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European Journal of Pharmacology 307 (1996) 1–6

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Propentofylline prevents neuronal dysfunction induced by infusion of anti-nerve growth factor antibody into the rat septum

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Received 5 October 1995; revised 6 February 1996; accepted 9 February 1996

Abstract

We have reported that the continuous infusion of anti-nerve growth factor (NGF) monoclonal antibody into the septum of rats produces neuronal dysfunction in the cholinergic system. Propentofylline has potent stimulatory effects on NGF synthesis/secretion in mouse astrocytes in vitro. To investigate the pharmacological effects of propentofylline, we used an animal model of dementia in which anti-NGF antibody was infused into the septum for 16 days via a mini-osmotic pump. The rats were treated with propentofylline orally once a day throughout the period during which performance in learning and memory tasks was observed. In the vehicle-treated dementia rats, learning and memory ability and choline acetyltransferase and cholinesterase activity were reduced compared to values in the control rats. The administration of propentofylline prevented the decreased learning capacity and the deficit in cholinergic marker enzyme activities. These results suggest that the use of NGF stimulators may provide a new approach to the treatment of dementia.

Keywords: NGF (nerve growth factor); Propentofylline; Learning; Memory; Choline acetyltransferase; Dementia model; (Rat)

1. Introduction

In Alzheimer's disease, learning and memory are impaired by the loss of neurons in the cholinergic neuronal system (Bartus et al., 1982; Sims et al., 1983). Several studies have shown that nerve growth factor (NGF) may be a trophic factor for the magnocellular cholinergic neurons of the basal forebrain (Hefti et al., 1984; Honegger and Lenoir, 1982). These neurons project from the septum-diagonal band and basal nucleus to the hippocampus and cerebral cortex, respectively. One case report has shown that the i.c.v. infusion of NGF in a patient with Alzheimer's disease resulted in an increase in nicotine binding in the frontal and temporal cortices, and in a persistent increase in cortical blood flow (Olson et al., 1992). This case report suggested that NGF counteracted the cholinergic deficits 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 a good therapeutic method. Further, NGF does not cross the blood-brain barrier, and it is readily metabolized by peptidases when administered peripherally. NGF itself, therefore, cannot be used for medical treatment, unless an appropriate drug delivery system can be developed (Friden et al., 1993).

It has been reported that propentofylline [3,7-dihydro-3-methyl-1-(5-oxohexyl)-7-propyl-1*H*-purine-2,6 dione], a xanthine derivative, stimulates NGF synthesis and secretion in quiescent astroglial cells (Shinoda et al., 1990). Since this molecule crosses the blood-brain barrier, it could be expected to stimulate NGF synthesis in the brain; indeed, we found that the drug ameliorated the reduced NGF content in the brains of aged rats (Nabeshima et al., 1993). Further, the drug ameliorated cognitive and muscarinic acetylcholine receptor dysfunction in rats with basal forebrain lesions (Fuji et al., 1993a,b).

We have described a new dementia model in rats induced by depleting endogenous NGF by immunological means; in this model, specific deficits are shown in cholinergic function (Nitta et al., 1993b). With this dementia model, we investigated whether propentofylline exerted its effects on cognitive dysfunction via the stimulation of NGF synthesis.

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PII S0014-2999(96)00133-1

2. Materials and methods

2.1. Animals and surgery

Male Kbl Wistar rats (Oriental Bioservice Co., Kyoto, Japan), weighing 280–320 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 a.m.) and had free access to food and water, except during the behavioral experiments.

Surgery was carried out as reported previously (Nitta et al., 1993b). Anti-NGF monoclonal antibody (10 μg/2 weeks per rat, Boehringer Mannheim, Germany) was infused continuously for 16 days via a cannula attached to a modified mini-osmotic pump filled with saline containing the antibody. Anti-digoxigenin monoclonal antibody was used as the control antibody, because this antibody was produced from the same clone as the anti-NGF antibody. The cannula was implanted into the septum (A 0.5, L 1.0, H −7.3) (Paxinos and Watson, 1986).

