Review

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Choline-containing phospholipids: relevance to brain functional pathways

Abstract

Choline participates in several relevant neurochemical processes. It is the precursor and metabolite of acetylcholine (ACh), plays a role in single-carbon metabolism and is an essential component of different membrane phospholipids (PLs). PLs are structural components of cell membranes involved in intraneuronal signal transduction. This paper reviews the roles of choline and of choline-containing phospholipids (CCPLs) on brain metabolism in health and disease followed by an analysis of the effects of exogenously administered CCPLs on the brain, a topic extensively investigated by literature. Based on the observation of decreased cholinergic neurotransmission in brain disorders characterized by cognitive impairment, cholinergic precursor loading therapy with CCPLs was the first approach used to attempt for relieving the cognitive symptoms of Alzheimer's disease. This therapeutic strategy was discontinued due to the negative clinical results obtained with choline or lecithin. Negative results obtained with some compounds cannot be generalized for all CCPLs, as CDP-choline (citicoline) and to a greater extent choline alphoscerate (GPC) displayed interesting effects documented in preclinical studies and limited clinical trials. We provide evidence in favor of CDP-choline and GPC activity in cerebrovascular or neurodegenerative disorders characterized by cholinergic neurotransmission impairment. Based on the results of the controlled clinical trials available, we suggest that due to the lack of novel therapeutic strategies, safe compounds developed a long time ago such as effective CCPLs could have still a place in pharmacotherapy. Therefore selected compounds of this class should be further investigated by new appropriate clinical studies.

Keywords: brain; CDP-choline; choline; choline alphoscerate; cholinergic system.

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Introduction

Among the components of different membranes (i.e., cellular, mitochondrial, endoplasmatic reticules, Golgi apparatus, peroxiomes and lysosomes), phospholipids (PLs; i.e., phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and sphingomyelin) play an important role in cellular life.

The cellular membrane integrity has been extensively studied. Consequently, it is clear that the cellular life is completely dependent on its external and internal partitioning. Undernutrition during the first weeks of life is associated with holdback maturation of cerebral areas and the reduction of brain PLs in rats and humans [1, 2].

However, the effect of undernutrition on the mature brain is not clear. In adolescents, the eating disorder, anorexia nervosa, represents a cause of severe weight loss. In addition, anorectic patients develop brain atrophy, enlargement of the external cerebrospinal fluid spaces, and cognitive dysfunctions [3]. The cellular and molecular correlates of these changes are unknown. A consistent increase of the choline-containing compounds signal (so-called choline peak) by proton magnetic resonance (PMR) spectroscopy was observed in anorectic patients [4, 5]. It is not clear what the 'choline peak' denotes [6].

Choline was officially recognized as an essential nutrient by the Institute of Medicine in 1998 [7]. Its role in the body is complex. It is needed for the synthesis of the neurotransmitter acetylcholine (ACh), cell-membrane signaling PLs, lipid transport (lipoproteins), and methyl-group metabolism (plasmatic homocysteine reduction) [8]. It is the major dietary source of methyl groups via the synthesis of S-adenosylmethionine (AdoMet) [9]. At least 50 AdoMet-dependent reactions

have been identified in mammals [9]. These methylation reactions play major roles in the biosynthesis of lipids, regulation of several metabolic pathways, and detoxification in the body [9]. Choline is required for the biosynthesis of PLs, phosphatidylcholine (PC), lysophosphatidylcholine, choline plasmalogen, and sphingomyelin which are essential components for all membranes [10]. It plays important roles in brain and memory development in the fetus and reduces the risk of neural tube defects [11, 12]. Due to its wide-ranging role in human metabolism, choline deficiencies have an impact on diseases such as non-alcoholic fatty liver disease, atherosclerosis (via lipoprotein secretion), and possibly neurological disorders [13]. Therefore, getting adequate choline in the diet is important throughout life for optimal health.

