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Research report

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine improves cognitive decline by enhancing long-term depression

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ABSTRACT

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPhtCho) (1 μM) enhanced long-term depression (LTD), a synaptic plasticity relevant to learning and memory, in the CA1 region of rat hippocampal slices, where expression of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunit GluR1 on the plasma membrane was decreased. In the water maze test, oral administration with POPhtCho (5 mg/kg) significantly shortened the prolonged retention latency for rats intraperitoneally injected with scopolamine (1 mg/kg), while the acquisition latency was not affected. For humans with mild cognitive impairment and dementia (average of Mini Mental State Examination score, 18), oral intake with POPhtCho (300 mg/day, once after breakfast) everyday raised the score to over 20, corresponding to normal cognitive functions, throughout 6 months after intake. The results of the present study, thus, indicate that POPhtCho could ameliorate cognitive disorders, possibly by enhancing LTD.

1. Introduction

Phosphatidylcholine, a component of the plasma membrane, is hydrolyzed into *cis*-unsaturated free fatty acid and lysophosphatidylcholine at the β position by phospholipase A_2 (PLA_2) [9]. Phosphatidylcholine, alternatively, is hydrolyzed into choline and phosphatidic acid at the γ position by phospholipase D (PLD), and phosphatidic acid is further hydrolyzed into *cis*-unsaturated free fatty acid and lysophosphatidic acid at the β position by PLA_2 [9]. Choline serves as a selective agonist of α7 acetylcholine (ACh) receptors [1] that are preferentially localized on presynaptic terminals and involve neurotransmitter release. In addition, choline produces ACh together with acetyl-CoA as catalyzed by choline acetyltransferase, to activate nicotinic and muscarinic ACh receptors. *cis*-Unsaturated free fatty acids such as arachidonic, linoleic, and linolenic acid induce a long-lasting facilitation of hippocampal synaptic transmission by targeting presynaptic nicotinic ACh receptors under the influence of protein kinase C (PKC) [5,6]. This, in the light of the fact that presynaptic nicotinic ACh receptors participate in the expression of long-term potentiation (LTP) [4,7], a cellular model of learning and memory, raises the possibility that *cis*-unsaturated free fatty acids enhance cognitive functions. Lysophosphatidic acid and lysophosphatidylcholine are also shown to potentiate nicotinic ACh receptor responses [3,8], suggesting a contribution to LTP expression. Phosphatidylcholine, thus, might serve as an enhancer of cognitive functions. In support of this note, we earlier found that 1,2-dilynoleoyl-*sn*-glycero-3-phosphocholine (DLPhtCho) improves scopolamine-induced learning and memory deficits by facilitating hippocampal synaptic transmission under the control of α7 ACh receptors [11]. The present study was conducted to assess the effect of the other phosphatidylcholine, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPhtCho), on cognitive functions.

We show here that POPhtCho improves scopolamine-induced impairment of spatial learning and memory for rats, possibly by enhancing long-term depression (LTD) in concert with decreased expression of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunit GluR1 on the plasma membrane and that the lipid ameliorates cognitive decline for humans.

2. Materials and methods

2.1. Animal care

All procedures have been approved by the Animal Care and Use Committee at Hyogo College of Medicine and were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Participants

The study for human was approved by Institutional Review Board at Hyogo College of Medicine and informed consent was obtained from all the participants.

2.3. Field excitatory postsynaptic potential (fEPSP) recording

The hippocampus was isolated from male Wistar rats (5–7 w) and hippocampal slices (400 μm in thickness) were prepared. fEPSPs were recorded from the CA1 area

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of hippocampal slices by electrically stimulating the Schaffer collateral at 0.033 Hz in artificial cerebrospinal fluid (ACSF) (in mM: 117 NaCl, 3.6 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 25 $NaHCO_3$ and 11.5 glucose) oxygenated with 95% O_2 and 5% CO_2 at 34 °C. LTD was induced by applying low frequency stimulation (LFS) (120 pulses at 2 Hz, five trains with an inter-train interval of 60 s) in the presence and absence of POPhtCho (Avanti Polar Lipids, Inc., Alabaster, AL, USA) (1 μM).