2.2. Drug administration and experimental design

Propentofylline (Nippon Hoechst, Tokyo, Japan), dissolved in distilled water, was administered orally for 19 consecutive days at doses of 10 and 25 mg/kg per day, (Fuji et al., 1993a,b; Nabeshima et al., 1993). One group consisted of nine to ten rats. Learning and memory capacity was measured by monitoring performance in two tasks: water maze and passive avoidance. The behavioral study was started 7 days after the surgery and the two tasks were carried out sequentially. After the behavioral studies were completed, the rats were killed and the brains were removed. Choline acetyltransferase and cholinesterase activity in the frontal cortex, parietal cortex and hippocampus was measured. 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. Water maze task

A circular water tank (140 cm in diameter and 45 cm high) was used (Morris, 1984). A transparent platform (10 cm in diameter and 25 cm high) was set inside the tank, and the tank was filled to a height of 27 cm with water. The temperature of the water was approximately 23°C, and 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 cues 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 session, a rat was placed 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 the 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 on 5 consecutive days, twice a day.

2.4. Step-through passive avoidance task

The experimental apparatus consisted of two compartments (25 × 15 × 15 cm high), one illuminated, and one dark, both equipped with a grid floor (Nitta et al., 1993a). The two compartments were separated by a guillotine door. In the acquisition trial, 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 (3.0 mA, 5 s) was delivered through the grid floor. In the retention test, given 24 h after the acquisition trial, 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.

2.5. Measurement of choline acetyltransferase and cholinesterase activity

Measurement of choline acetyltransferase and cholinesterase activity was carried out as reported previously (Ellman et al., 1961; Kaneda and Nagatsu, 1985; Nitta et al., 1993a).

2.6. Statistical analysis

The data from the water maze task were analyzed by a repeated-measure analysis of variance and Tukey's test.

In the passive avoidance task, data were expressed in terms of medians and interquartile ranges and were analyzed by the Kruskal-Wallis test followed by the two-tailed Mann-Whitney's U-test. Choline acetyltransferase and cholinesterase activities were analyzed by a one-way analysis of variance and Tukey's test. P values of < 0.05 were regarded as significant.

3. Results

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. 1. The latencies in the control group on the first training trial were not different from those of the anti-NGF antibody infusion

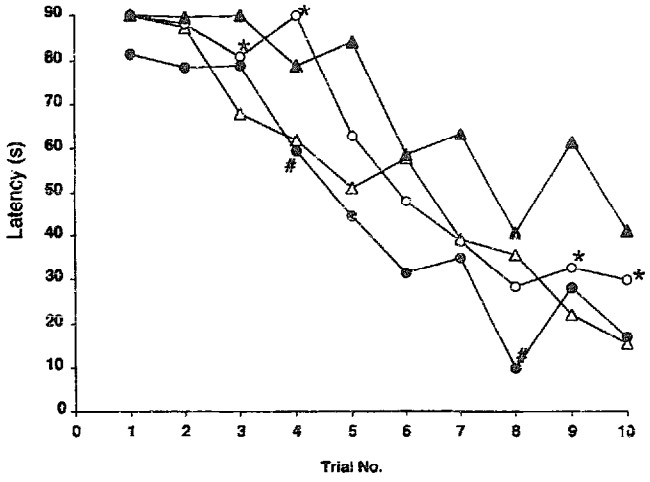

Fig. 1. Effects of propentofylline on the performance of the water maze task by rats that received a continuous infusion of anti-NGF antibody into the septum. (△) Control; (○) anti-NGF antibody + vehicle; (●) anti-NGF antibody + propentofylline (10 mg/kg); (▲) anti-NGF antibody + propentofylline (25 mg/kg); * $P < 0.05$ vs. control group, and # $P < 0.05$ vs. anti-NGF antibody-infused group.