Brain levels of free choline and ACh are very low (<30 nmol/g), and the large pools of them (>20 μmol/g) are invisible by nuclear magnetic resonance. The choline peak probably reflects tissue levels of phosphocholine (tissue level 200–300 nmol/g) and glycerophosphocholine (GPC; 500–600 nmol/g) [6, 14]. Free choline and choline metabolites were studied in rat brain [14]. Choline metabolism analysis in the central nervous system (CNS) represents a relevant issue because of the close interconnection of phospholipid metabolism, choline availability and the ACh synthesis [15–17]. However, the physiological relevance of the transfer of lipid-bound choline was controversial [18]. No or very sparse data have been reported on the possible transfer of phosphocholine or GPC through the blood-brain barrier (BBB) [19], although, some years ago, GPC was introduced as a probable and possible 'treatment' of age-associated dementia disorders [14].

Changes of phospholipid metabolites were detected in postmortem Alzheimer's disease (AD) brains [20] in an investigation aimed at assessing the hypothesis of brain cell membrane degeneration as a cause of AD pathophysiology [20]. Analysis included the evaluation in postmortem brain tissue from patients with AD and age-matched controls of: 1) levels of PLs; 2) their water-soluble metabolites; and 3) GPC choline-phosphodiesterase activity. Higher levels of the phospholipid catabolite GPC were noticeable in AD brains. In contrast, choline and ethanolamine levels were lower in AD, and phospholipid levels were slightly decreased. This study has documented an increase of membrane phospholipid catabolism in AD brains and a decrease of levels of initial phospholipid precursors [20]. These findings support the view that phospholipid turnover is elevated in neurodegenerative diseases [20].

Potential role of choline in AD or neurodegeneration

Crosstalk between phospholipases and amyloid during pathogenesis

A mutual interaction between amyloid formation and membrane phospholipid breakdown is suggested by several reports. Amyloid peptides are able to activate phospholipase A_2 in PCl2 cells [21]. Therefore, formation of amyloid peptide would be expected to accelerate membrane breakdown [22]. The role of calcium-dependent phospholipase A2 in phospholipid breakdown was investigated a long time ago [23, 24]. However, the cytosolic effect of different isoenzymes (isoforms) of PLA2 on AD was only recently reviewed [25]. Amyloid peptide increases choline conductance and choline fluxes in PCl2 cells. As free choline is more concentrated in the cytoplasm than in extracellular fluid (ca. 10-fold) [26], an increase of membrane permeability for choline would result in a loss of cellular choline counteracting any attempts of the cell for de novo-phospholipid synthesis [6]. Cellular membrane breakdown is a typical feature of neuronal degeneration in acute (stroke) and chronic (dementia) disorders [6].

Choline metabolism in cholinergic neurons: a pathogenetic factor?

In addition to its general role in neuronal cell loss, phospholipid breakdown may be involved in specific damage of central cholinergic neurons (e.g., as in AD). These neurons are unique in their requirement of choline which they use for synthesizing both PC and their neurotransmitter ACh. High affinity choline uptake (HACU) imports choline in cholinergic nerve ending for ACh synthesis while low affinity choline uptake (LACU) imports choline used for PC synthesis [26]. However, ACh and PC synthesis compete for free choline and PC synthesis is regulated by ACh concentration. The described phenomenon of PC breakdown during ACh synthesis has been termed 'autocannibalism' and it was suggested that it contributes to the central nervous neuron degradation in AD [27, 28].

These data provided the conceptual basis for proposing to treat neurodegenerative diseases such as AD with precursors of PLs. The working hypothesis was that an addition of choline could counter phospholipid breakdown normalizing not only PC but also

phosphatidylethanolamine and phosphatidylserine. These findings demonstrated that the supply of choline under certain conditions is not only rate-limiting for PC synthesis but also for the maintenance of other membrane PLs levels [27]. Therefore, the precursors ethanolamine and cytidine may also be rate-limiting for phospholipid biosynthesis under pathological conditions [29].

