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REVIEW

Ergothioneine in the brain

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Ergothioneine (ERGO) is a naturally occurring food-derived antioxidant. Despite its extremely hydrophilic properties, ERGO is easily absorbed from the gastrointestinal tract and distributed to various organs, including the brain. This is primarily because its entry into brain cells is mediated by the ERGO-specific transporter OCTN1/SLC22A4. *Octn1* gene knockout mice do not have ERGO in the brain, due to the absence of OCTN1 in neurons, neural stem cells, and microglia. The existence of OCTN1 and uptake of ERGO into the brain parenchymal cells may suggest that ERGO and its transporter play a pivotal role in brain function. Oral administration of ERGO has antidepressant activities in mice. Furthermore, repeated oral administration of ERGO and ERGO-containing food extract tablets enhance memory function in mice and humans, respectively. ERGO also protects against stress-induced sleep disturbance and neuronal injury induced by amyloid β in rodents. *In vitro* observations suggest that ERGO benefits brain function through both its antioxidative activity and by promoting neurogenesis and neuronal maturation. This review discusses the possible involvement of ERGO in brain function and its potential therapeutic properties.

Keywords: antioxidant; cognitive function; depression; ergothioneine; microglia; neural stem cells; neurodegenerative diseases; neurogenesis; neurons; transporter

The food-derived antioxidant ergothioneine (ERGO) is efficiently absorbed from the gastrointestinal tract after oral ingestion and thereafter distributed to various organs, including the brain [1–4]. It is intriguing that ERGO crosses the plasma membrane of cells in these organs despite its hydrophilic nature. This is primarily due to its transporter carnitine/organic cation transporter (OCTN1)/solute carrier (SLC) 22A4 being expressed ubiquitously [1–3,5–8]. OCTN1 plays an essential role in ERGO homeostasis at least in rodents and also accepts many other compounds as substrates *in vitro*, but the transport activity for those compounds is much lower than that for ERGO [3–5,9], suggesting that OCTN1 could be virtually an ERGO-specific transporter. ERGO is present in the brain of wild-type mice, probably because of the daily digestion of ERGO-containing feed, but it is instead absent in *octn1* gene knockout (*octn1*$^{-/-}$) mice [3]. This results from OCTN1 being expressed in neurons, neural stem cells (NSCs), and microglia in the mouse brain [2,10,11]. In mammals, ERGO is thought not to be synthesized in the body, but ingested from daily diet. Thus, the existence of a specific transporter for exogenous ERGO in brain parenchymal cells may imply that ERGO exerts a physiological role in brain function. Interestingly, ERGO levels in the bloodstream

Abbreviations
AD, Alzheimer's disease; Aβ, amyloid β; BBB, blood–brain barrier; BMECs, brain microvascular endothelial cells; ERGO, ergothioneine; hCMEC/D3, human cerebral microvascular endothelial cell line; LPS, lipopolysaccharide; MCI, mild cognitive impairment; mTOR, mammalian target of rapamycin; NSCs, neural stem cells; OCTN1, carnitine/organic cation transporter 1; *octn1*$^{-/-}$, *octn1* gene knockout; PD, Parkinson's disease; PTZ, pentylenetetrazole; ROS, reactive oxidative species; SDS, social defeat stress.

are decreased in both elderly subjects and those with mild cognitive impairment (MCI), as well as in patients suffering from several diseases, including Parkinson's disease (PD) and frailty [12–15]. Low plasma ERGO levels are also associated with neurodegeneration and cerebrovascular diseases in dementia [16]. Moreover, oral administration of ERGO enhances memory function in humans [17] and mice [18] and exhibits antidepressant activity in mice [19]. Oral administration of ERGO protects against stress-induced sleep disturbance [20] and neuronal injury induced by amyloid β (Aβ) in rodents [21]. Many *in vitro* studies have suggested that the effects of ERGO on brain function may be the result of its antioxidative activity, whereas promotion of neurogenesis and neuronal maturation are also beneficial effects of ERGO in the brain [2,10,18,21–24]. Such beneficial effects of ERGO after oral administration suggest that ERGO is an exogenous neurotrophic food-derived compound. This review will discuss the possible involvement of ERGO in brain function and its potential application as healthy food and therapeutic reagent.