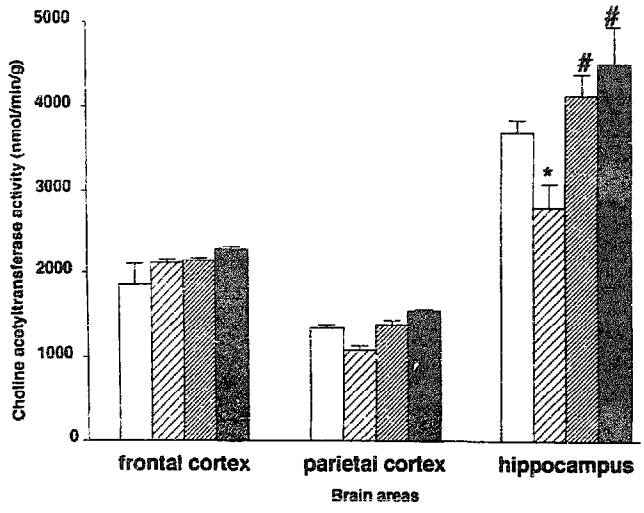

Fig. 3. Effects of propentofylline on choline acetyltransferase in th frontal cortex, parietal cortex and hippocampus of rats that received continuous infusion of anti-NGF antibody into the septum. Bar heigh are means ± S.E.M. Blank column, control; hatched column, anti-NG antibody + vehicle; black/white heavy-hatched column, anti-NGF ant body + propentofylline (10 mg/kg); white/black heavy-hatched colum anti-NGF antibody + propentofylline (25 mg/kg); * $P < 0.05$ vs. contr group. # $P < 0.05$ vs. anti-NGF antibody-infused group.

group. However, repeated training slowly shortened the latencies in the anti-NGF antibody infusion group, and rapidly shortened the latencies in the control group. Administration of propentofylline attenuated the anti-NGF antibody infusion-induced impairment of learning. In the 4th and 8th trials, the latencies of propentofylline (10 mg/kg)-treated rats were shorter than those of the vehicle-treated rats (Fig. 1).

As shown in Fig. 2, the control rats had a very long step-through latency during the retention test in the passive avoidance task. The anti-NGF antibody-infused rats had shorter step-through latency than the controls. Th propentofylline (10 mg/kg)-treated rats showed a signifi cantly longer step-through latency than the anti-NGF anti body-infused rats did.

As shown in Fig. 3, choline acetyltransferase activity i the hippocampus was reduced to 76% of that of the contr rats by the infusion of anti-NGF antibody. Treatment wit

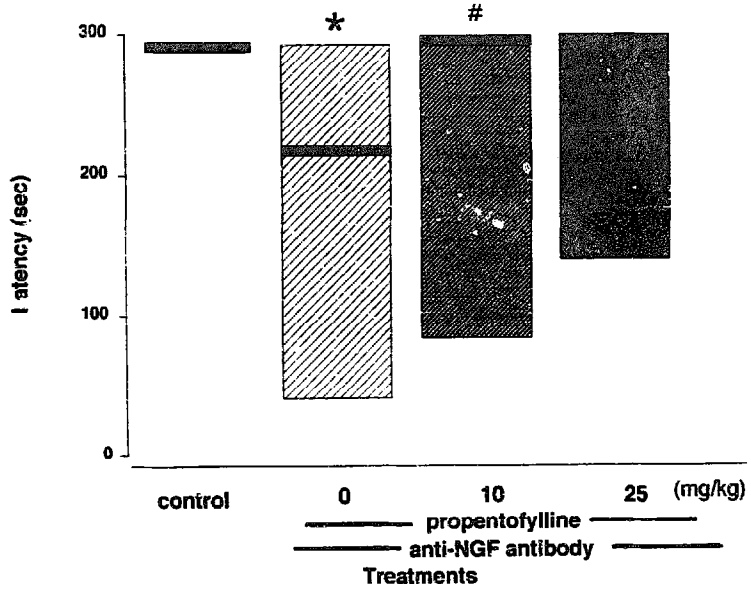

Fig. 2. Effects of propentofylline on the performance of the passive avoidance task by rats that received a continuous infusion of anti-NGF antibody int the septum. Horizontal bars show median values for step-through latency and vertical bars show the interquarile range. * $P < 0.05$ vs. control grou # $P < 0.05$ vs. anti-NGF antibody-infused group.