Choline effects on ACh

A direct role of choline on ACh release is controversial. In 1975, Cohen and Wurtman analyzed the effect of choline chloride in rats, reporting a dose-dependent increase in brain ACh concentrations [30]. A subsequent study demonstrated that choline treatment increased ACh release in rat brain slices [31]. In the same work it was shown that choline treatment decreased PLs content in striatal and cerebellar stimulated slices [31]. The results of this study are ambiguous because some treatments led to PLs increasing, whereas other (similar) treatments significantly decreased PLs [31]. The effect of choline introduced with diet or administered pharmacologically was investigated in rat hippocampus [32]. This study has shown that under stimulated conditions, hippocampal ACh release could be facilitated when the availability of choline for ACh synthesis was enhanced. Under certain conditions, significant effects of increased choline availability on ACh release can be revealed in the absence of an overall increase of extracellular choline [32].

Models of hypoxia-induced phospholipid breakdown were used to study the neuroprotective effects of compounds of natural origin (e.g., bilobalide). The breakdown of membrane PLs significantly contributes to hypoxia-induced neuronal degeneration and is accompanied by increases of free fatty acids following ischemia [33, 34] and electroconvulsive shock [33, 35]. The cascade effects of hypoxia/ischemia leads to hydrolysis of PC and a further breakdown of glycerophosphocholine. These changes increase free choline release [36]. The release of choline from tissues or cells is a sensitive indicator of an enhanced hydrolysis of PC and can be easily determined by chemiluminescence [37], HPLC [14] or radioactive labeling. In certain cells, choline release may reflect the activity of a specific receptor-activated enzyme catalyzing PC hydrolysis. This is the case of the release of free choline from HL60 cells evoked by the ADP-ribosylating factor (ARF) which is related to the activation of phospholipase D (PLD) [38]. These data collectively suggest that an increase of free choline does not always imply an ACh augmentation.

PC hydrolysis in cells of neural or glial origin activated by mAChRs

Several cholinergic muscarinic receptor agonists have been shown to elevate PLD activity in nervous tissue and glial cells [39]. In rat brain slices, PLD was activated by glutamate [40], noradrenaline [41], histamine and endothelin in a Ca^{2+}-dependent manner [42].

A muscarinic activation of hippocampal PLD could not be observed in mature slices [41–43], whereas high concentrations (1 mm) of carbachol stimulated PLD activity in hippocampal slices taken from 8-day-old rats. Similar data were also reported by other studies [44].

Synaptosomal PLD was investigated in synaptic terminals prepared from adult rat cortexes. In this preparation, basal PLD activity was present but did not respond to ACh, glutamate or aluminium fluoride [45], indicating that PLD activity of nerve terminals is not activated by agonists and not coupled to G proteins.

The muscarinic activation of PC hydrolysis has been investigated in different glial and neuronal cell culture systems. A muscarinic PLD activation has been described in human oligodendroglioma cells [46] as well as in 1321N1 astrocytoma cells [47]. In primary astrocytes from rat brain, Gustavsson et al. [48] have presented clear-cut data on muscarinic PLD activation whereas Bruner and Murphy [49] were unable to detect this effect. The reason for these inconsistencies is unclear. In cells of neural origin, muscarinic hydrolysis of PC has been described in human LA-N-2 neuroblastoma cells [50] and in SK-N human neuroblastoma cells [51]. In the latter case, PtdCho hydrolyses may have been due to a PtdCho-PLC activation. A muscarinic activation of PtdCho hydrolysis has also been described in adrenal chromaffin cells [52], but the mechanism of this effect is controversial [53]. Muscarinic activation of PLD in neuronal tissue is probably an exception rather than a rule. The same is true for non-neuronal tissues [54]. Hence, the general role of muscarinic activation of PLD in cellular activities should be reconsidered.

The role of the basal forebrain cholinergic system in cognitive processes is well-established, particularly in association with the functional decline accompanying normal as well as pathological aging (for a review see [55]). In a dated paper, the effect of a choline-containing phospholipid, GPC, on scopolamine-induced amnesia was investigated [56]. This study observed that treatment with GPC prevented impairment of acquisition of the passive avoidance response and disruption of consideration of previous acquired tasks induced by scopolamine [56]. Behavioral effects noticeable with the compound

under discussion are related to the marked increase of ACh production in some cognitively relevant brain area (e.g., hippocampus) it induces [56].

