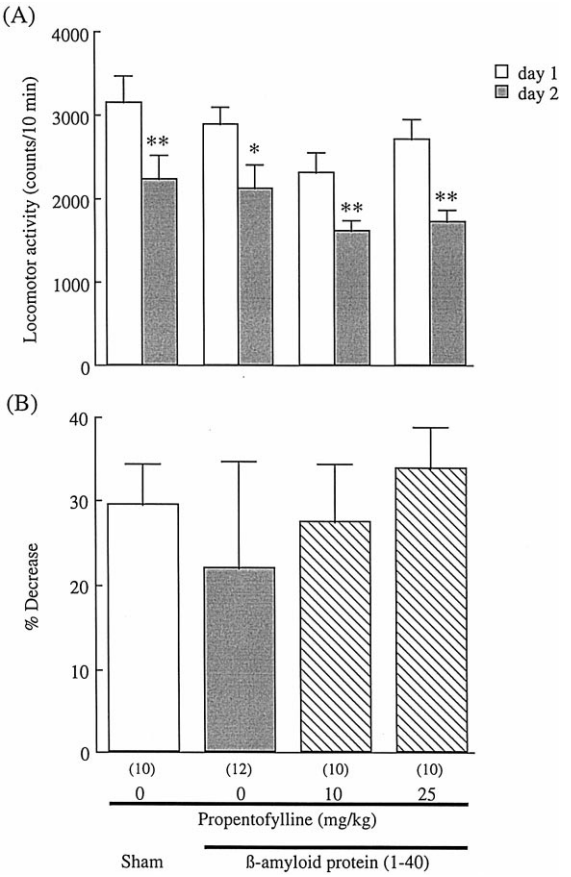

Fig. 1. Effects of propentofylline on performance of the β-amyloid protein-infused rats in the habituation task. The task was carried out on day 8 and day 9 after the start of β-amyloid protein infusion. Locomotor activity was measured for 10 min. Columns indicate mean ± S.E. The number of rats used in each group is shown in parentheses. * $P < 0.05$; ** $P < 0.01$ vs. day 1 of each treatment.

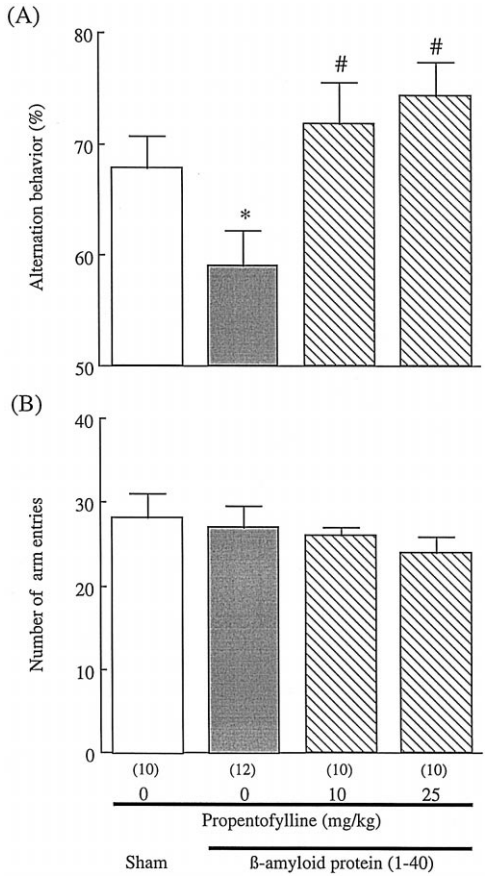

Fig. 2. Effects of propentofylline on spontaneous alternation behavior (A) and the number of arm entries (B) during an 8-min session in the Y-maze task of the β-amyloid protein-infused rats. The task was carried out on day 10 after the start of β-amyloid protein infusion. Columns indicate mean ± S.E. The number of rats used in each group is shown in parentheses. * $P < 0.05$ vs. sham, # $P < 0.05$ vs. β-amyloid protein.

ity on the second day in the β-amyloid protein-infused group was less than that in the sham-operated group (Fig. 1B). Propentofylline showed a tendency to improve the impairment of motor habituation caused by β-amyloid protein.

