Vitamin E stimulates phosphatidylserine synthesis in the hippocampus and improves cognitive function at old age

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Abstract

Aged humans and animals demonstrate cognitive decline. Improvement of short-term memory performance in aged beagles with a nutraceutical supplement containing phosphatidylserine (PS) and vitamin E has been demonstrated. The purpose of the present study was to examine if alpha-tocopherol acetate could alter PS metabolism in the hippocampus and to improve cognitive function of aged rats. Intragastric administration of alpha-tocopherol acetate to 24-month-old rats for 14 days resulted in increase of newly synthesized PS level in the hippocampus of experimental animals as compared with control rats. At the same time, there was a decrease in \[^{14}C\]\ phosphatidylcholine content in the hippocampus of experimental rats with respect to controls. The changes in phospholipids levels seen in the hippocampus of experimental animals due to alpha-tocopherol acetate administration were associated with increased number of active avoidances and decreased latent period of avoidances on acquisition of a conditioned reflex in a shuttle box. The data obtained in the work provide evidence that alpha-tocopherol acetate is a powerful modulator of phospholipids metabolism in the hippocampus and its function at old age.

Introduction

Phospholipids are not only important components of biological membranes but participate actively in cell signaling. Phosphatidylserine (PS) is the major acidic phospholipid of nerve cell membranes and plays an exclusively important role in supporting neural functions [1]. PS has been shown to participate in the regulation of acetylcholine, dopamine, and noradrenaline release in old age[2]. Its role in signal transduction became evident. PS acts as a binding site for the protein kinase C in the plasma membranes and as a regulator of diacylglycerol kinase, c-Raf-1 protein kinase , and nitric oxide synthase [1]. It is important, that protein kinase C is a critical component of memory and learning processes [3]. Moreover, PS promotes neuronal survival and facilitates serine threonine kinase Akt signaling. Significant changes of the asymmetrical positioning of PS in the plasma membranes of synaptosomes and the expression of proapoptotic proteins have been found in the brains of patients with Alzheimer’s disease and impaired cognitive functions [4].

The prominent decrease of PS content has been observed in the hippocampus of rats during aging [5]. The PS level decreased by 50% in the hippocampus of the 24-month-old animals and by 80% in the brain region of the 30–32-month-old animals as compared with the adults. Administration of exogenous PS to aged rats promotes the formation of synapses, dendrites, and surface receptors of nerve cells in different parts of the brain [6] and improves the cognitive functions of aged animals [7]. The dietetic fish oil mimics an exogenous PS action on PS content in the hippocampus of old animals [5]. Both, the n-3 polyunsaturated fatty acids (PUFA)-enriched diet and exogenous PS addition increased the PS level in the hippocampus of the 24-month-old rats. Increased PS concentration in the membrane initiates the interaction of the PH domain of the kinase Akt with the plasma membrane, facilitates translocation and phosphorylation of Akt and thus promotes cell survival [8]. Both the antiapoptotic effect of n-3 PUFA and Akt translocation are sensitive to n-3 PUFA-induced PS accumulation, strongly suggesting that the antiapoptotic effect of n-3 fatty acids depends on its ability to accumulate PS in neuronal membranes. The ability of dietetic fish oil to nullify the age-dependent disturbances of the PS turnover as well as hippocampus function suggests the critical role of PS in cognitive function decline during normal physiological aging."