Choline involvement for optimal brain health: role of CCPLs

The pivotal role of choline in synthesis of the PLs PC, lysoPC, choline plasmalogen and sphingomyelin (fundamental component for membranes) is well-established [10, 57]. Dietary choline is also essential for memory development of a fetus [11, 12] and could decrease the risk of the development of neural tube defects [11, 12]. The importance of choline intake for different pathological and/or not pathological status as pregnancy and lactation, neural tube defects, memory development, heart disease, inflammation, and breast cancer was reviewed [58]. In the conclusion of this careful paper, authors suggested the necessity of increasing the awareness among health professionals and consumers of choline as an essential, but currently suboptimal, nutrient. They also highlighted the critical role it plays throughout life, especially for pregnant and lactating women.

National Health and Nutrition Examination Survey (NHANES) data indicate that for the majority of the population choline consumption is below current dietary recommendations (<300 mg for normal subjects and <200 mg for heart-patients). Increasing awareness of the pervasiveness of suboptimal choline intake must become the focus of public health efforts in order to promote optimal health. Education regarding the richest food sources of choline can assist in reaching this goal [58].

Supplementation of free choline in the diet increases brain choline availability, but it is not demonstrated that this increases ACh synthesis and/or release. Several therapeutic studies with choline and lecithin in AD and vascular

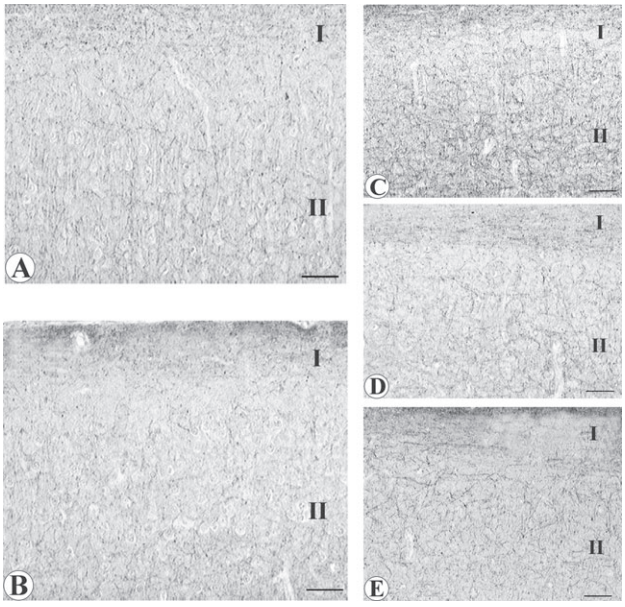

Figure 1 Sections of rat frontal cortex processed for neurofilament 200 kDa immunohistochemistry.
(A) Control normotensive Wistar Kyoto (WKY) rat; (B) control untreated spontaneously hypertensive rats (SHR); (C) SHR treated with galantamine; (D) SHR treated with choline alphoscerate; (E) SHR treated with galantamine and choline alphoscerate in association. Neurofilament 200 kDa immunoreactive axons in the neuropil of frontal cortex were remarkably decreased in SHR. The loss of neurofilament 200 kDa immunoreactive axons was countered by treatment with galantamine and galantamine plus choline alphoscerate. Choline alphoscerate alone was less active but was more active than galantamine by itself. Roman numerals indicate the cerebrocortical zones shown. Calibration bar: 50 μm. Adapted from [69].