3.2. Effects of propentofylline on spontaneous alternation behavior of the β-amyloid protein-infused rat in the Y-maze task

As shown in Fig. 2A, the spontaneous alternation behavior in the β-amyloid protein-infused group was significantly less than that in the sham-operated group ($P < 0.05$). Both doses of propentofylline significantly attenuated the impairment of this behavior induced by β-amyloid protein-infusion ($P < 0.05$). On the other hand, the number of arm entries did not differ significantly among the sham-operated, β-amyloid protein-infused and propentofylline-treated groups (Fig. 2B).

3.3. Effects of propentofylline on performance in the water-maze task of the β-amyloid protein-infused rats

The mean values of the latencies of the four groups to escape onto the hidden platform in each training session of the water maze task are shown in Fig. 3A. The latency in the β-amyloid protein-infused group on the first training trial was not different from that in the sham-operated group. Repeated training rapidly shortened the latencies in the sham-operated group, while in the β-amyloid protein-infused group it shortened the latencies more slowly. From the 6th to 10th training trials, the latencies in the β-amyloid protein-infused group were significantly longer than those in the sham-operated group. Daily administration of propentofylline attenuated the β-amyloid protein-induced impairment of spatial learning in the water maze task in a dose-dependent manner. In the 8th and 10th training trials, the latencies of the propentofylline (25 mg/kg)-treated

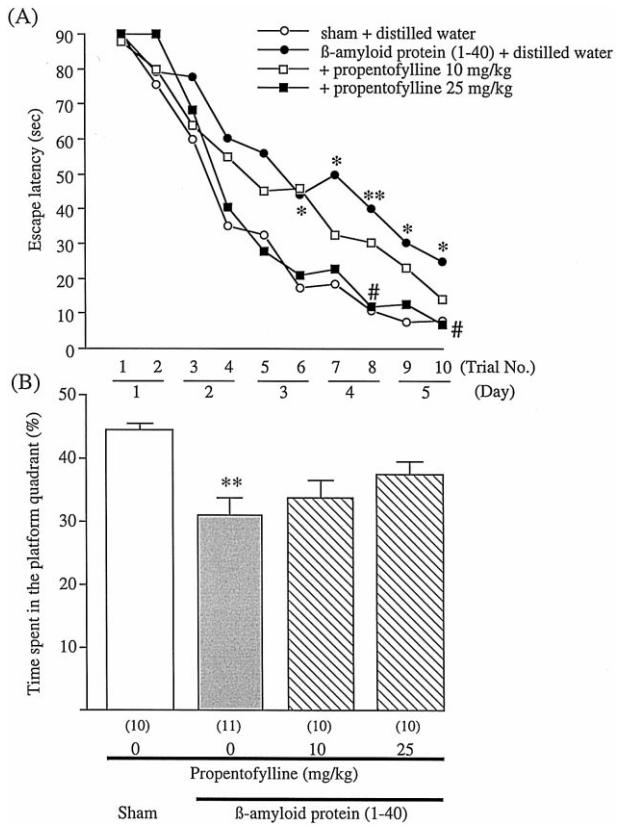

Fig. 3. Effects of propentofylline on performance in the training trials (A) and in the probe trial (B) of the water maze task of the β-amyloid protein-infused rats. The training trials were carried out on day 11–15 after the start of β-amyloid protein infusion. The probe trial was carried out on day 15 after the start of β-amyloid protein infusion, immediately after the 10th training trials. Columns indicate mean ± S.E. The number of rats used in each group is shown in parentheses. * $P < 0.05$; ** $P < 0.01$ vs. sham, # $P < 0.05$ vs. β-amyloid protein.

group were significantly shorter than those of the β-amyloid protein-infused group Fig. 5.

