

Review Article

Vitamin K, an emerging nutrient in brain function

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Abstract.

Historically discovered for its role in blood coagulation, there is now convincing evidence that vitamin K has important actions in the nervous system. As a unique cofactor to the γ -glutamyl carboxylase enzyme, vitamin K contributes to the biological activation of proteins Gas6 and protein S, ligands for the receptor tyrosine kinases of the TAM family (Tyro3, Axl, and Mer). Functionally, Gas6 has been involved in a wide range of cellular processes that include cell growth, survival, and apoptosis. In brain, vitamin K also participates in the synthesis of sphingolipids, an important class of lipids present in high concentrations in brain cell membranes. In addition to their structural role,

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sphingolipids are now known to partake in important cellular events such as proliferation, differentiation, senescence and cell–cell interactions. In recent years, studies have linked alterations in sphingolipid metabolism to age-related cognitive decline and neurodegenerative diseases such as Alzheimer's disease (AD). Emerging data also point to unique actions of the K vitamers menaquinone-4 (MK-4) against oxidative stress and inflammation. Finally, there is now data to suggest that vitamin K has the potential to influence psychomotor behavior and cognition. This review presents an overview of what is known of the role of vitamin K in brain function.

Keywords: vitamin K, cognition

1. Introduction

A member of the lipid-soluble vitamins, vitamin K comprises a group of compounds that possess a common 2-methyl-1,4-naphthoquinone ring but differ in their structure at the 3-position. Vitamin K occurs naturally in two forms. Phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) or vitamin K₁ (K₁), is synthesized in plants and represents the main source of dietary vitamin K in the Western world [1]. The second form, the menaquinones or vitamin K₂ (VK₂), are of bacterial origin and form a family of vitamers with repeating unsaturated 5-carbon (prenyl) side chains at the 3 position. They are collectively referred to as menaquinone-n (MK-n) based on the number of prenyl units they contain, menaquinones 6–13 being the predominant forms in animal tissues [2]. One of the menaquinones, menaquinone-4 (MK-4) is not a common product of bacteria but is now known to be synthesized

from K₁ [3,4]. Very recently, it was established that the human UbiA prenyltransferase containing 1 (UBIAD1) enzyme is responsible for MK-4 biosynthesis [5]. The principal K vitamers are presented in Fig. 1.

Phylloquinone is present in high amounts in green leafy vegetables (*e.g.*, swiss chard, spinach, broccoli, cabbage, etc) and certain oils (*e.g.*, soybean, canola, olive) [6]. Menaquinones are not widely distributed in commonly consumed foods but can be found in animal-based foods (*e.g.*, chicken, meats) and some cheeses [7,8]. Current dietary recommendations for phylloquinone in Canada and the US are 120 and 90 $\mu\text{g day}^{-1}$ for adult males and females, respectively [1].

Reports published in the last 15 years have confirmed the presence of vitamin K in brain homogenates [9]. Interestingly, while in the majority of extrahepatic tissues VK is present in the forms of phylloquinone (K₁) and menaquinone-4 (MK-4), vitamin K in the brain occurs predominantly as MK-4. Specifically, it was recently established that MK-4 represents >98% of total vitamin K in brain in both 6-month [10] and 21-month-old rats [11]. In brain, MK-4 is present in highest concentrations in midbrain and pons medulla, and in lowest concentrations in cerebellum, olfactory bulb, thalamus, hippocampus, and striatum [10]. Menaquinone-4

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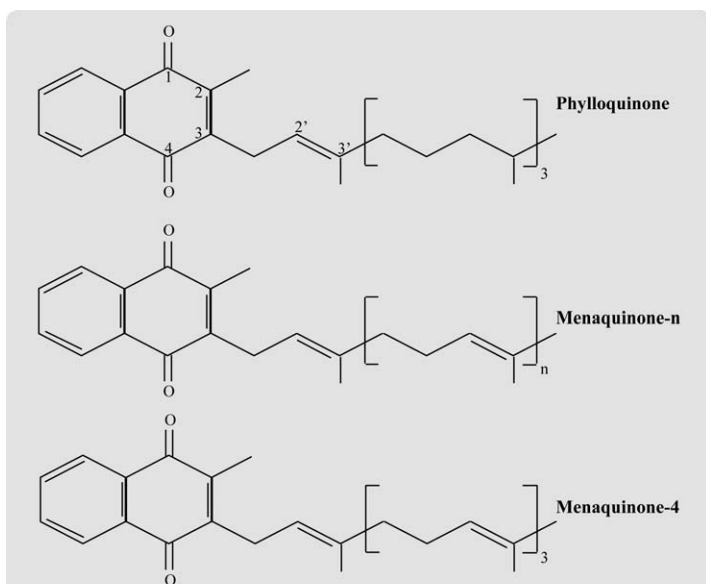


Fig. 1. Chemical structures of the principal K vitamers.

concentrations in brain have been shown to be higher in female than in male rats, to decrease as a function of age, and to increase with phylloquinone intake [10,12,13].