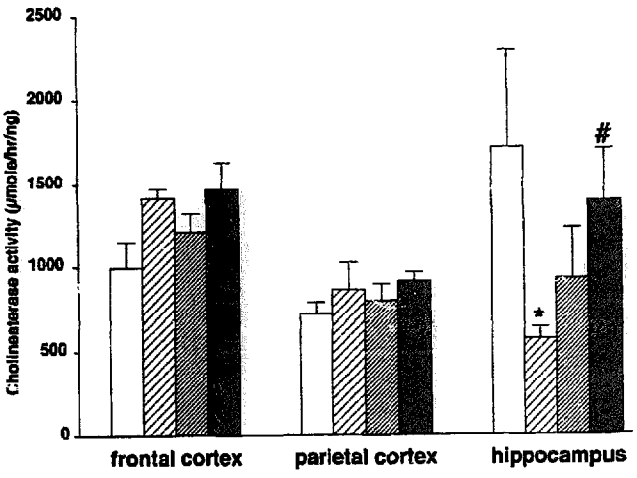

Fig. 4. Effects of propentofylline on cholinesterase activity in the frontal cortex, parietal cortex and hippocampus of rats that received a continuous infusion of anti-NGF antibody into the septum. Blank column, control; hatched column, anti-NGF antibody + vehicle; black/white heavy-hatched column, anti-NGF antibody + propentofylline (10 mg/kg); white/black heavy-hatched column, anti-NGF antibody + propentofylline (25 mg/kg): * $P < 0.05$ vs. control group. # $P < 0.05$ vs. anti-NGF antibody-infused group.

propentofylline (10 and 25 mg/kg) prevented the reduction in choline acetyltransferase activity in the hippocampus ($P < 0.05$). However, choline acetyltransferase activity in the frontal and parietal cortices was not changed by the infusion of anti-NGF antibody or treatment with propentofylline.

As shown in Fig. 4, cholinesterase activity in the hippocampus of rats infused with anti-NGF antibody was markedly reduced, to 33% of the control value. Treatment with propentofylline (25 mg/kg) prevented the reduction in cholinesterase activity in the hippocampus of rats infused with anti-NGF antibody. However, cholinesterase activity in the frontal and parietal cortices was not changed by infusion of anti-NGF antibody or treatment with propentofylline.

4. Discussion

The important finding in this study is that propentofylline, an agent that stimulates NGF synthesis in vitro, when administered orally, ameliorated the behavioral deficits and prevented the reduction in cholinergic activity induced in rats by the infusion of anti-NGF antibody into the septum.

In the present study, we used a rat model of a dementia prepared by infusing anti-NGF antibody into the septum; endogenous NGF was thus removed by immunological means. With this model, we were able to investigate whether the ameliorative effects of propentofylline on the impairment of learning and memory and on the dysfunction of the cholinergic system were related to the stimulation of NGF synthesis.

In the two behavioral tasks, propentofylline ameliorated the behavioral deficits in the rats infused with anti-NGF antibody. Furthermore, propentofylline prevented the severe damage observed in the cholinergic neuronal system in this model. These results suggest that the ameliorative effects of propentofylline on the impairment in learning and memory and on the deficits of cholinergic function in this dementia model may be related to the stimulatory effects of propentofylline on NGF synthesis. However direct evidence that the induction of NGF by propentofylline ameliorated the neuronal dysfunction in this model could not be obtained, because we measured NGF in the septum by using an enzyme immunoassay method, in which a monoclonal antibody was used (Nabeshima et al. 1993; Nitta et al., 1993c). Infused antibody interrupts the immune reaction in enzyme immunoassays. Therefore, we measured the NGF content in the frontal cortex, parietal cortex, striatum and hippocampus, which were non-infused areas. In these areas, the NGF content was not changed significantly among the control, anti-NGF antibody and propentofylline-treated groups (data not shown). An increase in the NGF content of the septum would be expected after the administration of propentofylline, since endogenous NGF was removed by immunological means. Hence, the use of the Northern blot technique or an in situ hybridization study would be more appropriate.