A spatial probe trial was carried out after the 10th training trial to examine whether the rats had learned the position of the platform. The β-amyloid protein-infused group, compared with the sham-operated group, showed a significant decrease in the time spent in the quadrant in the which the platform had been located (platform-quadrant) during training (sham-operated group: 44.3 ± 1.2%, β-amyloid protein-infused group: 30.9 ± 2.8%) (Fig. 3B). Propentofylline (at 25 mg/kg) treatment showed a tendency to reverse the decrease in time spent in the platform quadrant in the β-amyloid protein-infused group (propentofylline 10 mg/kg: 33.7 ± 2.8%, 25 mg/kg: 37.3 ± 2.0%).

3.4. Effects of propentofylline on performance in the multiple-trial passive avoidance task of the β-amyloid protein-infused rats

The step-through latencies and numbers of trials in the acquisition trial did not differ among the 4 groups (Fig. 4A,B). The step-through latency in the β-amyloid protein-infused group in the retention trial was significantly shorter than that in the sham-operated group (Fig. 4A). Propentofylline at a dose of 10 mg/kg markedly ameliorated the decrease in the step-through latency in the retention trial in the β-amyloid protein-infused group Fig. 5.

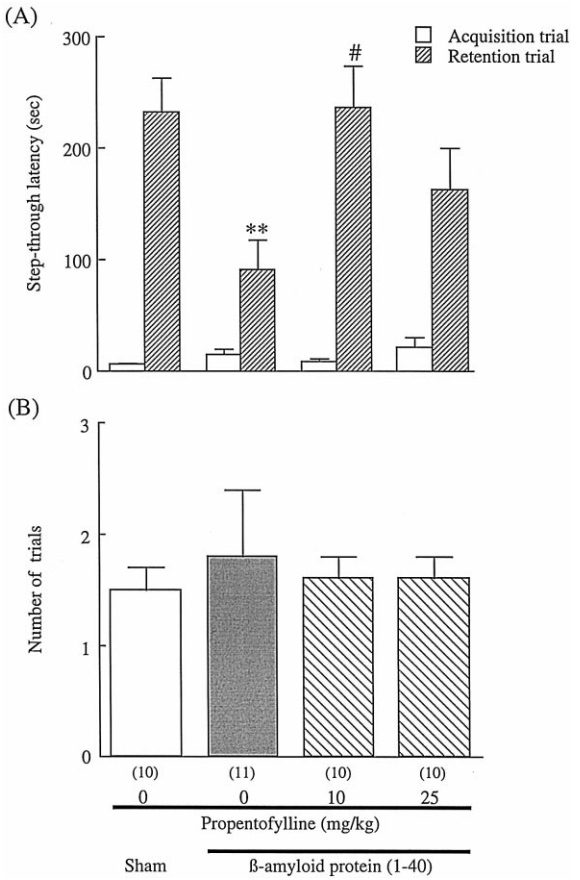

Fig. 4. Effects of propentofylline on step-through latency (A) and the number of training trials (B) in the multiple-trial passive avoidance task of the β-amyloid protein-infused rats. The task was carried out on day 16–17 after the start of β-amyloid protein infusion. Columns indicate mean ± S.E. The number of rats used in each group is shown in parentheses. ** $P < 0.01$ vs. sham, # $P < 0.05$ vs. β-amyloid protein.

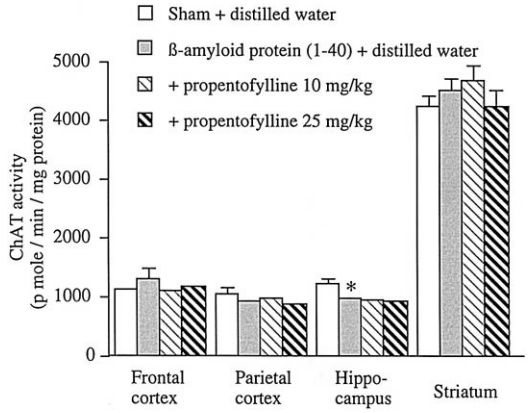

Fig. 5. Effects of propentofylline on the choline acetyltransferase activity in the β-amyloid protein-infused rats. Rats were killed on day 18 after the start of β-amyloid protein infusion. Columns indicate mean ± S.E. ($n = 6$). * $P < 0.05$ vs. sham.