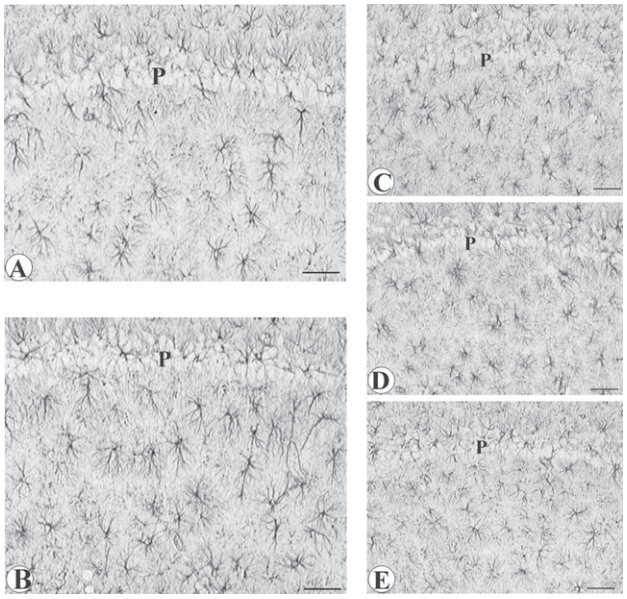

Figure 2 Sections of the CA1 subfield of hippocampus processed for glial fibrillary acidic protein (GFAP) immunohistochemistry to stain astrocytes.
(A) Control normotensive WKY rat; (B) control untreated SHR; (C) SHR treated with galantamine; (D) SHR treated with choline alphoscerate; (E) SHR treated with galantamine plus choline alphoscerate. Note in SHR the numerical and size increase of GFAP-immunoreactive astrocytes. This phenomenon was countered by treatment with choline alphoscerate and with galantamine plus choline alphoscerate but not with treatment with galantamine alone. P, pyramidal neurons. Calibration bar: 50 μm. Adapted from [70].

cognitive impairment demonstrated that even at high doses and over extended time periods, the results were clearly negative indicating that the supplemental choline cannot antagonize chronic cellular degeneration [56, 59–61].

In addition to choline, the administration of some choline precursors, CDP-choline and GPC (choline alphoscerate) has been tested as a possible therapeutic approach to treat neurodegenerative disorders. Both recent and relatively old studies evidenced the CDP-choline and GPC efficacy in several animal models of stroke and/or vascular cognitive impairment during not treated hypertensive disorders (Figures 1, 2 and 3) [56, 62–71].

Treatment with CDP-choline and GPC: pre-clinical and clinical data

The use of choline as ACh synthesis precursor is controversial. Several studies denied a precursor role of choline in the biosynthesis of ACh [56, 64], whereas others provided opposing results [72].

Dietary CDP-choline supplementation protects against development of memory deficit in aging rats [73]. Early-aged rats display a selective impairment in hippocampal-dependent long-term memory, and CDP-choline dietary supplementation may prevent this deficit [73]. Another paper published more recently by the same group reported that CDP-choline treatment had no memory-improving effect in enriched food (with CDP-choline) treated rats, nor did it prevent memory impairment caused by impoverished environmental conditions, in the case of treatments lasting less than the entire period of observation (3 months) [74]. Actually, these findings indicate that long-, but not short-term CDP-choline administration could improve memory impairment [75]. Hence, this time-dependent effect is probably mediated by membrane phosphatide synthesis that successively could lead to a memory enhancement.

After several years in which studies on these compounds were sparse, more recent investigations have re-evaluated them using different approaches [66, 67, 69–71]. These papers in general assessed the different effects of CCPLs on cerebral function and its preservation.