A recent study has shown that only acetylcholine is required for the amelioration of learning deficits (Winkler et al., 1995). We found that propentofylline dramatically prevented the reduced enzyme activity in the cholinergic neuronal system, indicating that the recovery of cholinergic neuronal system function may be an important factor in the propentofylline-induced amelioration of the impairment of learning and memory. It appears that NGF in the brain, induced by orally administered propentofylline, prevented the degeneration of cholinergic neurons that occurred as a result of the NGF deficiency induced by the infusion of anti-NGF antibody.

We have reported on the ameliorative effect of orally administered propentofylline on the impairment of learning and memory and on the dysfunction of muscarinic acetylcholine receptors in rats with basal forebrain lesions (Fuji et al., 1993a,b). However, it was not clear whether the effects of propentofylline on the impairment of learning and memory, and on the dysfunction of the cholinergic neuronal system, were based on its stimulatory effects on NGF synthesis/ secretion only, as propentofylline exhibits various pharmacological effects in the brain, e.g., preventing cerebral metabolic disorder during anoxia (Stefanovich and Nagata, 1983), improving cerebral edema (Mrsulja et al., 1983), and rescuing microglia from cytotoxicity (Banati et al., 1993).

The prevention by propentofylline of the reduction in the level of marker enzymes in cholinergic neurons was

dose-dependent; however, a low dose of propentofylline (10 mg/kg) was more effective than a high dose (25 mg/kg) in the behavioral experiments. The discrepancy between the effects of propentofylline on biochemical and behavioral parameters may be due to differences in the duration of administration. In the present experiment, we selected the doses of propentofylline on the basis of our previous studies (Fuji et al., 1993a,b; Nabeshima et al., 1993). In those papers and the present study, we used quite different types of dementia models such as basal forebrain-lesioned, aged and anti-NGF antibody-infused rats for the estimation of the pharmacological effects of propentofylline. In the basal forebrain-lesioned and aged rats, both 10 and 25 mg/kg of propentofylline were effective. However, in the present study, it seemed that the high dose (25 mg/kg) had either no effect or may have worsened the effect on learning and memory in the water maze and the passive avoidance task. The dose of 25 mg/kg of propentofylline might be an overdose for anti-NGF antibody-infused rats. The discrepancy would depend on the type of dementia model used in each study, since in vivo pharmacological effects can be influenced by the absorption, distribution, and metabolism of the drug, and these factors might be different among basal forebrain-lesioned, aged and anti-NGF antibody-infused rats. Another possible explanation for the bell-shaped dose-response curve is that a high dose of the drug may affect not only the cholinergic neuronal system, but also other neuronal systems and thereby cause an imbalance of various neuronal systems. Memory impairment may be produced by the imbalance of various neuronal systems, because learning and memory ability is supported by various neuronal systems in the brain.

As stated above, in one case report, it was found that NGF, delivered by catheter into the brain, had some ameliorative effects on Alzheimer's disease (Olson et al., 1992). This study suggested that neurotrophic factors such as NGF could be useful for the treatment of Alzheimer's disease; however, ethically, i.c.v. administration is not feasible in terms of the quality of life of the patient.

Our present study has shown that the peripheral administration of propentofylline, an NGF stimulator, prevented the reduction of cholinergic function in the NGF-depleted dementia model. We therefore believe that clinical trials of this drug for the treatment of Alzheimer's disease are warranted.

Acknowledgements

This work was supported, in part, by an SRF Grant for Biomedical Research, by a Grant-in-Aid for Science Research from the Ministry of Education, Science, and Culture of Japan (No. 07557009), by a grant for Gerontological Science Research from the Ministry of Health and Welfare of Japan (No. 91A-2406), and by a grant from th Uehara Memorial Foundation.