Vitamin E is a well known singlet oxygen quencher and antioxidant, and a modulator of lipid metabolism. There are a number of potential mechanisms by means of which vitamin E supplementation can change levels of phospholipids in cell membranes. Vitamin E has been shown to influence on gene expression and modulate the activities of a number of enzymes involved in signal transduction, including
phospholipase A2 [9], and diacylglycerol kinase [10]. Recently it has been demonstrated that alpha-tocopherol and gamma-tocopherol regulate the sphingolipid metabolism in the liver and hippocampus of old rats [11, 12]. Alpha-tocopherol supplementation nullified the age-dependent disturbances of sphingomyelin (SM) turnover linked to the increased state of oxidative stress in the liver in old age[11]. Alpha-tocopherol, as well as gamma-tocopherol, targeting the neutral and acid sphingomyelinases (SMase) activities and key enzyme of sphingolipid synthesis de novo, serine palmitoyl transferase, normalized the content of pro-apoptotic and pro-inflammatory lipid ceramide in the aged isolated hepatocytes. Besides, alpha-tocopherol or N-acetylcysteine (NAC) administration to old rats prevented ceramide accumulation and increased SM levels in the hippocampus of 24-month-old rats [12]. The oxidative stress-induced ceramide accumulation in the keratinocytes, neurons, and astrocytes can be blocked by a pretreatment of cells with the vitamin E, too [13-16].

Taking into account that the close metabolic relationship between PS and sphingolipids exist[17] and the fact that PS administration to old rats significantly decreased the neutral SMase-dependent ceramide production in the hippocampus of 24-month old animals[5] and improved acquisition and retention of passive and active avoidance tasks in aged Wistar rats[18], in the present study the effects of alpha-tocopherol on PS content and cognitive function of old animals were investigated.

In the present paper a significant increase of the newly synthesized PS content has been observed in the hippocampus of alpha-tocopherol-treated 24-month-old rats. Alpha-tocopherol could ameliorate the age-dependent decline of cognitive function in the old animals. Manipulating of the PS level in the hippocampus of the aged animals with alpha-tocopherol may be a strategy for improvement learning and memory abilities.

Methods

Animals

Experiments on rats were carried out according to the International Principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985) and National General Ethical Principles for Experiments on Animals (Ukraine, 2001). In the experiments 24-month-old male Wistar rats (n = 20) weighing 450-480 g were used. The rats which had a free access to a standard chow diet and drinking water ad libitum were divided into two groups. Rats of vitamin E-treated group were fed with the alpha-tocopherol acetate (100 mg/100g body weight) intragastrically daily for 14 days. Control 24-month-old rats were fed with corn oil. Prior to experiment the animals were starved overnight. Their hippocampuses were used for lipid analysis as described below.

Behavioral Characteristics of Experimental Animals

CAAR induced by pain stimulation was studied in 24-month-old rats trained in a shuttle chamber with two sections and an electrified floor, as described in [7]. Electric bulb (30 W) switching on 5 sec before application of unconditioned electrical pain stimulation of the limbs (0.8 to 1 mA current was supplied via the electrode-equipped floor alternately in one section or another) served as a conditioned stimulus. Passages of the animals from one section of the chamber to another caused by light switching were interpreted as conditioned reflex phenomena when the latency of such locomotor reaction was shorter than 5 sec. The daily training session for each animal included 30 presentations of the conditioning signal with randomized intervals from 30 to 90sec. Training lasted until the rat demonstrated a clear realization of CAAR (nine passages in response to 10 presentations of the conditioning signals). We daily recorded the number of conditioned reflex avoidances and unconditioned reactions within the training session, measured the delays of these reflexes (sec), and also estimated the total duration of the learning period (until reaching the selected criterion of reproducibility).

Phospholipid synthesis

Tissue samples were incubated in Krebs–Ringer bicarbonate buffer (pH 7.4) with the addition of 0.1 % albumin for 90 min at 37°C in the presence of [14?]H3COONa (10 µCi/ml). After inclusion of the label, tissues were washed in bicarbonate buffer (pH 7.4) with 0.1% albumin and used for lipid extraction and separation as described below.

Extraction and Separation of Lipids

Extraction of lipids was performed according to the Bligh–Dyer technique [19]. For phospholipids
separation thin-layer chromatography was used in the systems of solvents consisting of chloroform, methanol, acetic acid, formic acid, and water 70:30:12:4:2 by volume). Phosphatidylcholine (PC), phosphatidylinositol (PI) and sphingomyelin (SM) spots were detected in iodine vapor. Phosphatidylserine (PS) and phosphatidylethanolamine (PE) spots were detected by treating chromatograms with 3% ninhydrin solution in water-saturated butanol and identified by comparison with standards Sigma, USA). Phospholipid contents were estimated by the Bartlett method [20]. The gel spots containing [14C] lipids were scraped and transferred to scintillation vials. The radioactivity of gel sports were measured using a BETA radioactivity counter.

