Current Topics

Membrane Transporters as Targets for the Development of Drugs and Therapeutic Strategies

Review

Physiological Roles of Carnitine/Organic Cation Transporter OCTN1/SLC22A4 in Neural Cells

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Dysfunction in neurotransmission mediated by neurotransmitters causes various neurological disorders. Therefore, receptors and reuptake transporters of neurotransmitters have been focused on as a therapeutic target in neurological disorders. These membrane proteins have high affinity for a specific neurotransmitter and are highly expressed on synaptic membranes. In contrast, xenobiotic transporters have relatively lower affinity for neurotransmitters but widely recognize various organic cations and/or anions and are also expressed in brain neurons. However, it has been largely unknown why such xenobiotic transporters are expressed in neurons that play a key role in signal transduction. We have therefore attempted to clarify the physiological roles of one such xenobiotic organic cation transporter (OCT) in neural cells with the aim of obtaining new insight into the treatment of neurological disorders. Carnitine/organic cation transporter OCTN1/SLC22A4 is functionally expressed in neurons and neural stem cells. In particular, OCTN1 is expressed at much higher levels compared with other OCTs in neural stem cells and positively regulates their differentiation into neurons. OCTN1 accepts the naturally occurring food-derived antioxidant ergothioneine (ERGO) as a good *in vivo* substrate. Because ERGO is highly distributed into the brain after oral ingestion, OCTN1 may contribute to the alleviation of oxidative stress and promotion of neuronal differentiation *via* the uptake of ERGO in the brain, perhaps abating symptoms of neurological disorders. In this review, we introduce current topics on the physiological roles of OCTs with a focus on OCTN1 in neural cells and discuss its possible application to the treatment of neurological disorders.

Key words carnitine/organic cation transporter 1 (OCTN1); ergothioneine; neuron; neural stem cell; neurological disorder

1. INTRODUCTION

Neurotransmission mediated by neurotransmitters regulates brain function, which is the origin of various biological activities including thoughts, feelings, breathing, heartbeat, and digestion. Dysfunction in neurotransmission causes various neurological disorders, including Alzheimer's and Parkinson's diseases and depression. Therefore, the concentration of neurotransmitters in the synaptic cleft is strictly controlled by their uptake transporters and/or inactivating enzymes. The uptake of neurotransmitters is generally mediated by specific transporters that are expressed on neuronal plasma membranes and have high affinity for the neurotransmitters (Fig. 1). The uptake of neurotransmitters into neurons by those transporters controls their concentration in the synaptic cleft and is helpful in their recycling. Thus, such membrane transporters are recognized as "physiological" transporters that play a key role as the regulatory system of neurotransmission. On the other hand, efflux transporters are also expressed in neurons[1] and involved in pumping out intracellularly invasive xenobiotics and intracellularly produced metabolites to the extracellular space (Fig. 1). These transporters are considered to be the system that protects neurons from xenobiotics and unwanted metabolites. In addition to these two types of transporters, "xenobiotic" uptake transporters have recently been recognized to be expressed in brain neurons.[1] Xenobiotic uptake transporters have relatively lower affinity for neurotransmitters but widely recognize various organic cations and/or anions as their substrates (Fig. 1). These transporters principally contribute to the systemic absorption and/or elimination of therapeutic agents and xenobiotics in peripheral organs, including the small intestine, liver, and kidney, and it has been largely unknown why such xenobiotic transporters are expressed in neurons that play a key role in signal transduction. Gene knockout mice for these transporters generally show no remarkable phenotype in the central nervous system, at least under normal conditions, and therefore it has been difficult to clarify the physiological roles of these transporters in the brain. However, on the basis of their transport properties, it can be speculated that these transporters protect neurons from neurotoxicity and certain diseases by the uptake of excessive neurotransmitters existing in the synaptic cleft under pathological conditions. It was reported that several organic cation transporters (OCTs) are low-affinity transporters of monoamines,[2,3] and the inhibition of OCTs as well as the high-affinity monoamine transporters including the serotonin