Physiologically, vitamin K is historically known for its role as a cofactor for an endoplasmic enzyme named γ -glutamyl carboxylase (GGCX) involved in the posttranslational synthesis of gamma-carboxyglutamic acid (Gla) from glutamic acid (Glu) residues contained in vitamin K precursor proteins. Vitamin K hydroquinone (KH_2) is the active coenzyme for the reaction that also requires carbon dioxide and oxygen. Although this amino acid modification first targeted proteins involved in blood coagulation, gamma-carboxyglutamic acid is common to all vitamin K-dependent proteins (VKDP) and increases their affinity for calcium [14]. In the course of the catalytic sequence, hydroquinone is oxidized to VK 2,3-epoxide (KO), which in turn is recycled to the quinone and hydroquinone forms by a vitamin K oxidoreductase (VKOR). Activity of VKOR is inhibited by 4-hydroxycoumarin derivatives such as warfarin. Collectively, these reactions make up the vitamin K cycle (Fig. 2).

2. The vitamin K-dependent proteins in brain

Two vitamin K-dependent proteins (VKDP) have been closely linked to the brain namely Gas6 and protein S. Although they have not been directly associated with cognition or cognitive impairment, their cell signaling actions in neurons (both Gas6 and protein S), the glia (Gas6) as well as antithrombotic activity (protein S) would have the potential to influence the underlying cognitive process.

Gas6 was discovered after it was found to be the product of the growth-arrest-specific gene 6 [15] while protein S is a well characterized vitamin K-dependent anticoagulant factor [16]. Both proteins are structurally related with 44%

amino acid homology [15]. Using biochemical and histological techniques, Prieto et al. [17] showed that Gas6 is widely expressed in the rat central nervous system (CNS), beginning at late embryonic stages with levels remaining high through adulthood. In the adult rats, Gas6 is expressed in cerebral and piriform cortex, hippocampus (areas CA1, CA3, and the dentate gyrus), thalamic and hypothalamic structures, midbrain, and in motor and trigeminal nuclei. It is also present in the cerebellum where it is present at high levels in the Purkinje neurons and deep cerebellar nuclei. Of interest, when investigated in rat synaptosomes from striatum, hippocampus and frontal cortex, Gas6 was found to decline with age in a tissue-specific manner. The observed decline was most dramatic in the frontal cortex, levels in 24-month-old rats being >84% lower than those aged 6 months, while in striatum and hippocampus, the age-associated decline was of the order of 55% [18]. In contrast to Gas6, protein S is much less present in the brain with reported expression in the locus coeruleus and choroid plexus [17], and astrocytes [19].

Functionally, both Gas6 and protein S are ligands for the receptors tyrosine kinases of the TAM family (Tyro3, Axl, and Mer) which they activate by inducing their phosphorylation [20]. In the nervous system, Gas6 has been involved in functions such as cell survival, chemotaxis, mitogenesis, cell growth, and myelination. Specifically, Gas6 has been shown to prevent apoptosis of gonadotropin-releasing hormone (GnRH) neurons via the recruitment of the phosphatidylinositol 3-kinase (PI3-K) signaling pathway and subsequent stimulation of the extracellular signal-regulated (ERK) and the serine-threonine (Akt) kinases [21]. Regarding this population of neurons, Gas6 contributes to their migration from the