Statistical analysis

Statistical analysis of the effect of alpha-tocopherol acetate on the amount of newly synthesized phospholipids in the hippocampus of old animals, was carried out by using the dispersion one (Newman–Keuls test). To estimate the reproduction of the active avoidance conditioned reflex in the shuttle chamber, the nonparametric Mann–Whitney test was used. The P < 0.05 was taken as the critical significance level. Numerical data were analyzed using STATISTICA 6.0 software.

Results and Discussion

Previous studies have demonstrated that hippocampal PS contents in 24-month-old animals decrease as compared with those in young adult rats [5]. However, our results demonstrated that administration of alpha-tocopherol acetate to aged rats leads to increase of the newly synthesized PS and decrease of PC contents in the hippocampus (Fig.1). At the same time, there were no changes in the contents of [14C]PE, [14C]PI, [14C]SM and total [14C]phospholipids in the hippocampus of experimental rats as compared with controls (165732±24377 and 160859±37894 cpm/g, respectively). Alpha-tocopherol acetate did not change the content of PC and increase of PS level in the hippocampus of 24-month-old rats. PC contents in the hippocampus of alpha-tocopherol acetate-treated and control animals were 70.9±16.4 and 60.1±8.25 nmol/mg protein, respectively. PS contents in the hippocampus of alpha-tocopherol acetate-treated and control rats were 40.4±4.79 and 59.9±1.87 nmol/mg protein (p< 0.05), respectively.

PS synthesis in rodent tissues occurs primary via base exchange pathway [21]. Serine incorporation into PS through the serine base exchange reaction takes place in microsomes and needs PC as substrate. Taking into account that alpha-tocopherol acetate increased the newly synthesized PS content and reduced the newly synthesized PC level, the activation of base exchange pathway of phospholipid turnover in the hippocampus of old animals could be supposed. However, vitamin E can induce the PC degradation [22]. Vitamin E supplementation influences on the phospholipase A2-dependent phospholipid turnover, increasing the contents of a variety of lysophosphatidylcholine species in human plasma. These data suggested that alpha-tocopherol acetate could decrease PC content in aged hippocampus not only due to stimulation of base exchange reaction and synthesis of PS, but via PC degradation, too.

PS could be converted to PE in the mitochondria due to phospholipid decarboxylation. PS decarboxylase activity has been determined in cerebral cortex, cerebellum, retina, hippocampus and other brain regions [23, 24]. Although this pathway of PS turnover exists in the hippocampus, the fact that alpha-tocopherol acetate did not change the newly synthesized contents of [14C]PE, the contribution of PS decarboxylation in phospholipid increase could be excluded.

It was well documented that oxidative stress is a common feature of different brain structures in aged mice and rats [25, 26]. Hippocampus and frontal cortex mitochondrial dysfunction and higher content of oxidation products of phospholipids and proteins has been determined in aged rats as compared with young ones [27]. Dietary supplementation with vitamin E restored the mitochondrial respiration as well as increase complexes I and IV and mitochondrial nitric oxide synthase activities up to the values obtained for 4-month-old rats. Vitamin E prevented the increases in oxidation products and decreases the hippocampus mitochondrial mass in synaptic areas. It is worth noting that vitamin E could prevent PS oxidation whereas other more abundant classes of phospholipids, such as PC and PE, remained resistant to treatments during anti-Fas-induced apoptosis in Jurkat T cells [28]. These data suggest that the alpha-tocopherol acetate in the hippocampus of old rats could, at least partly, increase PS level due to decreased lipid oxidation.