2.4. Distribution of GluR1 and GluR2 in rat hippocampal slices

Rat hippocampal slices were taken out 10 min before and 60 min after LFS, that induced LTD, in the absence and presence of POPhtCho (1 μM). Then, fractions of the CA1 area around an electrode to record fEPSPs were cut off from slices and homogenized in 100 μl of a lysate solution (210 mM mannitol, 70 mM sucrose, 1 mM EDTA, 10 mM HEPES, and 1% protease inhibitor cocktail, pH 7.5). Homogenates were centrifuged at 11,000 rpm for 15 min, and the supernatant was further super-centrifuged at 100,000 × *g* for 1 h. The pellet and the supernatant were used as a plasma membrane-enriched and cytosol-enriched component, respectively. Each sample (20 μg protein) was loaded on 10% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. After blocking with 0.1% Tween-20 containing 5% BSA, blotting membranes were reacted with an antibody against GluR1 (1:5000) (Upstate, Lake Placid, NY, USA) or GluR2 (1:800) (CHEMICON, Temecula, CA, USA) followed by an HRP-conjugated anti-rabbit IgG antibody (1:5000) or anti-mouse IgG antibody (1:5000), respectively. Immunoreactivity was detected with an ECL kit (GE Healthcare Biosciences, Piscataway, NJ, USA) and signal density was measured with an Image Gauge software (FUJIFILM, Tokyo, Japan).

2.5. Water maze test

For animal model of learning and memory deficits, male Wistar rats (7 w) were intraperitoneally injected with scopolamine (1 mg/kg). A circular plastic water tank with 180 cm in diameter and 45 cm in deep was used for a water maze test. The entire inside of the pool was painted black, and the pool was filled up to 25 cm from the bottom with muddy water containing India ink at 22 °C. A platform (11 cm in diameter) painted black was placed into water, the top sinking 1 cm below water surface. The pool was put in a test room, where there were several marks that rats were able to see from the pool. The position of the marks remained unchanged throughout testing. A platform was located in the constant position, i.e., in the middle of one quadrant, equidistant from the center and edge of the pool. Rats facing the wall of the pool were placed into water at one of the five positions selected at random, and time from start to escape onto the platform (acquisition latency) was measured. When succeeded, rats were allowed to stay on the platform for 10 s. POPhtCho dissolved with polyethylene glycol (PEG) (final volume, 0.1 ml) or PEG alone (final volume, 0.1 ml) was orally administered to rats everyday throughout experiments from 7 days prior to injection with scopolamine or saline. Water maze task was performed two trials per day, and the second trial began 2 min after the end of the first trial. Rats received the task for consecutive 8 days and the acquisition latency (time form the start to arrival onto the plate) was measured. Seven days later, the platform was removed and the retention latency (time from the start to arrival to the place where the platform had been set, 30 s in maximum) was measured.

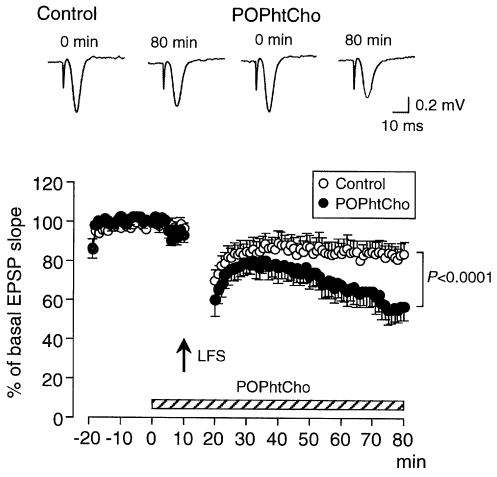

Fig. 1. POPhtCho enhances LTD. fEPSPs were monitored from the CA1 region of rat hippocampal slices, and LFS, to induce LTD, was applied to slices in the absence (control) and presence of POPhtCho (1 μM). In the graph, each point represents the mean (±SEM) percentage of basal EPSP slope (0 min) ($n = 8$).

2.6. Mini Mental State Examination (MMSE) test

MMSE test was performed on 67 subjects (31 males and 36 females) ranging in age from 59 to 93 years (average, 77.1 ± 0.8 years old), who had age-related cognitive disorders before and after oral intake with POPhtCho (300 mg/day, once after breakfast), that was extracted from egg lecithin, as a POPhtCho group and on 9 subjects (4 males and 5 females) ranging in age from 74 to 90 years (average, 79.4 ± 2.8 years old) without taking POPhtCho as a control group. Full marks are 30, and less than 20 corresponds to mild cognitive impairment and dementia.