Distribution of ERGO to the brain cells after gastrointestinal absorption

OCTN1/SLC22A4 is expressed in the small intestine, kidney, and brain of humans and rodents [1–3,5–8]. Pharmacokinetic analysis after oral administration of [^{3}H]ERGO revealed the highest distribution to the small intestine followed by the liver and extensive renal reabsorption in wild-type mice (cumulative urinary excretion for 2 weeks after oral administration of [^{3}H]ERGO was < 10% of the dose), whereas such distribution and reabsorption are significantly reduced in *octn1*$^{-/-}$ mice [3], suggesting that OCTN1 plays an essential role in maintaining ERGO homeostasis in the body. After being absorbed from the gastrointestinal tract, ERGO is highly distributed in the liver, and its distribution is rate-limited by the hepatic blood flow rate, at least in rodents [1]. This means that most orally absorbed ERGO is taken up by the liver, implying that this organ plays a physiological role in ERGO homeostasis. After oral administration, the plasma concentration of [^{3}H]ERGO in *octn1*$^{-/-}$ mice was transiently higher than that in wild-type mice, which was probably due to the efficient hepatic uptake of [^{3}H]ERGO in wild-type mice, but almost no uptake in the liver of *octn1*$^{-/-}$ mice [1,3]. Quantification of the putative metabolites of ERGO in several tissues in wild-type mice showed that the ratio of the major metabolite hercynine to ERGO in the liver and whole blood was approximately 1/100, and those of other metabolites, such as ERGO-SO_3H and S-methyl ERGO, were less than that of hercynine [4]. Thus, metabolism of ERGO in the body could be quite slow.

After the hepatic first-pass uptake, ERGO is distributed ubiquitously to organs, including the brain, which also expresses OCTN1 [1,3]. ERGO needs to cross the blood–brain barrier (BBB) to facilitate ERGO distribution into the brain parenchyma. Several quantitative proteomic analyses using liquid chromatography–mass spectrometry in isolated human brain microvascular endothelial cells (BMECs), which are essential components of the BBB, and the human cerebral microvascular endothelial cell line (hCMEC/D3) showed that protein expression of OCTN1 was below the lower limit of quantification [25–27]. On the other hand, the expression of mRNA and the gene product of OCTN1 was previously assessed in cultured human BMECs using reverse transcription polymerase chain reaction and western blotting, respectively [24]. OCTN1 knockdown by transfection with small interfering RNA suppresses ERGO uptake [24], suggesting the functional expression of OCTN1, at least in cultured human BMECs. Western blot analysis has also revealed expression of OCTN1 in the brain endothelial cells isolated from BALB/C mice [28]. Notably, [^{3}H]ERGO was observed in the plasma after oral administration, even in *octn1*$^{-/-}$ mice [3], suggesting that transporters other than OCTN1 are involved in gastrointestinal absorption. Thus, ERGO may pass through the BBB via OCTN1 and/or other transporters. Recently, SLC22A15 and SLC22A5 were reported to accept ERGO as a substrate *in vitro* [9]. They are expressed ubiquitously in organs, but their exact role in ERGO disposition has not yet been clarified.

In the brain, the concentration of food-derived ERGO and the distribution of intravenously administered [^{3}H]ERGO were correlated with the expression of OCTN1 mRNA [2]. OCTN1 was immunostained in neurons, and the uptake of [^{3}H]ERGO was observed in primary cultured mouse neurons [2], whereas OCTN1 mRNA was not detected in rat astrocytes, which constitutes a major cell population in the brain [29]. Thus, OCTN1 may be involved in ERGO uptake by neurons. OCTN1 mRNA and ERGO concentrations in the cerebellum were highest among brain regions [2], which might be due to the higher population of neurons than astrocytes in the cerebellum. OCTN1 mRNA and ERGO concentrations in the midbrain, medulla, and pons, and hypothalamus are relatively higher than other brain regions, whereas OCTN1 is also expressed in the other regions

including the hippocampus [2]. The existence of uptake transporters for food-derived ERGO in the brain may imply that ERGO plays a pivotal role in brain function. Information on the actual presence of ERGO in human brains is limited, but ERGO was detected in the postmortem brain of infants [30].