3.5. Effects of propentofylline on choline acetyltransferase activity in the β-amyloid protein-infused rats

Choline acetyltransferase activity in the frontal cortex, parietal cortex, hippocampus and striatum in the sham-operated rats was 1106.7 ± 45.6, 1039.6 ± 95.0, 1209.1 ± 75.1 and 4229.5 ± 190.8 pmol/min per mg protein, respectively. The activity in the hippocampus in the β-amyloid protein-infused rats was reduced significantly compared to that in the sham-operated rats. Treatment with propentofylline (10 and 25 mg/kg) did not affect the reduction caused by β-amyloid protein infusion. Choline acetyltransferase activity in the frontal cortex, parietal cortex and striatum did not change among the sham-operated, β-amyloid protein-infused and propentofylline (10 and 25 mg/kg)-treated rats.

4. Discussion

We have previously demonstrated that continuous infusion of β-amyloid protein-(1-40) into the cerebral ventricle of rats causes learning and memory deficits, reduction in choline acetyltransferase activity in the frontal cortex and hippocampus (Nabeshima and Nitta, 1994; Nitta et al., 1994; Nabeshima and Itoh, 1997), impairment of the nicotine- and KCl-induced stimulation of dopamine and acetylcholine release in vivo (Itoh et al., 1996), changes in ciliary neurotrophic factor (CNTF) levels in the brain (Yamada et al., 1995), and lack of appearance of long-term

potentiation in the CA1 field of the hippocampus (Nabeshima and Itoh, 1996). Therefore, this animal model of Alzheimer' disease may be useful for screening the compounds for Alzheimer's disease and investigating the mechanism of action of the drugs (Nabeshima and Nitta, 1994; Nitta et al., 1994).

Exogenous NGF has been shown to ameliorate brain dysfunction in Alzheimer's disease (Olson et al., 1992). Propentofylline, a prototype of NGF stimulators (Nabeshima and Itoh, 1997; Yamada et al., 1997), is also reported to be efficacious for the treatment of patients with Alzheimer's disease and vascular dementia (Marcusson et al., 1997). To support the clinical effects of propentofylline and clarify the mechanism of action, we investigated the effects of this drug on learning and memory impairment and dysfunction of the cholinergic system in the β-amyloid protein-infused rat.

Infusion of β-amyloid protein-(1-40) caused learning and memory deficits, as assessed by spontaneous behavior in Y-maze, and performance in water maze and passive avoidance tasks. On the other hand, habituation of locomotor activity to novelty in the β-amyloid protein-infused rat did not differ from that in sham-operated rats. Since the habituation task was carried out on day 8 and 9, like the first behavioral task, the accumulation of β-amyloid protein may be not have been sufficient to demonstrate behavioral neurotoxicity. In the other 3 behavioral tasks, Y-maze, water maze and multiple-trial passive avoidance tasks, the rats, which were continuously infused with β-amyloid protein, exhibited significant learning and memory impairments. Since these behavioral tasks utilized different reinforcements and need different motor skill for performance, it is unlikely that the impairment of performance was due to changes in motivation and other sensorimotor functions. Furthermore, we have confirmed that the reversed β-amyloid protein-(40-1) has no effect on performance in these behavioral tasks (unpublished observation). Therefore, it is considered that learning and memory function in rats is impaired by the continuous infusion of β-amyloid protein into the cerebral ventricle.

In the present study, we found that repeated administration of propentofylline at doses of 10 and 25 mg/kg ameliorated the learning and memory impairments in β-amyloid protein-infused rats. Previous studies have shown that successive administration of propentofylline ameliorates the basal forebrain lesion- and anti-NGF antibody-induced impairment of performance in several behavioral tasks in rats (Fuji et al., 1993b; Nitta et al., 1996). These experiments showed that the improving effects of propentofylline on learning and memory impairment are dose-dependent. However, a low dose of propentofylline (10 mg/kg) was more effective than a high dose (25 mg/kg) in the passive avoidance test in β-amyloid protein- and anti-NGF antibody-infused rats (Nitta et al., 1996). In the basal forebrain-lesioned rats, both 10 and 25 mg/kg of propentofylline were effective to ameliorate the deficits in the passive avoidance test. Since in vivo pharmacological effects can be influenced by the absorption, distribution and metabolism of the drug, these factors might be different among basal forebrain-lesioned, anti-NGF antibody-infused and β-amyloid protein-infused rats. Another possible explanation for the bell-shaped dose-response curve is that a high dose of propentofylline affects inhibitory neuronal systems and thereby causes an imbalance of various neuronal systems. Also, it has been reported that in vitro, stimulation of NGF synthesis/secretion by propentofylline shows a bell-shaped dose-response curve (Shinoda et al., 1990).