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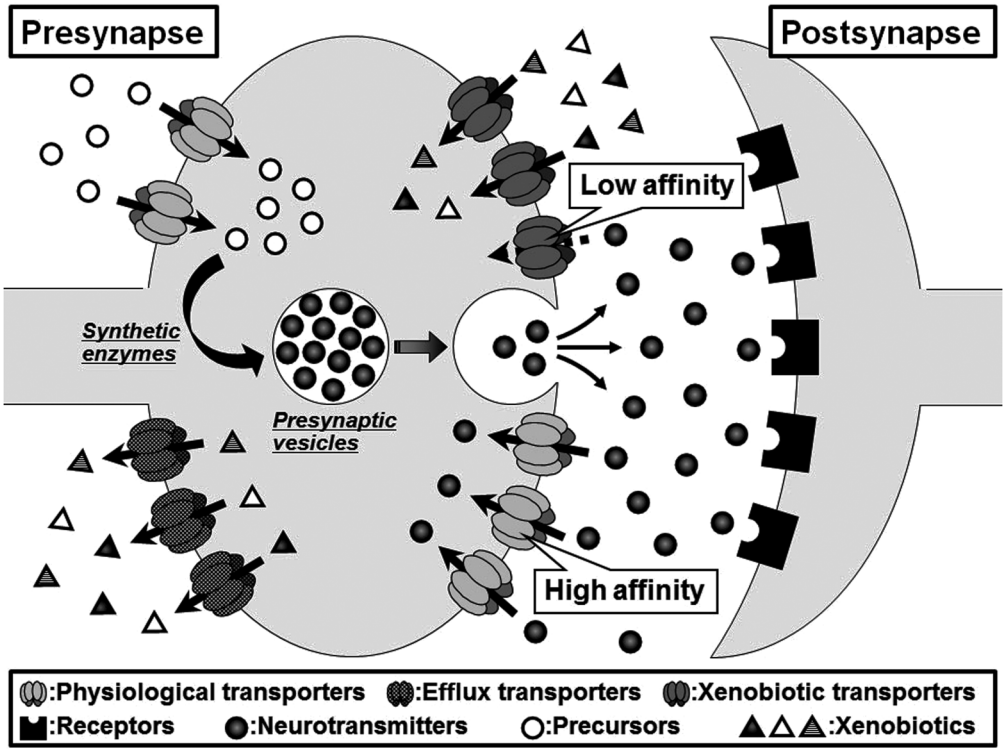

Fig. 1. Transporters Expressed in Neurons during Neurotransmission

Physiological transporters are involved in the uptake of physiologically necessary substances including neurotransmitters and their precursors into the intracellular space and have high affinity for a specific neurotransmitter. Efflux transporters are involved in pumping out physiologically unnecessary substances including xenobiotics and metabolites from the intracellular to extracellular space. Xenobiotic uptake transporters have relatively lower affinity for neurotransmitters but widely recognize various organic cations and/or anions. Neurotransmitters are synthesized from precursors by their synthetic enzymes, stored in presynaptic vesicles, released to the synaptic cleft, bound to their receptors expressed on the postsynaptic membrane to perform neurotransmission, and promptly retrieved by physiological transporters to terminate neurotransmission.

transporter (SERT) and norepinephrine transporter (NET) may be in part involved in the pharmacological effects of antidepressants.[4,5] OCTs include OCT1/SLC22A1, OCT2/SLC22A2, and OCT3/SLC22A3 and are polyspecific transporters for various types of organic cations. Carnitine/organic cation transporters (OCTNs) are another type of OCT and include OCTN1/SLC22A4 and OCTN2/SLC22A5. OCTN3 is not present in humans but expressed in rodents. Some of the physiological roles of OCTs in the brain have been recently reported, whereas information on OCTNs in the brain is largely unavailable. OCTN1 and -2 are responsible for the transport of ergothioneine (ERGO) and carnitine in the body, respectively.[6,7] ERGO is an antioxidant biosynthesized in fungi and mycobacteria, but not in mammals, and ingested from the daily diet. Carnitine is mainly ingested from the diet but also synthesized in mammals and is a vitamin-like substance essential for the beta-oxidation of fatty acids. Dysfunction of OCTN2 causes systemic primary carnitine deficiency, resulting in various symptoms, including cardiomyopathy, skeletal muscle weakness, and fatty liver.[7–9] In contrast, dysfunction of OCTN1 does not cause significant phenotypes, at least in peripheral organs under normal conditions in mice, and thereby we considered the possibility that OCTN1 may be a brain transporter and contribute to brain function. Thus, this review discusses whether OCTN1/SLC22A4 and OCTs are functionally expressed in the brain and involved in the onset or development of neurological disorders.