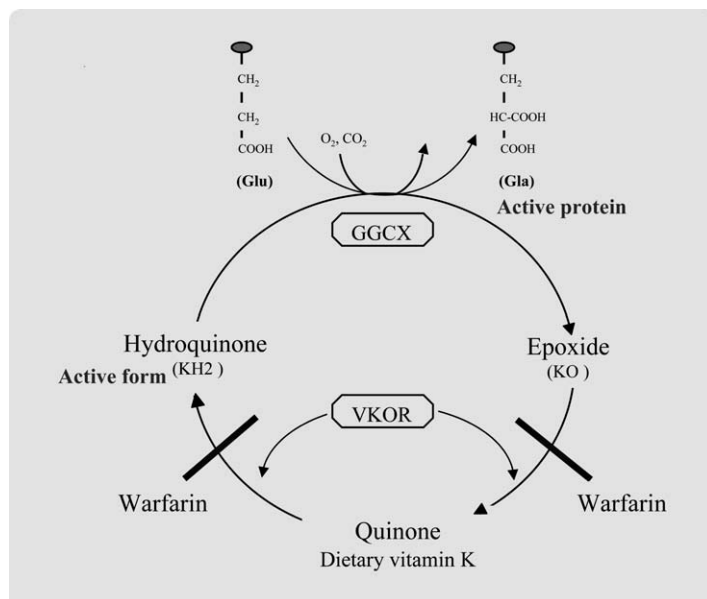


Fig. 2. A simplified depiction of the vitamin K cycle. GGCX refers to the γ -glutamyl carboxylase and VKOR to the vitamin K oxidoreductase enzymes, respectively. Glu refers to glutamic acid and Gla to gamma-carboxyglutamic acid.

olfactory bulb to the hypothalamus an essential step in their function [22]. A prosurvival effect for Gas6 has also been observed for hippocampal neurons [23], an action that involves the activation of the mitogen-activated protein kinase (MAPK) and PI3-K signaling pathways and their downstream effectors. Using primary cultures of cortical and hippocampal neurons, Prieto et al. [24] provided evidence that the Gas6-mediated activation of MAPK results in the recruitment of ERK, RSK90 (ribosomal protein S6 kinase), and CREB (cAMP response element-binding protein). Similarly, the Gas6 activation of PI3-K leads to the phosphorylation of Akt and the subsequent recruitment of mTOR (mammalian target of rapamycin) and P70S6K (P70S6 kinase). Of pertinence to the pathology of Alzheimer's disease (AD), Gas6 has been shown to rescue cortical neurons from amyloid β protein ($A\beta$)-induced apoptosis. A hallmark of AD [25], $A\beta$ induces Ca^{2+} influx via L-type voltage-dependent calcium channels, a feature that leads to its neurotoxicity. Addition of Gas6 to primary cultures of rat cortical neurons prevented cell apoptosis by inhibiting Ca^{2+} influx and ameliorated $A\beta$ -induced apoptotic features namely condensation of chromatin and fragmentation of DNA [26].

In addition to its signaling actions in neurons, Gas6 modulates survival and functions of the glia and microglia. Specifically, Gas6 has been shown to promote the survival of human oligodendrocytes *in vitro* and to protect them from tumor necrosis factor alpha (TNF α)-induced apoptosis through activation of the Axl receptor and the PI3-K/Akt signaling pathway [27,28]. In a recent study of Cuprizone-induced demyelination, the absence of Gas6 signaling (Gas6⁻¹⁻¹ mice) was associated with decreased oligodendrocytes survival, greater cell loss, and a reduction in overall myelination. In a subsequent study, Gas6 was found to suppress the microglial phenotype following a lipopolysaccharide (LPS) challenge, suggesting an antiinflammatory role for Gas6 [29]. These results are in line with those of Grommes et al. [30] who using a murine microglia cell line, observed reduced expression of the proinflammatory mediators nitric oxide synthase (iNOS) and interleukin-1 β (IL-1 β) in Gas6-treated cells following a LPS challenge. Importantly, two independent reports recently provided evidence for a modulatory role of Gas6 in remyelination. In the report by Binder et al. [31], Gas6 was shown to stimulate myelin synthesis by oligodendrocytes *in vitro* while the absence of Gas6 as assessed in knockout mice (Gas6^{-/-}) resulted in a delay in remyelination in the recovery phase of cuprizone treatment. In a separate report, direct administration of Gas6 in the brains of cuprizone-treated C57B16J mice resulted in increased maturation of oligodendrocyte progenitor cells and enhanced remyelination in the 2 weeks following cessation of treatment [32].

Although its actions in brain have been less investigated and are not as well characterized, protein S has been shown to offer neuronal protection during ischemic/hypoxic injury, through its antithrombotic functions and TAM-related signaling actions. In a murine *in vivo* model of stroke, protein S was found to significantly reduce brain infarction and edema volumes and to improve postischemic cerebral blood

flow in treated animals. Protein S treatment was also associated with less fibrin deposition and infiltration with neutrophils, and fewer apoptotic neurons, an effect also observed in cultured neurons. These effects of protein S at the cellular level resulted in improved motor performance of the protein S-treated animals [33]. Recently, the team further showed that protein S protected neurons from NMDA-induced toxicity and apoptosis through the Tyro3-PI3-K-Akt pathway [34].