There are previous reports that improvement by propentofylline of behavioral deficits is associated with changes in choline acetyltransferase activity or muscarinic acetylcholine receptors in the hippocampus of rats with basal forebrain lesions or an infusion of anti-NGF antibody in the septum (Fuji et al., 1993a,b; Nitta et al., 1996).

In the β-amyloid protein-infused rats, the reduction of choline acetyltransferas activity in the hippocampus is rather slight, and not related to doses of β-amyloid protein infused into the cerebral ventricle (Nitta et al., 1994). Behavioral deficits in β-amyloid protein-infused rats, therefore, may not be directly related to the reduction of choline acetyltransferase activity. In fact, propentofylline did not prevent the reduction of choline acetyltransferas activity in the hippocampus although it improved behavioral deficits in the β-amyloid protein-infused rats. Since release of acetylcholine and dopamine evoked by nicotine or potassium depolarization in vivo is markedly impaired in the β-amyloid protein-infused rats (Itoh et al., 1996), failure of the stimulus-induced increase in acetylcholine and/or dopamine release may be a possible mechanism for the learning and memory deficits induced by β-amyloid protein. To clarify the mechanism of action of propentofylline, it is important to examine whether it improves acetylcholine and dopamine release in the β-amyloid protein-infused rats. Furthermore, it should be determined whether propentofylline increases NGF protein and its mRNA levels, in the brains of rats infused with β-amyloid protein into the cerebral ventricle.

Propentofylline is known to enhance some actions of adenosine as an uptake inhibitor, and to increase cerebral blood flow (Hadlicka et al., 1981; Wu et al., 1984; Fredholm and Lingström, 1986). Therefore, it is also possible that propentofylline ameliorates learning and memory impairments in the β-amyloid protein-infused rats by increasing cerebral blood flow or potentiating the action of adenosine. In this regard, we have previously reported that coadministration of theophylline, an adenosine receptor antagonist, with propentofylline failed to inhibit the improving effects of propentofylline on impairment of performance in the passive avoidance task in basal forebrain-lesioned rats (Fuji et al., 1993b). The use of a more specific adenosine receptor antagonist than theophylline would be more appropriate for investigating whether anti-amnesiac

effects of propentofylline in β-amyloid protein-infused rats are caused by the inhibition of adenosine uptake.

5. Conclusion

We have demonstrated that the repeated administration of propentofylline ameliorates learning and memory deficits in rats continuously infused with β-amyloid protein-(1-40) into the cerebral ventricle, the findings being consistent with the recently reported clinical effects for Alzheimer's disease. Based on these experimental and clinical effects, we believe that expanded clinical trials of propentofylline for the treatment of Alzheimer's disease are warranted. Further studies are necessary to clarify the mechanism of action of propentofylline.

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References

Banati, R.B., Gehrmann, J., Schubert, P., Kreutzberg, G.W., 1993. Cytotoxicity of microglia. Glia 7, 111–115.

Bartus, R.T., Dean, R.L., Beer, B., Lippa, A.S., 1982. The cholinergic hypothesis of geriatric memory dysfunction. Science 217, 408–417.

Fredholm, B.B., Lingström, K., 1986. The xanthine derivative 1-(5′-oxohexyl)-3-methyl-7-propyl xanthine (HWA 285) enhances the actions of adenosine. Acta Pharmacol. Toxicol. 58, 187–192.

Friden, P.M., Walus, L.R., Watson, P., Doctrow, S.R., Kozarich, J.W., Backman, C., Bergman, H., Hoffer, B., Bloom, F., Granholm, A.C., 1993. Blood–brain barrier penetration and in vivo activity of an NGF conjugate. Science 259, 373–377.