2. TRANSPORTERS EXPRESSED IN NEURONS

Neurons regulate brain function *via* neurotransmission mediated by neurotransmitters. Physiological transporters are expressed on neuronal plasma membranes and involved in the uptake of physiologically necessary substances including neurotransmitters and their precursors into the intracellular space to perform proper neurotransmission (Fig. 1). Neurotransmitters are synthesized from precursors by their synthetic enzymes, stored in presynaptic vesicles, released to the synaptic cleft, bound to their receptors expressed on postsynaptic membranes to perform neurotransmission, and promptly retrieved by their specific transporters, *i.e.*, physiological transporters, to terminate neurotransmission (Fig. 1). The uptake of neurotransmitters by physiological transporters is presumably the system that prevents excess neurotransmission and recycles neurotransmitters. The following physiological transporters are expressed in neurons: the excitatory amino acid transporter (EAAT), which transports excitatory neurotransmitters including glutamic acid and aspartic acid; γ-butyric acid (GABA) transporter (GAT), which transports the inhibitory neurotransmitter GABA; SERT, NET, and dopamine transporter (DAT), which transport monoamine neurotransmitters including serotonin, norepinephrine, and dopamine; glutamine transporter, which transports glutamine useful for the synthesis of glutamic acid and GABA; and choline transporter, which transports choline useful for the synthesis of acetylcholine. SERT and NET are target molecules for the treatment of depression, and their selective inhibitors including paroxetine,

sertraline, milnacipran, and duloxetine are clinically used as antidepressants.

Efflux transporters are also expressed in neurons and transport physiologically unnecessary substances including xenobiotics and metabolites from the intracellular to extracellular space (Fig. 1). The following efflux transporters are expressed in neurons[1,10–12]: the ATP-binding cassette transporter A1 (ABCA1), which eliminates cholesterol; P-glycoprotein (P-gp), which eliminates various anticancer drugs, hydrophobic cations, and neutral compounds; and multidrug resistance-associated protein 1, which eliminates conjugates of glutathione, sulfate, and glucuronic acid. These efflux transporters are expressed at lower levels under normal conditions because neurons are nonproliferative cells, show low metabolic capacity, and minimally produce unnecessary compounds in the intracellular space. On the other hand, the expression of efflux transporters is increased under pathological conditions including Alzheimer's disease and epileptic seizures,[10,11] possibly because neurons produce many unnecessary compounds in the intracellular space under such conditions.

Other than the aforementioned two types of transporters, xenobiotic uptake transporters have also been recently recognized to be expressed in brain neurons[1] (Fig. 1). These transporters have relatively broad substrate specificity and accept various organic cations and/or anions. The following xenobiotic uptake transporters are expressed in neurons[13–18]: OCT2 and OCT3, which transport cationic compounds; OCTN1, OCTN2, and OCTN3, which transport cationic and zwitterionic compounds; and organic anion transporter 1, which transports anionic compounds. These transporters generally contribute to the systemic absorption and elimination of xenobiotics in peripheral organs, including the small intestine, liver, and kidney, but their roles in neurons are largely unknown. Several xenobiotic uptake transporters as well as physiological transporters recognize neurotransmitters, but the affinity for neurotransmitters of xenobiotic uptake transporters is much lower than that of physiological transporters.[2,3] Therefore, xenobiotic uptake transporters may not contribute greatly to the uptake of neurotransmitters in the synaptic cleft under normal conditions. Possible physiological roles of xenobiotic OCTs and OCTN1 in neurons are discussed in the following section.