Roles of ERGO in brain cells

Neurons

Neurons play central roles in brain function, and exposure to ERGO promotes neuronal maturation and protect against neurotoxicity [2,18,21,22,31,32] (Table 1). Regarding neuronal maturation, oral administration of ERGO significantly increased the number of mushroom-type spines, which form stable synaptic contacts in the hippocampal dentate gyrus [18]. In an *in vitro* study, exposure of primary cultured hippocampal neurons to ERGO (50–500 μM) elevated the mRNA and protein levels of the synapse formation marker Synapsin I in a dose-dependent manner [18]. These findings indicate that ERGO promotes neuronal maturation in the hippocampus.

ERGO also has protective capacity against neurotoxicity. Pretreatment with ERGO (500 and 1000 μM) attenuated the increase in $A\beta_{25-35}$-induced apoptotic death assessed using TUNEL staining in PC12 cells [22]. Furthermore, ERGO has shown protective effects against anticancer drug-induced neurotoxicity [31,32]; oral administration of ERGO (2 and 8 $mg \cdot kg^{-1} \cdot day^{-1}$ for 42 and 56 days) restored cisplatin-induced deficits in learning and memory, as evaluated with active and passive avoidance tests [31], and co-administration of ERGO (15 $mg \cdot kg^{-1}$, i.v., twice per oxaliplatin administration) decreased oxaliplatin accumulation in dorsal root ganglion neurons and ameliorated mechanical allodynia in rats [32].

Neural stem cells

Neural stem cells are essential for neurogenesis owing to their self-renewal ability and pluripotency to differentiate into neurons, astrocytes, and oligodendrocytes [33]. Neurogenesis occurs in limited regions of the brain such as the hippocampal dentate gyrus and subventricular zone and is indispensable for sustaining normal brain functions [33]. Suppression of neurogenesis leads to the development and progression of several neuropsychiatric disorders, such as major depression and dementia [34], whereas the promotion of neurogenesis is a potential therapeutic strategy for neuropsychiatric disorders [35]. Maintaining an ERGO-containing diet (120 mg/100 g diet, plausibly achievable level of daily ingestion) for 2 weeks in mice significantly increased the number of neurons positive for doublecortin, a newborn neuron marker in the hippocampal dentate gyrus [19], and the ERGO-induced neurogenesis may be related to the association between levels of plasma ERGO and hippocampal volume in dementia [16]. Exposure of NSCs to ERGO in primary cultures derived from wild-type mice promoted neuronal differentiation, but this did not occur in NSCs

Table 1. Expression and functions of OCTN1 and ERGO in brain parenchymal cells, BMECs, and choroid plexus cells. –, unknown.

Cells in the brain	Expression of OCTN1	Function of OCTN1 and/or ERGO
Neurons	(mRNA) cerebellum, cerebral cortex, hippocampus, hypothalamus, medulla and pons, and striatum in mice [2] (Protein) cerebellum, cerebral cortex, hippocampus, hypothalamus, midbrain, olfactory bulb in mice [2,8]	(OCTN1) Promotion of neurite outgrowth in Neuro2a [2]; (ERGO) Promotion of neuronal maturation [18], protection against cytotoxicity induced by Aβ [21,22], cisplatin [31], oxaliplatin [32]
Astrocytes	(mRNA) not detected in rat primary cultured cells [29]	–
Oligodendrocytes	–	–
NSCs	(mRNA and protein) murine primary cultured cells [10]	(OCTN1 and ERGO) Promotion of neuronal differentiation and suppression of proliferation [10,23]
Microglia	(mRNA) murine primary cultured cells [11]	(ERGO) Suppression of LPS-induced cellular hypertrophy [11]
BMECs	(mRNA and protein) human cultured cells [24]; (Protein) isolated murine brain endothelial cells [28]; (Protein) below the lower limit of quantification in the isolated human BMECs and hCMEC/D3 [25–27]	(ERGO) Suppression of ROS production and cell death induced by pyrogallol, xanthine oxidase plus xanthine, and high glucose [24], suppression of 7-ketocholesterol-induced endothelial injury [39]
Choroid plexus cells	(Protein) murine choroid plexus [8]	–