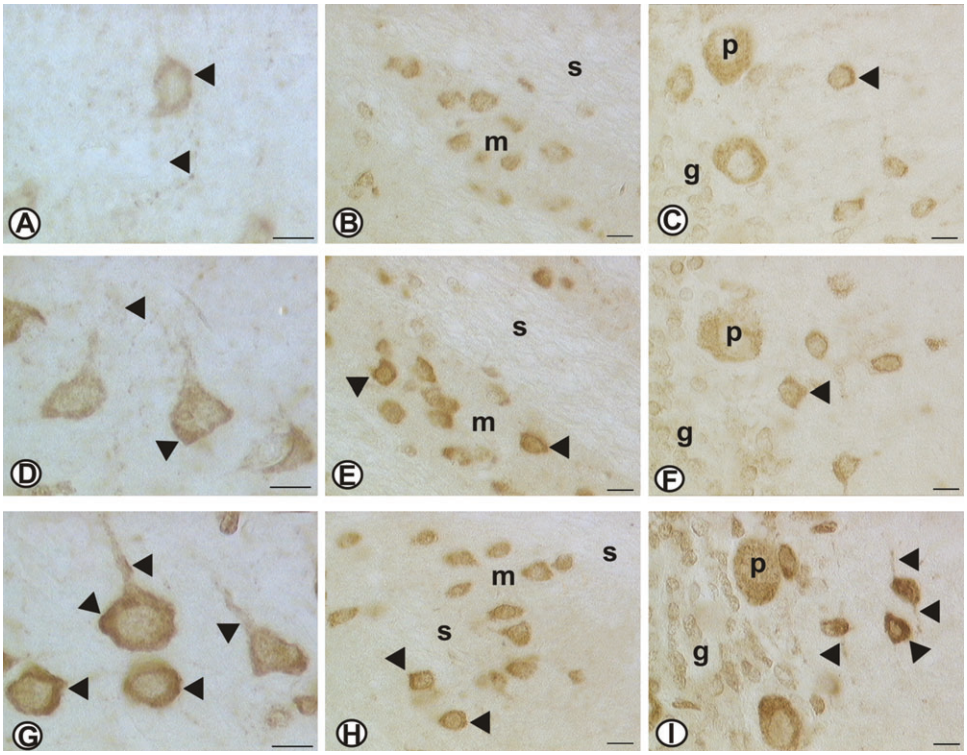

Figure 3 Micrographs of VAChT immunoreactivity in different cerebral areas investigated (A, D, G) frontal cortex; (B, E, H) striatum; (C, F, I) cerebellum.
Note varicosities (puntactes of immunostaining) surrounding cerebrocortical (A, D and G) and Purkinje neuron (C, F and I) cell bodies (arrowheads). In the striatum VAChT immunoreactive neurons were observed in the matrix. Treatment with CDP-choline (D, E, and F) and to a greater extent with choline alphoscerate (G, H and I) increased VAChT immunoreactivity compared to control rats (A, B and C). g, granular layer of cerebellar cortex; m, matrix; p, Purkinje neurons; s, striosome. Calibration bar: 10 μm. Adapted from [69].

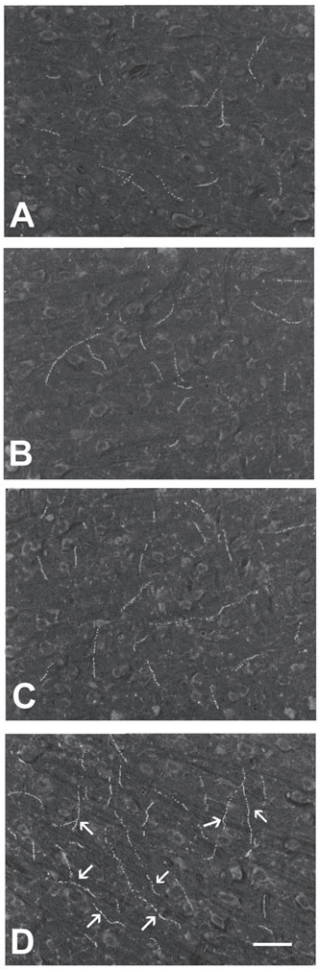

Figure 4 Dark-field sections of layer V of area 4 of frontal cortex. Acetylcholine immunohistochemistry.
(A) Control rat; (B) rat treated for 1 week with 150 mg/kg/day choline alphoscerate; (C) rat treated for 1 week with 2.5 mg/kg/day rivastigmine; (D) rat treated for 1 week with 150 mg/kg/day choline alphoscerate plus 2.5 mg/kg/day rivastigmine; (E) section next to that shown in A exposed to a pre-adsorbed acetylcholine antibody to verify the specificity of immune reaction. Note the localization of immune staining in nerve fiber-like structures and the increased intensity of immunostaining induced by treatment with choline alphoscerate or rivastigmine. A further increase of immunoreactivity was found in the rivastigmine plus choline-alphoscerate-treated rats (arrows). Calibration bar: 70 μm. Adapted from [66].