Administration of antioxidant, such as N-acetylcysteine (NAC), to 2-cyclohexene-1-one-treated rats prevented the glutathione depletion in striatum, hippocampus and...
frontal cortex tissues and disruption of the short-term spatial recognition memory in Y-maze test [29]. NAC may protect the brain of aged mice against glutathione depletion, and reverse age-induced deficits in hippocampal long-term potentiation [30] and Aβ-induced disturbances of learning and memory of animals [31]. Improvement of short-term memory performance with a nutraceutical supplement containing phosphatidylserine (PS) and vitamin E has been demonstrated in aged beagles [32].

In another set of experiments, cognitive functions of alpha-tocopherol acetate-treated and control 24-month-old rats were studied. The results obtained demonstrated that there were no differences in the rats’ spontaneous activity levels in the control and experimental groups during adaptation to the shuttle box. The numbers of spontaneous transfers in the box were 6.7 ± 0.78 and 6.2 ± 0.65 in the control and alpha-tocopherol acetate-treated groups, respectively. However, the number of combinations of stimuli needed to achieve the criterion of the conditioned active avoidance reflex in the group of rats receiving alpha-tocopherol acetate was significantly lower than that in control animals (82.00 ± 7.90 and 46.51 ± 5.45 (p < 0.05), respectively). The latent period of avoidance in experimental rats treated with alpha-tocopherol acetate decreased, while the number of active avoidances in the shuttle box increased on the second and third days of the experiment as compared with controls (Fig. 2, A, B).

Although the exact cause of age-dependent cognitive dysfunction is not known the impact of oxidative stress, neuroaxonal degeneration, and beta-amyloid has been identified. The diffuse loss of neurons and synapses in the neocortex, hippocampus and other subcortical regions of the brain at Alzheimer’s disease neuropathology [33] is associated with a ceramide accumulation [34, 35]. Ceramide can regulate both the amyloid precursor protein processing and beta-amyloid peptide generation [36]. SM turnover in the striatum, hippocampus and frontal cortex was more active in the aged rats than in the adult ones [5, 37]. The ceramide accumulation in the hippocampus and neocortex occurs in aging as a consequence of neutral SMase activation [5] and can be ameliorated with the antioxidants, NAC and alpha-tocopherol acetate [12]. In addition, neutral SMase could be inhibited with PS. PS addition to the culture media significantly decreased the daunorubicin-induced neutral SMase activity and accumulation of the ceramide due to the stimulation of PKC activity in the human monocytic leukemia cell lines U937 and HL-60 [38]. Based on these results, reasonable assumption can be made that the alpha-tocopherol acetate prevents ceramide accumulation in the hippocampus and cognitive dysfunction of old rats, at least in part, by the PS-induced SMase inhibition.

Conclusions

In summary, the data obtained demonstrated that alpha-tocopherol acetate is a powerful modulator of the phospholipid turnover in the hippocampus in old rats. The long-term treatment of old rats with the alpha-tocopherol acetate increased the reduced production of PS and decreased the content of newly synthesized PC in the hippocampus. This suggests that alpha-tocopherol acetate could restore the PS turnover in the hippocampus of old animals probably via stimulation of the base exchange reaction. However, other pathways of PS and PC turnover could be the targets of alpha-tocopherol acetate action in the hippocampus of old rats, too. The changes in phospholipids levels seen in the hippocampus of experimental animals due to alpha-tocopherol acetate administration were accompanied by increases in the number of active avoidances and decreases in the latent period of avoidances on acquisition of a conditioned reflex in a shuttle box. Taking into account an extremely important role of PS in brain functioning and results obtained in the present work it can be supposed that alpha-tocopherol acetate ameliorates the age-dependent decline of cognitive function due to increase of newly synthesized PS levels in the hippocampus.

References

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Illustrations

Illustration 1

Figure 1

Effect of alpha-tocopherol acetate on the phospholipids contents in the hippocampus of the 24-month-old rats. Here and in the Fig. 2 p < 0.05, alpha-tocopherol acetate-fed vs. control.
Illustration 2

Effects of alpha-tocopherol acetate on the number of active avoidances (A) and latent period (B) during acquisition of the conditioned active avoidance reflex in the 24-month-old rats