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MINIREVIEW ARTICLE

Neuroprotective properties of the excitatory amino acid carrier 1 (EAAC1)

Koji Aoyama · Toshio Nakaki

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Abstract Extracellular glutamate should be maintained at low levels to conserve optimal neurotransmission and prevent glutamate neurotoxicity in the brain. Excitatory amino acid transporters (EAATs) play a pivotal role in removing extracellular glutamate in the central nervous system (CNS). Excitatory amino acid carrier 1 (EAAC1) is a high-affinity Na^+-dependent neuronal EAAT that is ubiquitously expressed in the brain. However, most glutamate released in the synapses is cleared by glial EAATs, but not by EAAC1 in vivo. In the CNS, EAAC1 is widely distributed in somata and dendrites but not in synaptic terminals. The contribution of EAAC1 to the control of extracellular glutamate levels seems to be negligible in the brain. However, EAAC1 can transport not only extracellular glutamate but also cysteine into the neurons. Cysteine is an important substrate for glutathione (GSH) synthesis in the brain. GSH has a variety of neuroprotective functions, while its depletion induces neurodegeneration. Therefore, EAAC1 might exert a critical role for neuroprotection in neuronal GSH metabolism rather than glutamatergic neurotransmission, while EAAC1 dysfunction would cause neurodegeneration. Despite the potential importance of EAAC1 in the brain, previous studies have mainly focused on the glutamate neurotoxicity induced by glial EAAT dysfunction. In recent years, however, several studies have revealed regulatory mechanisms of EAAC1 functions in the brain. This review will summarize the latest information on the EAAC1-regulated neuroprotective functions in the CNS.

K. Aoyama · T. Nakaki (✉)
Department of Pharmacology, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi, Tokyo 173-8605, Japan
e-mail: nakaki@med.teikyo-u.ac.jp

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Introduction

Glutamate is the major excitatory amino acid and is widely distributed in the brain (Danbolt 2001). The main role of glutamate is considered to be acting as a major excitatory neurotransmitter in the central nervous system (CNS) (Fonnum 1984). The extracellular glutamate is maintained at low levels, not only for enhancing the signal-to-noise ratio in synaptic neurotransmission, but also for preventing excessive activation of *N*-methyl-D-aspartate (NMDA) receptors, which are toxic to neurons (Meldrum 2000). Glutamate can neither be degraded extracellularly nor penetrate the cell membrane. Therefore, glutamate uptake from the synaptic cleft by transporters is the most rapid way to decrease the extracellular glutamate level. In the previous studies, dysfunction of glial, but not neuronal, glutamate transporters has been thought to increase extracellular glutamate levels, leading to neurodegeneration. Some neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), or amyotrophic lateral sclerosis (ALS), have been suggested to have a pathogenesis related to glutamate neurotoxicity (Choi 1988; Olanow and Tatton 1999; Olney and de Gubareff 1978; Perry and Hansen 1990; Pomara et al. 1992; Rothstein et al. 1992). Moreover, selective loss of a glial glutamate transporter was found in patients with ALS (Fray et al. 1998; Milton et al. 1997; Rothstein et al. 1995). These findings have called attention to dysfunction of glial, but not neuronal, glutamate transporters in the etiology of neurodegenerative diseases. However, recent studies show that dysfunction of a neuronal glutamate

transporter, excitatory amino acid carrier 1 (EAAC1), is also involved in neurodegeneration by a mechanism separate from glutamate neurotoxicity (Aoyama et al. 2006; Berman et al. 2011; Cao et al. 2012). In this review, we will discuss the neuroprotective role of EAAC1 along with recent achievements, including clarification of the regulatory mechanisms of EAAC1 functions.

Excitatory amino acid transporters in the central nervous system

Glutamate is present intracellularly in the brain with a concentration of approximately 1–10 mM (Persson and Ronnback 2012), which is much higher than that of other amino acids (Danbolt 2001), while the extracellular glutamate is maintained at ~3–4 μM (Danbolt 2001). Glutamate is synthesized from glutamine in neuronal mitochondria and stored in the pre-synaptic terminals (Daikhin and Yudkoff 2000). The glutamate concentration inside synaptic vesicles is thought to be at least 60 mM (Burger et al. 1989; Shupliakov et al. 1992). Once stimulated, neurons release glutamate in the synaptic cleft at a peak concentration of 1.1 mM (Danbolt 2001). The glutamate released by pre-synaptic vesicles acts on glutamate receptors postsynaptically on the plasma membrane. To optimize glutamatergic neurotransmission and avoid glutamate neurotoxicity, the extracellular glutamate is cleared from the synaptic cleft within 1 ms after the release (Clements 1996; Clements et al. 1992; Diamond and Jahr 1997) by glutamate transporters. In the brain, there are high- and low-affinity glutamate transport systems of which the Km values are 1–100 μM and above 500 μM, respectively (Danbolt 2001). In particular, the high-affinity Na^+-dependent glutamate transporters known as excitatory amino acid transporters (EAATs) play a pivotal role in removing extracellular glutamate in the CNS. To date, five EAATs have been cloned: EAAT1 through 5 (Arriza et al. 1997; Fairman et al. 1995; Kanai and Hediger 1992; Pines et al. 1992; Storck et al. 1992). EAAT1–3 are expressed widely throughout the brain; EAAT1 (also called GLAST) and EAAT2 (also called GLT-1) are expressed by glia cells, while EAAT3 (also called EAAC1) is exclusively expressed by mature neurons (Kanai et al. 1995; Rothstein et al. 1994). EAAT4 and EAAT5 are expressed by Purkinje neurons in the cerebellum and neurons in the retina, respectively. EAAT forms a trimeric complex, which co-transports anionic amino acid with three Na^+ and one H^+ while counter-transporting one K^+ (Had-Aissouni 2012a, b). This transport system can maintain more than a 10^6-fold (glutamate) gradient across the plasma membrane under equilibrium conditions (Zerangue and Kavanaugh 1996b).