In sum, this brief review clearly points to important roles for Gas6 and protein S in the central nervous system.

3. Vitamin K and sphingolipid metabolism

Years prior to the discovery of Gas6 and protein S, a role for vitamin K had been demonstrated in sphingolipid metabolism. Sphingolipids are a group of complex lipids present in all mammalian cells where they are major components of cell membranes. They are present in particularly high concentrations in cells of the central nervous systems with the major sphingolipids consisting in ceramide, sphingomyelin, cerebroside, sulfatide, and ganglioside [35]. Initially appreciated for their structural role, sphingolipids are now viewed as key players of important cellular events such as proliferation, differentiation, senescence, cell-cell interaction, and transformation [36]. Furthermore, research conducted in recent years have linked alterations in sphingolipid metabolism to the aging process [37] and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases [38,39].

A role for vitamin K in sphingolipids was initially demonstrated in bacteria, where it was found to be essential to cell membrane structure [40,41]. The action of vitamin K resided in its induction of 3-ketodihydrosphingosine (3-KDS) synthase, the enzyme involved in the initial step of sphingolipid biosynthesis [42]. A role for vitamin K in sphingolipid metabolism was then confirmed in rodents with a report that warfarin treatment was associated with decreased 3-KDS synthase activity and significant reductions in brain sulfatides (42%), sphingomyelin (17%) and cerebroside (12%) [43]. In a subsequent study, warfarin also associated with decreased sulfotransferase activity, the enzyme responsible for sulfatide synthesis [44]. Interestingly, most of the observed warfarin-induced changes could be reversed by subsequent administration of vitamin K [43,44]. In a report that followed, the team provided evidence that sulfatide synthesis could also be modulated by vitamin K intake. Rats administered a vitamin K deficient diet resulted in a significant decrease in brain sulfatides while feeding them with an excess of vitamin K for 1-2 weeks lead to increased sulfotransferase activity and brain sulfatide concentrations. The vitamin K response was observed with either phyloquinone or MK-4 as the source of the vitamin [45].

More recently, our group reported that in brain tissues of 6-month-old rats having been fed a low (L: 80 $\mu\text{g kg}^{-1}$ diet), adequate (A: 500 $\mu\text{g kg}^{-1}$ diet) or high (H: 2,000 $\mu\text{g kg}^{-1}$ diet) phyloquinone containing diet since weaning, MK-4 was positively correlated to sulfatides and sphingomyelin,

and negatively correlated to gangliosides, the strength of the relationships decreasing as a function of phylloquinone intake. Positive correlations between MK-4 and sulfatide concentrations were also recently observed in the hippocampus and cortex of 12- and 24-month-old Fisher 344 male rats [46]. In our study, vitamin K intake did not influence sphingolipid concentrations in the brains at 6 month of age, a finding that may have been due to the duration of diet exposure [10]. However, when we investigated the animals later in their lives (20 months), rats which had consumed the low phylloquinone diet had higher concentrations of ceramides in their hippocampus (a regions associated with learning and memory) and lower concentrations of gangliosides in their pons medulla and midbrain, compared to the other two groups. Interestingly and as discussed in a future section, this sphingolipid profile was associated with cognitive impairment [11]. The observed higher levels of ceramides in the hippocampus of the rats fed the L diet is of particular interest as ceramides have been shown to mediate processes such as differentiation, growth arrest, apoptosis, and senescence [36]. When present in high concentrations, ceramides have notably been involved in inflammatory processes [47] and in the generation of reactive oxygen species from mitochondria [38]. They have also been shown to inhibit the neuronal survival pathway regulated by PI3-K/Akt [48] and activate the caspase-9/caspase-3 pathway [49,50]. Finally, numerous studies have reported elevated levels of ceramides in neurodegenerative disease such as Alzheimer's disease [51]. Likewise, the fact that vitamin K intake could modulate ganglioside and sulfatide concentrations in pons medulla and midbrain of old animals is physiologically compelling. Gangliosides are abundant on neuronal cell surfaces and are actively involved in the maintenance and repair of the nervous tissues [52] while sulfatides partake in oligodendrocyte differentiation and regulate the myelination process [38]. Furthermore, both gangliosides and sulfatides possess signaling functions [39]. In a recent report, sulfatides and the ganglioside subtype GM1 were implicated in the modulation of uptake of the neuronal mediator dopamine by striatal neurons [53].