derived from *octn1*$^{-/-}$-mice [23], suggesting that OCTN1-mediated ERGO uptake is essential for ERGO-induced neuronal differentiation.

The mechanisms underlying ERGO-induced neuronal differentiation were examined [10,23]. ERGO increased phosphorylation of mammalian target of rapamycin (mTOR), an amino acid sensor, and tropomyosin receptor kinase B (TrkB), which is a receptor for neurotrophins such as brain-derived neurotrophic factor and neurotrophin 4/5 [23]. Moreover, exposure to rapamycin, an inhibitor of mTOR complex 1 (mTORC1), or GNF5837, an inhibitor of TrkB, suppressed ERGO-induced neuronal differentiation [23]. These results suggest that ERGO promotes neuronal differentiation via the mTORC1/TrkB signaling pathway.

Microglia

Microglia are immune cells that play fundamental roles in the immune response in the brain. Chronic overactivation of microglia is involved in the onset and exacerbation of several neuropsychiatric disorders [36]. Therefore, regulation of microglial activation is believed to be essential for brain homeostasis and a therapeutic approach for neuronal disorders [37]. Exposure of primary cultured murine microglia to ERGO suppresses the lipopolysaccharide (LPS)-induced production of reactive oxidative species (ROS) and cellular hypertrophy [11], and mRNA expression of interleukin-1β in the microglia of *octn1*$^{-/-}$ was higher than that in wild-type microglia. Thus, OCTN1-mediated ERGO may prevent the activation of microglia by xenobiotics. This may be consistent with the observation that ERGO uptake and OCTN1 expression were markedly upregulated by LPS-induced activation [11]. In contrast, in the cultured *octn1*$^{-/-}$ microglia, exposure to LPS slightly increased cellular hypertrophy [11]. The deletion of the *octn1* gene may cause downregulation of such hypertrophy-promoting factors, or OCTN1 may transport unidentified compounds that positively regulate microglial hypertrophy [11]. Hence, the negative regulation of microglial activation by ERGO would be involved in suppression of chronic overactivation of microglia in the pathology of several neuropsychiatric disorders although further studies are required to precisely evaluate its beneficial activity.

Brain microvascular endothelial cells

Brain microvascular endothelial cells are major components of the BBB, and endothelial cell dysfunction has been implicated as a crucial event in the onset of several neurodegenerative diseases [38]. BMECs are more sensitive to oxidative stress than peripheral endothelial cells [38]. Exposure of cultured human BMECs to ERGO (100 or 1000 μM) suppressed ROS production and protein expression of NADPH-oxidase 1 and protected against cell death induced by pyrogallol, xanthine oxidase plus xanthine, and high glucose [24]. Exposure of ERGO (100 μM, 1, or 10 mM) to hCMEC/D3 also protects against cytotoxicity induced by 7-ketocholesterol, a cholesterol oxidation product [39]. Since the ERGO concentration used in these studies is much higher than that observed in plasma *in vivo*, further studies may be needed to assess the potential effect of ERGO on the BBB. Nevertheless, ERGO can exert antioxidant effects in multiple cells in the brain, including not only parenchymal cells but also BMECs.