References

Banati, R.B., J. Gehrmann, P. Schubert and G.W. Kreutzberg, 199 Cytotoxicity of microglia, Glia 7, 111.

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

Ellman, G.L., K.D. Courtney, V.A. Andres, Jr. and R.M. Featherston 1961, A new and rapid colorimetric determination of acet cholinesterase activity, Biochem. Pharmacol. 7, 88.

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

Fuji, K, M. Hiramatsu, S. Hayashi, T. Kameyama and T. Nabeshim 1993a, Effects of propentofylline, a NGF synthesis stimulator, o alterations in muscarinic cholinergic receptors induced by basal for brain lesion in rats, Neurosci. Lett. 150, 99.

Fuji, K., M. Hiramatsu, T. Kameyama and T. Nabeshima, 1993b, Effec of repeated administration of propentofylline on memory impairmer produced by basal forebrain lesion in rats, Eur. J. Pharmacol. 23(411.

Hefti, F., A. Dravid and J. Hartikka, 1984, Chronic intraventricul injections of nerve growth factor elevate hippocampal choline acety transferase activity in adult rats with partial septohippocampal lesion Brain Res. 293, 305.

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

Kaneda, N. and T. Nagatsu, 1985, Highly sensitive assay for cholin acetyltransferase activity by high-performance liquid chromatograph with electrochemical detection, J. Chromatogr. 341, 23.

Morris, R.G.M., 1984, Development of water-maze procedure for study ing spatial learning in the rat, J. Neurosci. Meth. 11, 47.

Mrsulja, B.B., D.V. Micic and B.M. Djuricic, 1983, Gerbil stroke mode an approach to the study of therapeutic aspects of postischemic brai edema, Adv. Biosci. 43, 45.

Nabeshima, T., A. Nitta and T. Hasegawa, 1993, Impairment of learnin and memory and the accessory symptom in aged rats as senil dementia model (3): oral administration of propentofylline produce recovery of reduced NGF content in the brain of aged rats, Jpn. . Psychopharmacol. 13, 89.

Nitta, A., K. Hayashi, T. Hasegawa and T. Nabeshima, 1993a, Develo ment of plasticity of brain function with repeated trainings an passage of time after basal forebrain lesions in rats, J. Neural Transn [G-Sect] 93, 37.

Nitta, A., K. Murase, Y. Furukawa, K. Hayashi, T. Hasegawa and T Nabeshima, 1993b, Memory impairment and neural dysfunction aft continuous infusion of anti-nerve growth factor antibody into th septum in adult rats, Neuroscience 57, 495.

Nitta, A., K. Murase, Y. Furukawa, K. Hayashi, T. Hasegawa and T Nabeshima, 1993c, Effects of oral administration of a stimulator f nerve growth factor synthesis in basal forebrain-lesioned rats, Eur. . Pharmacol. 250, 23.

Olson, L., A. Nordberg, H. Von Holst, L. Bäckman, T. Ebendal, Alafuzoff, K. Amberla, P. Hartvig, A. Herlitz, A. Lilja, H. Lundqvis B. Långström, B. Meyerson, A. Persson, M. Viitanen, B. Winblad an A. Seiger, 1992, Nerve growth factor affects ^{11}C-nicotine bindin blood flow, EEG, and verbal episodic memory in an Alzheim patient (Case Report), J. Neural Transm. [P-D Sect] 4, 79.

Paxinos, G. and C. Watson, 1986, The Rat Brain in the Stereotaxi Coordinates (Academic Press, North Ryde).

Shinoda, I., Y. Furukawa and S. Furukawa, 1990, Stimulation of ner

growth factor synthesis/secretion by propentofylline in cultured mouse astroglial cells, Biochem. Pharmacol. 49, 1813.

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

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

Winkler, J., S.T. Suhr, F.H. Gage, L.J. Thal and L.J. Fisher, 1995, Essential role of neocortical acetylcholine in spatial memory, Nature 375, 484.