In light of the cellular actions of sphingolipids, the K vitamers have the potential to influence brain homeostasis through yet another mechanism.

4. Other actions of vitamin K

Recent studies suggest that the K vitamers, notably MK-4, have specific actions independent of their role in protein carboxylation and sphingolipid metabolism. Both *in vitro* and animal studies point to protective roles for MK-4 against oxidative stress and inflammatory processes, two phenomenon associated with age-associated neurodegenerative diseases.

In a study by Tsang et al. [54], both phylloquinone and MK-4 were shown to promote neurite outgrowth on PC12D cells in the presence of nerve growth factor, an action that proved mediated by the protein kinase A and MAPK signaling pathways. These findings concord with results from an

older study in which the two K vitamers were shown to have survival-promoting effects on different neuronal cell types (cortex, hippocampus, and striatum) in the later stages of embryogenesis [55]. In these two reports, the action of the K vitamers was not linked to any of the VKDPs.

Recently, MK-4 and to a lesser extent K₁, were shown to prevent glutathione depletion-mediated oxidative injury as defined by free radical accumulation and cell death in primary culture of oligodendrocyte precursors and immature fetal cortical neurons [56]. Treating the cell cultures with warfarin had no effect on the protective role of MK-4 against oxidative injury, strongly pointing to an action independent of the VKDPs. In a subsequent study, it was shown that MK-4 acted, at least in part, through inhibition of the enzyme 12-lipoxygenase [57]. The neuroprotective effect of MK-4 was recently confirmed in the context of methylmercury-induced cell death, another glutathione depletion model [58].

Some reports also point to protective actions of MK-4 in inflammatory processes. *In vitro*, MK-4 has been shown to limit the production of IL-6 in cultured human fibroblasts [59] and of prostaglandins [60]. In animal studies, MK-4 has been observed to limit inflammation in models of encephalomyelitis [61] while phylloquinone has been reported to suppress lipopolysaccharide-induced inflammation in the rat [62]. In a recent study, the antiinflammatory activity of vitamin K, notably MK-4, was shown to be mediated via the inhibition of the nuclear factor κ B signaling pathway [63]. Furthermore, recent epidemiological cohort studies have reported that a high vitamin K nutritional status is associated with lower levels of the proinflammatory markers IL-6, intracellular adhesion molecule-1, tumor necrosis factor receptor 2, and C-reactive protein [64,65].

Taken together, these studies strongly point to protective effects of vitamin K, notably MK-4, against oxidative stress and potential antiinflammatory actions.

5. Vitamin K, behavior and cognition

In light of its key actions on the different cell types making up the nervous system, vitamin K would have the potential to influence psychomotor functions and the cognitive process. Unfortunately, these aspects of the vitamin K role have been largely neglected thus far, with only a few reports currently available. Below, is a summary of the animal and human studies that have addressed this topic.

5.1. Animal studies

Evidence suggesting that vitamin K could influence psychomotor functions came from the study of Cochetto et al. [66] in the mid 1980s. In this report, vitamin K deficiency induced by administration of a vitamin K deficient diet or warfarin treatment was associated with hypoactivity in rats. When assessed with the open field paradigm, locomotor activity of vitamin K deficient rats was 25% lower than in controls and warfarin treatment was associated with a shift from more to less exploratory behavior. In contrast, cognitive abilities as assessed with the radial arm maze, were not altered by

vitamin K status. In this report the observed psychomotor abnormalities were assumed to result from a vitamin K deficiency. As inhibitor of the VKOR, the enzyme responsible for the recycling of the vitamin in tissues, warfarin treatment leads to the accumulation of the K vitamers in their epoxide, inactive, forms, and to vitamin K deficiency over time. Unfortunately, in this study, information pertaining to the vitamin K status of the warfarin-treated rats was not provided.

More recently, our group reported that lifetime consumption of a low containing phyloquinone diet results in cognitive deficits in old age. When subjected to the Morris water maze test, 20-month-old rats which had received a low (L) ($80 \mu\text{g kg}^{-1}$ diet) containing phyloquinone diet since weaning, were found to acquire spatial learning more slowly (*i.e.*, longer latencies) than rats fed adequate (A) or high (H) (500 and $2,000 \mu\text{g kg}^{-1}$ diet, respectively) phyloquinone diets. Motor activity and exploratory behavior (open field test) were also assessed in these animals and did not vary as a function of diet. Similarly, anxiety (elevated plus maze test) was not affected by lifelong vitamin K intake. Thus, the impairment observed in the water maze was not likely attributable to differences in either motor ability or emotionality, but rather reflected a true cognitive deficit. Interestingly, the low vitamin K diet had no impact on psychomotor abilities or cognition in rats aged 6 and 12 months suggesting that vitamin K nutrition was particularly important to brain function in the more vulnerable aging state. Noteworthy, the vitamin K-induced cognitive alterations observed in the old animals were associated with higher concentrations of ceramides in the hippocampus and lower gangliosides in the pons medulla and midbrain [11]. As discussed in a previous section, some sphingolipids have been associated with age-associated cognitive decline and neurodegenerative diseases.

