Effect of Intracerebroventricular Administration of Soybean Lecithin Transphosphatidylated Phosphatidylserine on Scopolamine-Induced Amnesic Mice

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Received December 2, 1999 Accepted July 6, 2000

ABSTRACT—The effect of intracerebroventricularly administered soybean lecithin transphosphatidylated phosphatidylserine (SB-tPS) on memory impairment was evaluated by a passive avoidance task. SB-tPS significantly prolonged the step-through latency induced by scopolamine treatment as in our previous report where SB-tPS was orally administered. The same doses of soybean phosphatidylcholine were ineffective. This result indicates that SB-tPS can act on the brain without any peripheral modification.

Keywords: Phosphatidylserine, Intracerebroventricular administration, Scopolamine-induced amnesia

Phosphatidylserine (PS) is a ubiquitously occurring phospholipid, especially rich in brain cells, and its effects on brain function have been studied (1). Therapeutic benefits of bovine cortex-derived PS (BC-PS) on senile dementia have been demonstrated by several double-blind, placebo-controlled studies (1, 2). However, in terms of safety, bovine cortex is not suitable for food use, because of the risk of contamination by prion that causes certain encephalopathies such as bovine spongiform encephalopathy.

Recently, soybean lecithin transphosphatidylated phosphatidylserine (SB-tPS), which was enzymatically prepared from soybean lecithin and L-serine by a phospholipase D reaction, was shown to recover drug-induced amnesia in rodents by intraperitoneal or oral administration (3, 4). In addition, the soybean derived PS, whose fatty acid composition should be almost the same as SB-tPS, improved cognitive disorder of senile subjects (5).

However, whether peripherally administered SB-tPS directly acts on the brain is still obscure, while BC-PS was reported to restore the memory impairment of aged rats by intracerebroventricular (i.c.v.) administration (6). Therefore, it is probable that molecular species of orally administered SB-tPS in part are converted into similar composition to BC-PS during intestinal absorption and subsequent peripheral circulation. That PS may stimulate secretion of some active compounds from peripheral organs is also possible. Actually, lysophosphatidylserine was reported to induce the histamine release from mast cells to in-

crease glucose concentration in blood and brain (7).

The present experiment was performed to elucidate whether the peripheral events are necessary for SB-tPS to recover memory impairment. To answer this question, the nootropic effect of i.c.v. administration of SB-tPS to scopolamine-induced amnesic mice was examined by a passive avoidance task. Phosphatidylcholine (PC), which was reported to improve senile dementia when given together with other cholinomimetics (8), was also applied.

ddY male mice at 5 weeks of age (Shizuoka Laboratory Animal Center, Hamamatsu) were used. SB-tPS was prepared as previously reported (3) with slight modifications. SB-tPS and soybean PC (SB-PC; Avanti Polar-Lipids, Inc., Alabaster, AL, USA) were emulsified in 0.05 M Tris-HCl buffer (pH 7.4) by sonication. Scopolamine bromide (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in saline.

The passive avoidance test was done according to Furushiro et al. (4) using a step-through type apparatus (Muromachi Kikai, Tokyo). Briefly, in an acquisition trial, a mouse was placed in an illuminated chamber, and when the mouse entered a dark chamber, a 2.0-mA electrical shock (shock scrambler SGS-001, Muromachi Kikai) was given from floor grids until the mouse ran back into the lighted compartment. Scopolamine (0.5 mg/kg) was intraperitoneally administered 30 min before the acquisition trial. SB-tPS or SB-PC was given intracerebroventricularly soon after the acquisition trial under light anesthesia with ether. Twenty-four hours after the acquisition trial, a retention trial was done and latency time (LT) to reenter the dark

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chamber was measured up to 300 s.

The data were analyzed by the Kruskal-Wallis analysis of variance by ranks. When statistical significance was detected, the post hoc comparison was done by using the non-parametric Bonferroni multiple comparison test (two-tailed).

As shown in Fig. 1, treatment with 20 μ g of SB-tPS significantly extended the LT ($P < 0.05$ vs vehicle), although a larger amount of SB-tPS (50 μ g) became less effective. This manner is similar to our previous result where oral administration of SB-tPS was effective in the scopolamine-induced amnesic mice (4). In contrast, any significant recoveries of the shortened LT were not observed by SB-PC treatment.

These results indicate that peripheral modification or signal transduction would not be necessary for SB-tPS to express the nootropic effect as well as BC-PS (6). Toffano et al. reported that 0.25% of radiolabelled BC-PS intravenously injected to mice was detected in the brain (9). If this ratio is adaptable to the previous report (4) where orally administered PS (240 mg/kg) restored scopolamine-induced amnesia, approximately 20 μ g PS is accessible to the brain. This amount corresponds to the effective dose in the present study. Therefore, it is likely that SB-tPS can function on the central nerve system (CNS) even if it reached to the brain without modification through the peripheral tissues such as the intestine or liver. However, the possible modification of the SB-tPS molecule in glial cells or neurons remains to be investigated. How intracerebroventricularly administered PS restores the scopolamine-induced amnesia can be partly explained as follows: PS can promote the acetylcholine release from aged-rat cerebral

cortex (10) or synaptosome preparations of young mouse brain (11), probably by affecting exocytotic processes such as Ca^{2+} uptake (12) and/or subsequent membrane fusion (13). Therefore, an increase of acetylcholine release may contribute to the restoration of the scopolamine-induced amnesia by PS.

It is also proposed that PS can improve the status of membrane potential or receptor functions by modulating membranous microenvironments. Actually, PS increased the synaptosomal membrane fluidity and the activities of membrane intrinsic proteins such as Na^+ , K^+ -ATPase (14).

Furthermore, an increase in brain glucose concentration by PS administration was reported (1, 3). Since glucose is a major energy source in brain, it is possible that PS ameliorates brain function through the improvement of glucose supply. According to Chang et al., the increase in brain glucose concentration with PS was mediated by histamine release from mast cells (7). However, mast cells are absent in brain tissue. Therefore, another mechanism would be responsible for the recovery of memory impairment by intracerebroventricularly administered PS. This consideration, however, needs further investigation because glucose level in brain was not determined in this experiment.

PC has been reported to improve senile dementia when administered together with other cholinomimetics (8), although the effects of PC itself were controversial (15). In our preliminary experiment, an oral administration of PC did not restore the scopolamine-induced amnesia at the effective dose of SB-tPS (unpublished data). The present result that the i.c.v. administration of SB-PC at the effective doses of SB-tPS failed to restore the scopolamine-induced amnesia suggests that different mechanisms to

Fig. 1. Latency time of SB-tPS or SB-PC treated mice in the retention trial of passive avoidance test. Each column and bar indicates the median and interquartile range, respectively. Numbers of mice are given above the columns. ** $P < 0.01$ vs Control, # $P < 0.05$ vs Vehicle (Bonferroni's test).

modify the brain function should exist rather than a pharmacokinetic difference between PS and PC. Although, larger doses of PC may be effective in this study because clinical effective doses were more than 30 times that of PS (8).

In this report, we demonstrated that the i.c.v. administration of SB-tPS restored the scopolamine-induced memory impairment in mice and proposed the hypothesis that SB-tPS can function on the CNS without any modification through the peripheral tissues. To confirm this idea, further investigations, such as studying PS metabolism in brain cells and hormone secretion by PS treatment, are needed.

Acknowledgments

We thank Dr. Satoshi Kudo and Dr. Masashi Sakai in our laboratory for their critical reading of the manuscript and Mr. Hideyuki Yamatoya for his useful advice.

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