A choline-containing phospholipid recently revisited both in preclinical and clinical investigations is GPC. Treatment with GPC alone or in association with the cholinesterase inhibitor rivastigmine significantly increased ACh concentrations in different rat cerebral areas (cerebral cortex, hippocampus, and striatum) [66]. The results regarding cerebral cortex can be observed in Figure 4.

The neuroprotective effects of GPC were also demonstrated in different pre-clinical studies [67, 71]. However, CDP-choline and GPC ability to modify cholinergic transporters arrangement is demonstrated [70]. A recent paper of our group has shown an activity of GPC and CDP-choline treatment on cholinergic transporters in spontaneously hypertensive rats (SHR) as an animal model of vascular brain injury [71].

Recent clinical trials assessing the activity of CDP-choline and GPC are relatively sparse. However, both in the past and at present, some trials have investigated the clinical activity of these compounds in AD and vascular dementia (VaD) [76, 77]. A randomized dose-response trial of CDP-choline in acute ischemic stroke patient was published in 1997 [62]. The use of CDP-choline in cognitive decline (vascular or degenerative) improved some neuropsychological and/or cognitive tests such as Mini Mental State Examination (MMSE) and Alzheimer's Disease Assessment Scale-cognitive (ADAS-cog) [77]. In 1993, a multicenter study showed that GPC treatment improved most neuropsychological parameters in elderly patients with probable dementia [63]. Ten years later, the effect of GPC was studied in a multicenter, double-blind, randomized, placebo-controlled trial, patients affected by mild to moderate dementia of the Alzheimer's type [78]. A recent multicenter, double-blind, randomized, placebo-controlled trial 'Effect of association between a cholinesterase inhibitor and GPC on cognitive deficits in Alzheimer's disease associated with cerebrovascular injury' (ASCOMALVA) was designed to assess if the association of the cholinesterase inhibitor (ChE-I) donepezil with GPC has a more favorable clinical profile than monotherapy with donepezil alone. Donepezil plus GPC compared to donepezil alone improved the different items analyzed except the 'Basic Activities of Daily Living'. Interim data of ASCOMALVA suggest that the association of GPC with the standard treatment of a ChE-I may represent an option to prolong the beneficial effects of cholinergic therapies in AD with concomitant ischemic cerebrovascular injury [79].

The results of all of the clinical studies, although limited in terms of patients recruited are univocal in reporting that both GPC and CDP-choline using diverse mechanisms of action improve the cognitive abilities of patients affected by neurodegenerative diseases. Moreover, the two compounds did demonstrate good safety and tolerability [62, 78, 79].

Conclusions

CCPLs are compounds of clear interest in neuroscience and neurochemistry research and their use has clarified

several mechanisms of nerve cell membrane organization and activity. Their role in the biosynthesis of various neurotransmitters including ACh has been extensively demonstrated. The observation of a loss of the ACh biosynthetic enzyme choline acetyltransferase in the cerebral cortex of AD patients stimulated the development of cholinergic strategies to counter cognitive dysfunction typical of adult-onset dementia, including AD [64, 80–83]. Cholinergic precursor loading therapy was the first approach to attempt for relieving cognitive impairment in AD, although controlled clinical trials failed to show the relevant effects induced by choline or the choline-containing phospholipid, phosphatidylcholine (lecithin). The reasons for the lack of effect of this precursor strategy are unclear [64], but negative results obtained with choline or phosphatidylcholine [64, 84] cannot be generalized for all cholinergic precursors [68]. As discussed above CDP-choline and to a greater extent GPC displayed in preclinical studies and in clinical trials interesting effects worthy of being investigated further. In view of this and due to the lack of novel therapeutic strategies, safe compounds developed years ago such as CDP-choline and GPC could have still a place in pharmacotherapy and should be investigated by new clinical studies.

Conflict of interest statement

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