3. PHYSIOLOGICAL ROLES OF ORGANIC CATION TRANSPORTERS IN NEURONS

Concentrations of neurotransmitters are excessively increased in the synaptic cleft when dysfunction in the neurotransmitter elimination system by physiological transporters occurs due to the abuse of dependence-producing drugs or neurodegeneration, resulting in various types of psychiatric and motor dysfunction. Elimination systems for excessive neurotransmitters released to the synaptic cleft are needed to prevent such neuronal dysfunction. Among neurotransmitters, affect-related monoamines, including serotonin, norepinephrine, and dopamine, are basic compounds and substrates for OCT2 and -3. However, OCT2 and -3 have low affinity for monoamines, with the Michaelis constant (K_m) values being several hundred or thousand micromoles.[2,19] In contrast, SERT, NET, and DAT have much higher affinity for the corresponding monoamines, and the K_m values are several hundred or thousand nanomoles.[20–22] Therefore, monoamine neurotransmitters could be mainly eliminated from the synaptic cleft by SERT, NET, and DAT, but not by OCT2 and -3, under normal conditions. The low-affinity transporters would not contribute to the uptake of monoamines in the synaptic cleft owing to the existence of the high-affinity transporters. On the other hand, if dysfunction of the high-affinity transporters is caused by the abuse of dependence-producing drugs or neurodegeneration, OCT2 and -3 may play a significant role in the elimination of excessive neurotransmitters existing in the synaptic cleft. Alternatively, with excessive neurotransmitters in the synaptic cleft, the high-affinity transporters would be saturated with the corresponding neurotransmitters, resulting in a major role of low-affinity transporters in their uptake. Thus, OCT2 and -3 may play fundamental roles in preventing neurotoxicity by the uptake of excessive neurotransmitters in the synaptic cleft under pathological conditions and thus can be regarded as "disease" transporters.

Excessive release of the excitatory neurotransmitter glutamic acid to the synaptic cleft induces neurotoxicity, resulting in the onset and development of various neurodegenerative disorders including Alzheimer's disease and ischemic stroke.[23,24] On the other hand, dysfunction in neurotransmission mediated by the inhibitory neurotransmitter GABA is related to the onset and development of several neurological disorders including epilepsy and insomnia.[25,26] The high-affinity transporters for glutamic acid and GABA are EAAT and GAT, respectively, but the low-affinity transporters are unclear. Glutamic acid and GABA are amino acids and zwitterionic compounds. Certain amino acid transporters may act as their low-affinity transporters under pathological conditions. The OCTN family can also recognize zwitterionic compounds in addition to cationic compounds. However, there is little information on the possible transport of neurotransmitters by OCTNs. In 2012, Pochini *et al.* clarified that OCTN1 transports acetylcholine in both uptake and efflux directions *in vitro*.[27] Further studies are awaited to clarify the role of OCTN1 in the homeostasis of acetylcholine in neurons.

Recent findings have suggested the possible role of transporters other than in the system to maintain homeostasis of amino acid neurotransmitters in the brain. Various protective systems function to maintain cellular homeostasis in somatic cells including neurons. When neurons have been damaged by the onset of Alzheimer's disease or epileptic seizures, ABCA1 and P-gp are induced.[10,11] Because unnecessary and toxic compounds, which are not produced under normal conditions, are produced under pathological conditions, these efflux transporters may eliminate them from the intracellular space to protect neurons. OCTN1 may also be involved in the protection of cells from neurotoxicity by transporting neuroprotective compounds. The OCTN1 substrate antioxidant ERGO was reported to protect neurons from cytotoxicity induced by a variety of neurotoxins, including *N*-methyl-D-aspartate, β-amyloid, and cisplatin.[28–30] It was demonstrated that systemically administered ERGO is taken up by neurons *via* OCTN1 *in vivo*, supporting such a protective role of this transporter.[16] Interestingly, the expression of the OCTN1 gene product is increased in inflammatory tissues of peripheral organs.[31,32] Thus, OCTN1 may be induced as a system to delete reactive oxygen species, which are produced in high levels in inflammatory tissues, because the OCTN1 substrate ERGO is

a potent food-derived antioxidant. Although it has not yet been clarified whether OCTN1 is induced in neurons during cellular toxicity, the expression of OCTN1 is increased with neuronal maturation (unpublished data). Overall, regarding OCTs in the brain, OCT2 and -3 may be disease transporters to prevent neurotoxicity by eliminating excessive neurotransmitters in the synaptic cleft, whereas OCTN1 may protect neurons by the uptake of antioxidants under pathological conditions.