Fuji, K., Hiramatsu, M., Hayashi, S., Kameyama, T., Nabeshima, T., 1993a. Effects of propentofylline, a NGF synthesis stimulator, on alterations in muscarinic cholinergic receptors induced by basal forebrain lesion in rats. Neurosci. Lett. 150, 99–102.

Fuji, K., Hiramatsu, M., Kameyama, T., Nabeshima, T., 1993b. Effects of repeated administration of propentofylline on memory impairment produced by basal forebrain lesion in rats. Eur. J. Pharmacol. 236, 411–417.

Hadlicka, O., Kowarek, J., Wright, A.J.A., 1981. The effect of a xanthine derivative, 1-(5′-oxohexyl)-3-methyl-7-propylxanthine (HWA 285), on heart performance and regional blood flow in dogs and rabbits. Br. J. Pharmacol. 72, 723–730.

Hefti, F., Dravid, A., Hartikka, J., 1984. Chronic intraventricular injections of nerve growth factor elevate hippocampal choline acetyltransferase activity in adult rats with partial septo-hippocampal lesions. Brain Res. 293, 305–311.

Honegger, P., Lenoir, D., 1982. Nerve growth factor (NGF) stimulation of cholinergic telencephalic neurons in aggregating cell culture. Dev. Brain Res. 3, 229–238.

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.

Marcusson, J., Rother, M., Kittner, B., Rossner, M., Smith, R.J., Babic, T., Folnegovic-Smalc, V., Moller, H.J., Labs, K.H., 1997. A 12-month, randamized, placebo-controlled trial of propentofylline (HWA285) in patients with dementia according to DMS III-R. Dementia Geriatr. Cogn. Disord. 8, 320–328.

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

Mrsulja, B.B., Micic, D.V., Djuricic, B.M., 1983. Gerbil stroke model: an approach to the study of therapeutic aspects of postischemic brain edema. Adv. Biosci. 43, 45–62.

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

Nitta, A., Ogihara, Y., Onishi, J., Hasegawa, T., Furukawa, S., Nabeshima, T., 1996. Propentofylline prevents neuronal dysfunction induced by infusion of anti-nerve growth factor antibody into the rat septum. Eur. J. Pharmacol. 307, 1–6.

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.

Olson, L., Nordberg, A., von Holst, H., Bäckman, L., Ebendal, T., Alafuzoff, I., Amberla, K., Hartvig, P., Herlitz, A., Lilja, A., Lundqvist, H., Långstöm, B., Meyerson, B., Perssion, A., Viitanen, M., Winblad, B., Seiger, A., 1992. Nerve growth factor affects $^{11}C-nicotine$ binding, blood flow, EEG, and verbal episodic memory in an Alzheimer patient (case report). J. Neural Transm. Parkinson's Dis. Dementia Sect. 4, 79–95.

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

Platel, A., Porsolf, R.D., 1982. Habituation of exploratory activity in mice: a screening test for memory enhancing drugs. Psychopharmacology 78, 346–352.

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.

Sims, N.R., Bowen, D.M., Allen, S.J., Smith, C.C.T., Neary, D., Thomas, D.J., Davison, A.N., 1983. Presynaptic cholinergic dysfunction in patients with dementia. J. Neurochem. 40, 503–509.

Stefanovich, V., Nagata, K., 1983. Effects of propentofylline and cinepazide on cerebral energy metabolism in nitrogen breathing rats. IRCS Med. Sci. 11, 972.

Wu, P.H., Barraco, R.A., Phillis, J.W., 1984. Further studies on the

inhibition of adenosine uptake into rat brain synaptosomes by adenosine derivatives and methyl xanthines. Gen. Pharmacol. 15, 251–254.

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., Nitta, A., Hasegawa, T., Fuji, K., Hiramatsu, M., Kameyama, T., Furukawa, Y., Hayashi, K., Nabeshima, T., 1997. Orally active NGF synthesis stimulators: potential therapeutic agents in Alzheimer's disease. Behav. Brain Res. 83, 117–122.