Therapeutic implications of ERGO in brain diseases

Dementia

Cognition is an essential function of the brain. Recently, ERGO levels in patients with cognition-related disorders were reported to be lower than those in healthy subjects [12,13,15]; Cheah et al. [12] have shown that whole-blood ERGO levels in individuals over 60 years of age and a subset of these subjects with MCI is significantly lower (0.65-fold) compared with that of age-matched healthy subjects, suggesting that such lower levels of bodily ERGO may be a risk factor for neurodegeneration in the elderly. In addition, the results of whole-blood metabolomics analysis reported by another group also indicated that levels of ERGO (0.41-fold) and its putative metabolite *S*-methyl-ERGO (0.20-fold) in patients with dementia were significantly lower than those in healthy elderly subjects [13,15]. Lower plasma ERGO levels are also significantly associated with white matter hyperintensities and brain atrophy markers, such as reduced global cortical thickness and hippocampal volume in patients with dementia [16]. These findings do not necessarily demonstrate ERGO function in human brains, but strongly suggest an association between ERGO levels in circulation and cognitive function and/or neurodegeneration in humans.

Recently, more direct evidence for the cognitive stimulating effect of ERGO has been reported in humans: a randomized, placebo-controlled, double-blind, parallel-group study in humans (placebo, $n = 19$; ERGO, $n = 21$), including healthy volunteers and subjects with MCI, demonstrated that repeated

oral administration of ERGO-containing mushroom extract tablets for 12 weeks enhances cognitive function (Table 2) [17]. In that study, daily oral administration of 5 mg ERGO-containing mushroom extract tablets significantly improved verbal memory compared with that of the placebo group, and this was assessed using a test for cognitive function, Cognitrax, at week 12 after start of the administration. In the ERGO administration group, scores of composite memory, verbal memory, psychomotor speed, reaction time, working memory, complex attention, and sustained attention were significantly improved compared with the scores before the intervention, whereas these scores were not altered in the placebo group [17]. The difference in the improvement effect on brain function between ERGO-containing tablets and placebo may suggest beneficial effect of ERGO although possible effects of other ingredients in the mushroom cannot be excluded since mushrooms generally contain many bioactive compounds.

The improvement effect of ERGO on cognitive function in healthy conditions has been further supported by the recent finding in mice that oral administration of ERGO enhances learning and memory ability [18], and repeated oral administration of ERGO at 1–50 mg·kg^{-1} for 2 weeks improved learning and memory ability as evaluated by the novel object recognition test [18] (Table 2). ERGO administration significantly increased the number of matured spines, which are indispensable for cognitive function, in the hippocampus, suggesting that ERGO may enhance cognitive function, possibly via the maturation of spines in the hippocampus. In the brain diseased states, on the other hand, Song et al. [21,40] have demonstrated that oral administration of ERGO (0.5 mg·kg^{-1}) improves cognitive impairment induced by intracerebroventricular infusion of Aβ_{1-40} or subcutaneous administration of D-galactose (Table 2). ERGO administration significantly prevented the accumulation of Aβ in the hippocampus and brain lipid peroxidation, along with restoring acetylcholinesterase activity and maintaining the glutathione/glutathione disulfide ratio and superoxide dismutase activity in the brains of Aβ_{1-40}-treated mice [21]. Taken together with the lower levels of food-derived ERGO in cognitive impairment and neurodegeneration, ERGO may play fundamental roles in the promotion of cognitive function and/or protection against cognition-related diseases in the brain. Further studies are needed to clarify its preventive and therapeutic potential in dementia and Alzheimer's disease (AD).