5.2. Human studies

Whether vitamin K status has the ability to influence behavior and cognition in healthy individuals remains undetermined at this time, however, fetal exposure to warfarin derivatives during the first trimester of pregnancy has long been shown to result in anomalies of the central nervous system. Symptoms for this condition, referred to as warfarin embryopathy or fetal warfarin syndrome, includes dilatation of the cerebral ventricles, microcephaly, mental retardation, optic atrophy, and blindness [67].

More recently, our group published a detailed analysis of phyloquinone intakes of 31 community-dwelling patients in the early stages of Alzheimer's disease (AD) and compared them to those of 31 age- and gender-matched cognitively intact controls. Mean phyloquinone intakes were significantly lower in participants with AD ($63 \pm 90 \mu\text{g day}^{-1}$ vs. $139 \pm 233 \mu\text{g day}^{-1}$) even after adjusting for energy intakes. Vegetables, fats, and fruits contributed over 70% of total phyloquinone intakes in both groups and green vegetables, the main source of vitamin K contributed 33 and 49% to total intakes in patients and controls, respectively.

This lower consumption of green vegetables in participants with AD explained their overall lower vitamin K intakes [68]. Data pointing to a depressed vitamin K status in AD patients was also provided in an earlier report by Sato et al. [69]. In this study conducted in 100 women with AD and 100 age-matched community dwelling participants, plasma phyloquinone levels were found to be significantly lower in AD patients than in controls. Furthermore, serum phyloquinone concentrations correlated positively with cognitive abilities assessed with the mini-mental state examination test, and negatively with the uncarboxylated form of the VKDP osteocalcin, an indicator of low vitamin K status [69].

The lower phyloquinone concentrations observed in AD individuals is of particular interest as some studies have linked circulating phyloquinone to the ApoE genotype with concentrations decreasing in the order of $E2 > E3 > E4$, that is, individuals carrying the ApoE4 allele having the lowest circulating phyloquinone concentrations. This has been assumed to be related to a higher clearance of the vitamin K-rich intestinal lipoproteins from the circulation in apoE4 carriers [70]. Noteworthy, this genotype is also the one that has long been identified as a risk factor for AD [25]. In light of this and of the known actions of vitamin K in brain, Allison [71] hypothesized that chronically low vitamin K could represent a risk factor for AD. Although interesting, this hypothesis will have to be investigated in large prospective studies.

6. Conclusion and perspective

Work conducted in the past 25 years has clearly established a role for vitamin K in brain function. Although very much an emerging field of research, the role of vitamin K in cognition and neurodegenerative diseases is one that will likely attract much attention in the coming years. However, in light of their cellular actions, it can already be argued that both Gas6 and protein S would have the potential to influence neurocognitive processes in a significant manner. By virtue of its action in blood coagulation as an antithrombotic factor, protein S could contribute to the maintenance of the brain vasculature and support cerebral blood flow and oxygenation. Likewise, both Gas6 and protein S could support cognitive processes through their cell signaling effects. The pro-survival effect of Gas6 on neurons and glial cells has repeatedly been associated with the activation of the MAPK and PI3-K signaling pathways and their downstream effectors, notably ERK and CREB. This is of particular interest given that activation of CREB has been associated with long-term potentiation, a measure considered to be one of the major mechanism by which the brain acquires and stores information [72]. Transcription factor CREB has also been shown to modulate the activities of neurotrophins such as BDNF involved in synaptic plasticity [73,74]. Likewise, vitamin K could support neurocognitive functions through its role in sphingolipids. Brain sphingolipids have been shown to be responsive to vitamin K status and studies have linked these lipids to key cellular events such as inflammations

and oxidative damage. Finally, neurocognitive functions could benefit from what appears to be unique protective functions of the K vitamers, MK-4.

In light of our current knowledge of the actions of vitamin K in brain, much is to be expected from this nutrient in the future.

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