2.7. Statistical analysis

Statistical analysis was carried out using Fisher's Protected Least Significant Difference (PLSD) test and *t*-test.

3. Results

3.1. POPhtCho enhances LTD

Our first attempt was to see the effect of POPhtCho on synaptic plasticity relevant to learning and memory. We have earlier

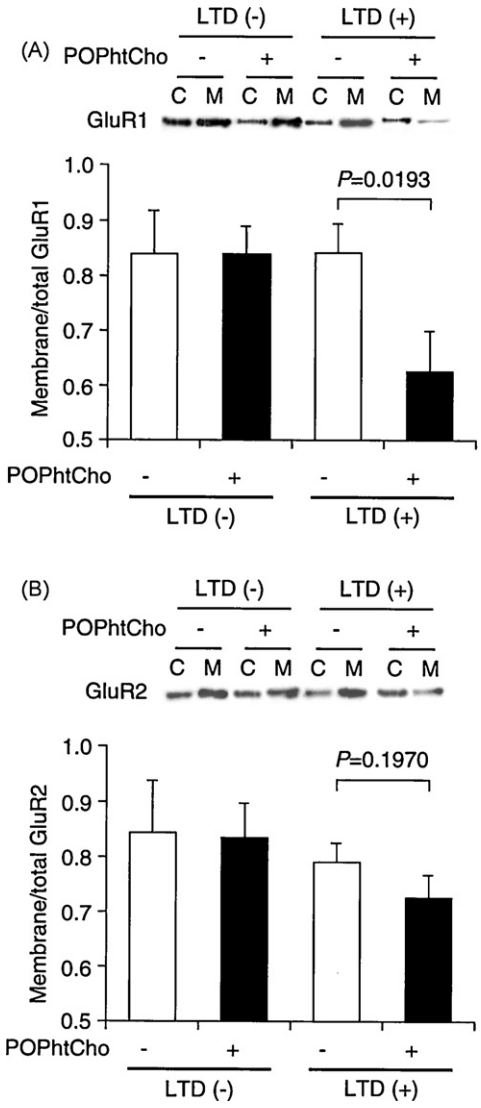

Fig. 2. POPhtCho reduces expression of AMPA receptors on the plasma membrane during LTD. Western blotting using an antibody against GluR1 (A) or GluR2 (B) was carried out in the plasma membrane and cytosolic component from rat hippocampal slices taken out 10 min before and 60 min after LFS, that induced LTD, in the absence and presence of POPhtCho (1 μM). In the graphs, each column represents the mean (±SEM) ratio of signal intensity for GluR1 or GluR2 in the plasma membrane component/signal intensity for each subunit in total cells ($n = 4$). *P* values, unpaired *t*-test.

confirmed that POPhtCho (1 μM) does not affect basal fEPSPs elicited from the CA1 region of rat hippocampal slices and Schaffer collateral-CA1 LTP [11]. In the present study, POPhtCho (1 μM) significantly enhanced LTD as compared with control LTD ($P < 0.0001$, Fisher's PLSD test) (Fig. 1).

3.2. POPhtCho decreases GluR1 expression on the plasma membrane during LTD

To understand the mechanism underlying POPhtCho-induced enhancement in LTD, we examined distribution of the AMPA receptor subunits GluR1 and GluR2 in rat hippocampal slices. POPhtCho (1 μM) had no effect on expression of GluR1 or GluR2 on the plasma membrane before LTD induction (Fig. 2A and B). POPhtCho (1 μM) significantly reduced GluR1 expression on the plasma membrane during LTD as compared with the expression in the absence of POPhtCho (Fig. 2A). Likewise, POPhtCho (1 μM) decreased GluR2 expression on the plasma membrane during LTD, although it was not significant (Fig. 2B). The reduced GluR1 expression on the plasma membrane during LTD could account for POPhtCho-induced enhancement in LTD.