4. PHYSIOLOGICAL ROLES OF ORGANIC CATION TRANSPORTERS IN NEURAL STEM CELLS

Neural stem cells have self-renewal ability, as well as pluripotentiality to differentiate into neurons, astrocytes, and oligodendrocytes. It has recently been reported that xenobiotic transporters are also expressed in neural stem cells.[33–35] Neural stem cells actively repeat proliferation and differentiation in the developing brain, and thereby brain function is normally constructed. On the other hand, their proliferation and neuronal differentiation abilities decrease with brain maturation. Even in the adult brain of mammals including humans, however, it was shown that neural stem cells exist and neurogenesis occurs in the hippocampal dentate gyrus and subventricular zone.[36] In the adult brain, the proliferation and neuronal differentiation abilities in neural stem cells are generally low under normal conditions. However, when neurons are damaged, the abilities are activated to recover neuronal function in the damaged area.[37] Thus, the proper regulation of proliferation and neuronal differentiation of neural stem cells is essential for the maintenance and recovery of normal brain function. Proliferation and neuronal differentiation are regulated by extracellular signal regulatory molecules including neurotransmitters. Various neurotransmitter receptors and physiological transporters involved in neurotransmitter uptake are expressed in neural stem cells and regulate their proliferation and differentiation.[38–41] However, the regulation of proliferation and differentiation in neural stem cells by xenobiotic uptake transporters has not been clarified. Xenobiotic uptake transporters that regulate membrane permeation of their substrates may presumably control the intracellular concentration of compounds essential for proliferation and/or differentiation in neural stem cells and could be important candidates as intracellular environment regulatory molecules expressed on the cellular membrane.

We have recently reported that the expression level of OCTN1 mRNA was the highest among OCTs in neural stem cells[35] (Fig. 2A): Expression levels of OCTN2 and -3 mRNA were much lower than those of OCTN1, and mRNA for OCT1-3, MATE1, and PMAT was not detectable (<60 copies/μg total RNA). These results suggest that OCTN1 is a primary OCT, implying a specific role of this transporter in the regulation of cellular function in neural stem cells. The OCTN1-mediated uptake of ERGO in neural stem cells suppresses cellular proliferation *via* regulation of oxidative stress and promotes cellular differentiation into neurons by modulating the expression of basic helix-loop-helix transcription factors including Math1 *via* an unidentified mechanism different from antioxidant action.[35] When neural stem cells were cultured in medium containing growth factors, the expression of OCTN1 was induced in a time-dependent manner (Fig. 2B), with a concomitant increase in the number of cells[35] (Fig. 2C). Neural stem cells show low proliferative ability in the adult brain, but their proliferation is enhanced when neurons are damaged. Therefore the *in vitro* proliferation of neural stem cells may reflect the activated cells at the onset of neurological disorders.