Table 2. Therapeutic effects of ERGO in the brain

Diseases or animal models	Therapeutic effects	Dose	Proposed mechanism[a]
Healthy participants and patients with MCI	Enhancement of cognitive function [17]	5 mg·day^{-1} for 4, 8, and 12 weeks (p.o. as tablets of mushroom extract)	Neurogenesis; neuronal maturation
Normal mice	Enhancement of cognitive function [18]	1–50 mg·kg^{-1}·day^{-1} three times a week for 2 weeks (p.o.)	Neurogenesis; neuronal maturation
AD model mice	Protection against neuronal injury induced by Aβ_{1-40} infusion into cerebral ventricle [21]	0.5 and 2 mg·kg^{-1}·day^{-1} from day 1 to 16 and from day 29 to 67 (p.o.)	Prevention of Aβ accumulation; restoration of acetylcholinesterase activity; anti-oxidative actions; lipid peroxidation inhibition
Brain aging model mice	Protection against deficits of learning and memory induced by D-galactose administration [40]	0.5 mg·kg^{-1}·day^{-1} for 88 days (p.o.)	Prevention of Aβ accumulation; anti-oxidative actions; lipid peroxidation inhibition
Normal mice	Antidepressant-like activity [19]	120 mg/100 g diet for 2 weeks	Neurogenesis
SDS model rats	Protection against stress-induced social avoidance and sleep disturbances [20]	0.25 mg·mL^{-1} in drinking water for 28 days	Anti-inflammatory effects; anti-oxidative actions; lipid peroxidation inhibition
PTZ-induced epilepsy model	Suppression of PTZ-induced seizure [50]	50 mg·kg^{-1}·day^{-1} for 12–22 days (p.o.)	Decrease in homostachydrine concentration in the brain; anti-oxidative actions
Normal mice	Protection against cisplatin-induced neuronal injury and deficit of learning and memory [31]	2 and 8 mg·kg^{-1}·day^{-1} for 42 and 56 days (p.o.)	Lipid peroxidation inhibition; anti-oxidative actions; restoration of acetylcholinesterase activity

[a]Underlying mechanisms as speculated by the authors.

Parkinson's disease

The serum ERGO levels in patients with PD, a neurodegenerative disease associated with the loss of dopaminergic neurons in the substantia nigra pars compacta, were reportedly lower than those in healthy participants (0.45-fold) [14]. The decline in ERGO levels in patients may imply elevated oxidative stress and/or insufficient ability to scavenge free radicals, which could contribute to PD pathogenesis [14] because many studies have indicated a relationship between ROS-induced mitochondrial dysfunction caused by α-synuclein and the onset of PD. Interestingly, OCTN1 is expressed not only in plasma membranes but also in mitochondria [41], and ERGO (1 mM) protects against mitochondrial DNA damage induced by hydrogen peroxide in HeLa cells [42]. ERGO (10 μM) protected against cisplatin-induced cytotoxicity in primary cultured cortical neurons [31]. OCTN1 is highly expressed in the midbrain, where the substantia nigra pars compacta is located, and the food-derived ERGO concentration is ~ 2.4 $\mu g \cdot g^{-1}$ tissue (~ 10 μM if the gravity of the brain is assumed to be unity) in mice [2], which is not very different from the protective concentration of ERGO in cultured cortical neurons [31]. Hence, ERGO may exert a potential protective effect on neurons against oxidative stress, and the decline in ERGO levels may be involved in the pathogenesis of several neurodegenerative disorders, including PD.

Depression and stress-induced sleep disturbance

Depression is a mental illness that approximately 5% of adults currently suffer from worldwide (http://ghdx.healthdata.org/gbd-2019). Although various types of antidepressants, such as selective serotonin reuptake inhibitors, are clinically available, these agents have limited therapeutic efficacy, delayed onset of response, and various side effects [43]. It is thus important to clarify the pathophysiology of depression and develop antidepressant drugs targeting novel molecules with limited side effects. To date, several possible mechanisms underlying the onset of depression have been suggested, including monoamine deficiency, decline in neurogenesis, disruption of neural plasticity, imbalance between glutamate and gamma-aminobutyric acid, and excess inflammation and oxidative stress [43–47]. Interestingly, ERGO has unique biological activities, such as the promotion of neuronal differentiation of NSCs and neuronal maturation via activation of mTORC1 and TrkB [10,18,23]. mTOR and TrkB are considered potential targets for the treatment of depression [48]; therefore, ERGO may exhibit antidepressant activity. In fact, oral ingestion of ERGO (120 mg/100 g diet) for 2 weeks in mice significantly decreased the immobility time, an index of depression-like behavior, in forced swimming and tail suspension tests with a concomitant increase in ERGO concentration in the brain (~ 20 $\mu g \cdot g^{-1}$ tissue, Table 2) [19], and the ingestion promotes neurogenesis in the hippocampus, indicating that oral ERGO ingestion exerts antidepressant-like activity, possibly via promotion of neurogenesis in mice [19].