3.3. POPhtCho improves scopolamine-induced impairment of spatial learning and memory for rats

We next examined the effect of POPhtCho on impairment of spatial learning and memory using water maze test. For control rats, oral administration with POPhtCho (1 mg/kg) had no effect on the acquisition and retention latency (Fig. 3A and B). Intraperitoneal injection with scopolamine markedly prolonged both the acquisition and retention latency (Fig. 3A–D), indicating scopolamine-induced impairment of learning and memory. No significant improvement against the prolonged acquisition and retention latency was obtained with oral administration with 1 mg/kg of POPhtCho (Fig. 3A and B). In contrast, oral administration with 5 mg/kg of POPhtCho significantly shortened the prolonged retention latency ($P = 0.0276$, unpaired t-test as compared with that for rats treated with scopolamine without POPhtCho) (Fig. 3D), while the prolonged acquisition latency was not affected (Fig. 3C). This implies that POPhtCho is capable of improving spatial learning and memory deficits induced by scopolamine.

3.4. POPhtCho ameliorates cognitive disorders for humans

We finally examined whether POPhtCho improves cognitive disorders for humans. Of 67 subjects for a POPhtCho group 43 ones belonged to mild cognitive impairment and dementia, and the average MMSE score before oral intake with POPhtCho was 18.1 ± 0.6 (Fig. 4). Oral intake with POPhtCho (300 mg/day, once after breakfast) everyday significantly raised MMSE scores, the average score reaching over 20, i.e., normal cognitive functions, throughout 6 months after intake (Fig. 4). In contrast, no increase in MMSE score was obtained with control subjects, who did not take POPhtCho, throughout 3 months (Fig. 4). These results indicate that POPhtCho is still capable of ameliorating cognitive disorders for humans.

4. Discussion

PhtCho is hydrolyzed into *cis*-unsaturated free fatty acid and lysophosphatidylcholine by PLA_2 or into choline and phosphatidic acid by PLD followed by further hydrolysis of phosphatidic acid into *cis*-unsaturated free fatty acid and lysophosphatidic acid