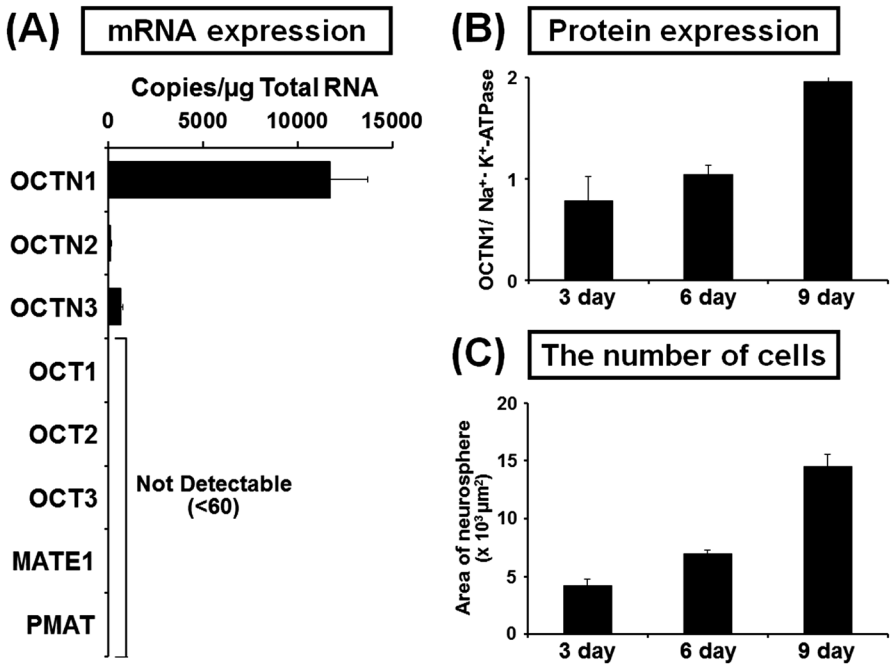

Fig. 2. Expression of OCTN1 in Cultured Neural Stem Cells

Neural stem cells derived from the mouse cerebral cortex were cultured in medium to which growth factors were added. (A) Total RNA was extracted from cortical neural stem cells cultured for 9 d *in vitro* (DIV) for absolute quantitative RT-PCR analysis. Each value represents mean±S.E.M. (n=3–5). (B) The membrane fraction was extracted by the centrifugation method, and the protein levels of OCTN1 in cortical neural stem cells cultured for 3 to 9 DIV were determined by Western blot analysis. Each value was normalized by the protein level of Na^+/K^+-ATPase and represents mean±S.E.M. (n=3). (C) Cortical neural stem cells were cultured for 3 to 9 DIV, and the area of neurospheres was quantified using ImageJ. Each value represents mean±S.E.M. (n=14). Figures were adapted from Ishimoto *et al.*[35]

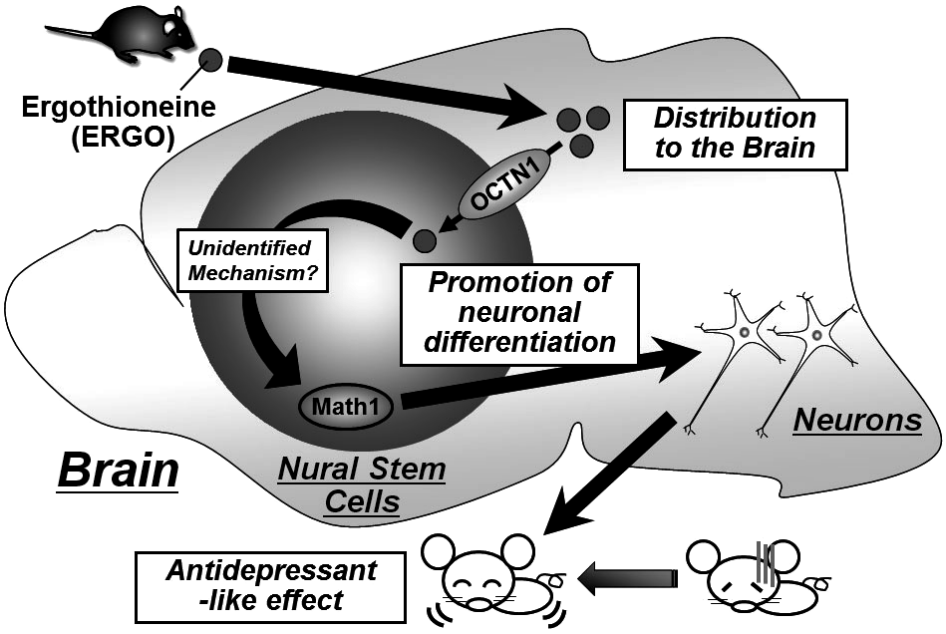

Fig. 3. Physiological Roles of OCTN1-Mediated Uptake of ERGO in the Brain

ERGO is highly distributed into the brain after oral ingestion, promotes cellular differentiation into neurons by inducing the expression of the neuronal differentiation activator gene Math1 *via* an unidentified mechanism in neural stem cells, and exerts an antidepressant-like effect.