Matsuda et al. [20] also reported that oral administration of ERGO prevented depressive behavior and depression-like sleep abnormalities in rats (Table 2). In their studies, preventive administration of ERGO (0.25 $mg \cdot mL^{-1}$) via drinking water corresponding to ~ 30 $mg \cdot kg^{-1} \cdot day^{-1}$ based on water intake and body weight, ameliorated social defeat stress (SDS)-induced social avoidance behaviors against other rats and sleep abnormalities such as an increase in rapid eye movement sleep [20]. Furthermore, they demonstrated that SDS substantially increases the levels of the fecal gut bacterium *Lactobacillus reuteri* in correlation with fecal ERGO level, implying a protective system against psychosocial stress in the microbiota–gut–brain axis [20]. ERGO in the cerebellum and plasma of the sleep-state was significantly reduced compared with that in the awake state, suggesting an association between ERGO and sleep [49]. The authors proposed that the reduction of the ERGO during sleep-state could be due to a reduced neuronal cerebellar activity and/or sleep-induced alterations of the ERGO synthesis in the gut microbiome. Hence, ERGO may also be involved in depression and sleep, and its administration would be helpful for the improvement of stress-induced sleep. Further studies are required to evaluate the potential effects of ERGO on patients with depression and stress-induced sleep disturbances.

Epilepsy

Epilepsy is a disorder of the central nervous system that causes seizures or periods of unusual behavior, sensations, and loss of awareness. In pentylenetetrazole (PTZ)-induced epilepsy model mice, repeated oral administration of ERGO suppressed the seizure score (Table 2) [50]. Unexpectedly, *octn1*$^{-/-}$ mice showed lower seizure scores than wild-type mice in this model, suggesting that OCTN1 may positively affect seizures possibly by transporting unidentified compounds and/or the resultant secondary effects. Further untargeted metabolomic analysis using the brains of wild-type and *octn1*$^{-/-}$ mice identified the plant alkaloid homostachydrine

as a novel substrate of OCTN1, which was found to increase seizure scores in a PTZ-induced epilepsy model [50]. This may be consistent with the fact that repeated oral administration of ERGO decreased the concentration of homostachydrine in the brain [50], supporting the partial association of this plant alkaloid with PTZ-induced seizure. However, transport activity of OCTN1 for homostachydrine was much lower than that for ERGO [50], and therefore, homostachydrine seems to be a weak substrate for OCTN1. In addition, homostachydrine concentrations in human plasma are much lower than those in mice [50], and there have been no reports showing changes in ERGO or homostachydrine levels in epilepsy patients to date. Thus, further studies are needed to clarify the association between ERGO and this disease.

Conclusions and perspectives

Food-derived ERGO is efficiently distributed to the brain via its specific transporter, OCTN1, and exerts multiple functions in different types of brain cells. Hence, the low level of ERGO in patients with cognition-related disorders may implicate that ERGO deficiency is pathogenic in neurodegenerative disorders. In fact, ERGO can regulate neuronal differentiation, neurogenesis, and microglial activation, and also protects against neurotoxicity induced by pathogenic proteins or chemicals, including Aβ and anticancer drugs. Moreover, as oral administration of ERGO or ERGO-containing food extract enhances cognitive function in healthy humans and mice, ERGO may thus be applicable to functional foods that help maintain human health. Further studies are required to discover the potential therapeutic and preventive properties of ERGO and to clarify the underlying mechanisms.

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Author contributions

IT and KY designed and wrote the review.

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