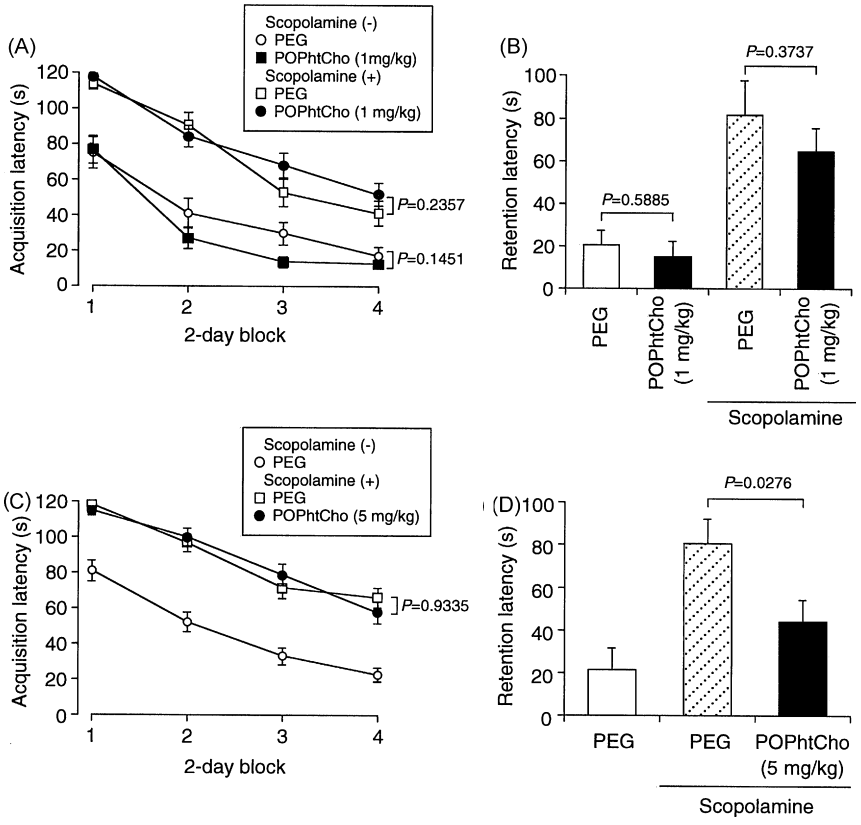

Fig. 3. POPhtCho improves scopolamine-induced spatial learning and memory deficits. Water maze task was performed two trials per day for consecutive 8 days and the acquisition latency was measured. Seven days later, the retention latency was measured. Rats were orally administered with PEG or POPhtCho (POPC) (1 and 5 mg/kg) everyday throughout experiments from 7 days prior to treatment with saline [scopolamine (−)] or scopolamine (1 mg/kg) [scopolamine (+)]. Saline or scopolamine was intraperitoneally injected 30 min prior to water maze task. (A and C) Each point represents the mean (±SEM) acquisition latency from consecutive 2 days ($n = 15$). P value, Fisher's PLSD test. (B and D) Each column represents the mean retention latency ($n = 15$). P value, unpaired t-test.

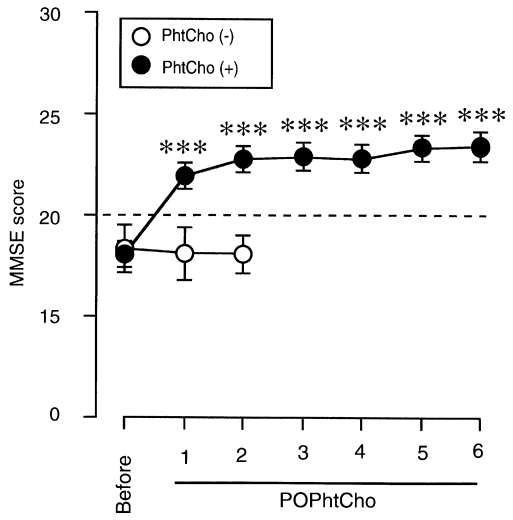

Fig. 4. POPhtCho ameliorates cognitive disorders. MMSE test was carried out once a month on 67 subjects before and after oral intake with POPhtCho (300 mg/day, once after breakfast) everyday [POPhtCho (+)] and 9 subjects without taking POPhtCho [POPhtCho (−)]. In the graph, each point represents the mean (±SEM) MMSE score at the periods as indicated. ***$P < 0.0001$, paired t-test.

by PLA_2. *cis*-Unsaturated free fatty acids and lysophosphatidylcholine induce a long-lasting facilitation of hippocampal synaptic transmission [5,6,10]. Choline, an agonist of α7 ACh receptors [1], and lysophosphatidic acid, that enhances activity of nicotinic ACh receptors [8], would facilitate hippocampal synaptic transmission, since presynaptic nicotinic ACh receptors are implicated in the expression of LTP [4,7].

In the present study, POPhtCho significantly enhanced hippocampal LTD, while it had no effect on basal hippocampal synaptic transmission and LTP [11]. This implies that the POPhtCho action on LTD is due to POPhtCho by itself, but not to its metabolites. Interestingly, POPhtCho significantly reduced expression of the AMPA receptor subunit GluR1, but not GluR2, on the plasma membrane during LTD in rat hippocampal slices. Overall, POPhtCho appears to enhance LTD by decreasing GluR1 expression on the plasma membrane. What signals underlie GluR1 trafficking under the control of POPhtCho during LTD, however, remains to be explored.

How LTD contributes to cognitive functions is not fully understood. A study suggests that hippocampal LTD is linked to spatial reversal learning [2], but it is presently unknown whether POPhtCho improves reversal learning impairment by enhancing LTD. In the water maze test, POPhtCho shortened the prolonged retention latency for rats treated with scopolamine, although the prolonged acquisition latency was not affected. This implies that POPhtCho improves scopolamine-induced learning and memory deficits, possibly by enhancing LTD. This also suggests that LTD is related to retention but not acquisition for spatial learning and memory. The most striking finding in the present study is that POPhtCho raised lowered MMSE scores, corresponding to mild cognitive impairment and dementia, to normal levels for humans. This represents that POPhtCho could serve as an enhancer of cognitive functions.

In conclusion, the results of the present study demonstrate that POPhtCho improves spatial learning and memory deficits for rats, possibly by enhancing LTD in association with decreased expression of the AMPA receptor subunit GluR1 on the plasma membrane, and that POPhtCho ameliorates cognitive disorders for humans. This provides evidence that POPhtCho exerts its beneficial action on cognitive functions.

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