5. POSSIBLE APPLICATION OF OCTN1 TO THE TREATMENT OF NEUROLOGICAL DISORDERS

The OCTN1 substrate ERGO is a potent food-derived antioxidant and present in mammal brain *in vivo* at the level of 0.2 to 1 mg per 100 g of tissue.[6,42] Systemically administered ERGO can be distributed widely to different brain regions, including the cerebellum, medulla and pons, hypothalamus, striatum, midbrain, hippocampus, and cerebral cortex.[16] The concentration of ERGO in each brain region is compatible with expression levels of OCTN1 in the brain,[16] and the distribution of ERGO to body organs including the brain is mainly governed by OCTN1.[6,43] The K_m values for ERGO of OCTN1 obtained in human embryonic kidney 293 cells overexpressing mouse and human OCTN1 were 4.68 and 21 μM, respectively.[6,44] In peripheral organs, *OCTN1* gene mutation is associated with susceptibility to Crohn's disease and rheumatoid arthritis,[6,8,9] strongly suggesting that OCTN1 may play a role in these inflammatory disorders. In addition, ERGO concentrations in erythrocytes and systemic blood of patients with rheumatoid arthritis and Crohn's disease, respectively, are different from those in individuals without these diseases,[6,45] suggesting that ERGO is primarily important in the association of OCTN1 with these diseases. In brain neurons, on the other hand, the OCTN1-mediated uptake of ERGO could play a protective role against the oxidative stress related to the development of various neurological disorders, including Alzheimer's, Parkinson's, and Huntington's diseases.[23,46] In addition, OCTN1 is functionally expressed in neural stem cells as well as neurons, and the OCTN1-mediated uptake of ERGO promotes differentiation of neural stem cells into neurons.[35] Taken together, the induction of OCTN1 and/or the ingestion of ERGO are conceivable as treatments for neurological disorders associated with OCTN1. We have recently reported that ERGO is highly distributed into the brain after the oral ingestion of an ERGO-containing diet, promotes neuronal differentiation, and exerts an antidepressant-like effect in mice[43] (Fig. 3). Song *et al.* showed that the oral ingestion of ERGO improves learning and memory abilities impaired by the administration of D-galactose in mice.[47] Furthermore, it has recently been reported that the concentration of ERGO is decreased in the serum of patients with Parkinson's disease[48] and in the blood of elderly individuals with mild cognitive impairment.[49] A decrease in the concentration of the antioxidant ERGO or change in the function/expression of OCTN1 may be in part involved in neurological disorders. Further studies are needed to clarify whether the OCTN1-mediated uptake of ERGO may alleviate symptoms of neurological disorders, including Alzheimer's and Parkinson's diseases and depression, and whether dysfunction of OCTN1 may be involved in these neurological disorders.

6. FUTURE PERSPECTIVE

We have discussed the possibility that xenobiotic uptake transporters including OCTN1 may be disease transporters in neurons and neural stem cells to play a neuroprotective role under pathological conditions. These transporters may contribute to the avoidance of neurotoxicity by eliminating excessive neurotransmitters in the synaptic cleft, the protection of neurons by antioxidant uptake, and the promotion of neuronal differentiation in neural stem cells, and consequently prevent the onset and/or development of neurological disorders. An exact understanding of the xenobiotic uptake transporters in the brain still requires experimental support. In particular, it is necessary to clarify which substances the xenobiotic uptake transporters transport as their endogenous substrates. The identification of such endogenous substrates would promote understanding of neurological disorders since they could contribute to the maintenance or breakdown of homeostasis in the brain. Future analyses using various neuropathological animal models and neurological disorder patients are expected to

clarify the physiological roles of xenobiotic uptake transporters expressed in neural cells and propose new points of view in the treatment of neurological disorders.

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Conflict of Interest The authors declare no conflict of interest.

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