Neuronal vulnerability to glutamate toxicity is 100-fold greater in astrocyte-poor culture than in astrocyte-rich culture (Rosenberg and Aizenman 1989). Astrocytes take up approximately 50 % of human brain volume (Tower and Young 1973) and exert a critical influence on extracellular glutamate clearance, which is one of the most important role of astrocytes for neuronal survival under insulted conditions (Chen and Swanson 2003). In the brain, astroglial EAATs, such as GLAST and GLT-1, play a central role in removing interstitial glutamate. In particular, most of the uptake activity depends on GLT-1 in the CNS (Grewer and Rauen 2005; Holmseth et al. 2012; Tanaka et al. 1997). The glutamate uptake activity in liposomes prepared from GLT-1-knockout mouse brains was only ~2 % of that from the wild type, suggesting an absolute contribution of GLT-1 to glutamate uptake in the synaptic terminals (Holmseth et al. 2012). Although microglia also express messenger RNA for both GLAST and GLT-1 (Kondo et al. 1995), they express EAATs only under pathological conditions, and not under physiological conditions, in vivo (Persson and Ronnback 2012). Mature neurons in vivo do not express either GLAST or GLT-1, but do express EAAC1. The EAAC1 protein level is the highest in the hippocampus, followed by the cortex, striatum, thalamus, cerebellum, and midbrain, and is lowest in the spinal cord (Rothstein et al. 1994). In the hippocampus, the EAAC1 protein level is almost double that in the striatum or thalamus, and three to four times higher than that in the cerebellum or midbrain (Holmseth et al. 2012). The distribution pattern of EAAC1 in the cells is different from that of glial EAATs (Furuta et al. 1997; Rothstein et al. 1994). The analysis of subcellular localization revealed that EAAC1 was present in the neuronal soma and dendrites, but not in the axons or synaptic terminals (Coco et al. 1997; Holmseth et al. 2012; Rothstein et al. 1994; Shashidharan et al. 1997). This finding suggests a negligible contribution of EAAC1 to glutamate transport in the synaptic terminals, although it is plausible that EAAC1 has non-synaptic functions in neurons.

High extracellular glutamate levels would cause epilepsy and neuronal cell death (During and Spencer 1993; Tanaka et al. 1997). The loss of either GLAST or GLT-1 protein using antisense oligonucleotide (ODN) was shown to be toxic to neurons in vitro and caused markedly elevated extracellular glutamate levels in rat brains intraventricularly administered antisense ODNs; the rats showed some abnormal behaviors mainly related to motor symptoms, but not seizures (Rothstein et al. 1996). In contrast to GLAST or GLT-1 antisense ODN, EAAC1 antisense ODN was not toxic to neurons in vitro and did not affect extracellular glutamate levels by intraventricular administration to the rat brains (Rothstein et al. 1996). Interestingly, the rats administered EAAC1 antisense ODN showed seizures

(Rothstein et al. 1996). Subsequent studies have reported mild motor discoordination in GLAST-deficient mice (Watase et al. 1998), and lethal spontaneous seizures in GLT-1-deficient mice (Tanaka et al. 1997), whereas neither motor discoordination nor epilepsy was observed in EAAC1-deficient mice (Peghini et al. 1997). In the rat seizure models, the expression of EAAC1 mRNA or protein increased (Doi et al. 2009; Lu et al. 2008; Ross et al. 2011) or decreased (Simantov et al. 1999) in the brains. Even in patients with epilepsy, the expression of EAAC1 has varied widely among studies (Crino et al. 2002; Mathern et al. 1999; Proper et al. 2002; Rakhade and Loeb 2008). It is still unclear whether EAAC1 dysfunction is involved in the mechanisms of epilepsy and glutamate-induced neurotoxicity.

Ischemic preconditioning, which is a brief period of sublethal ischemia, induces adaptive responses to protect the brain from subsequent, otherwise lethal ischemic injury (Kirino 2002; Nandagopal et al. 2001). Previous studies have demonstrated several mechanisms different from the cell types (Garnier et al. 2003; Kato et al. 1994; Trendelenburg and Dirnagl 2005). EAATs might be also involved in these mechanisms showing down-regulation of GLAST and GLT-1 expressions in ischemic preconditioning (Douen et al. 2000). Indeed, reversal of glutamate transport via EAATs during ischemia exacerbates neurotoxicity in the brain (Phillis et al. 2000; Rossi et al. 2000). Some studies also showed reducing ischemia-evoked glutamate release and glutamate-induced neurotoxicity by the pretreatment with EAAT inhibitors (Marini and Novelli 1991; Phillis et al. 2000). However, other studies showed conflicting results of EAAT expressions in ischemic preconditioning (Bigdeli et al. 2008, 2009). Moreover, the rats administered GLT-1 antisense ODN exacerbated transient focal ischemia-induced neuronal damage (Rao et al. 2001) and EAAC1-deficient mice subjected to transient cerebral ischemia exhibited twice as much neuronal death as wild-type mice (Won et al. 2010). Precise mechanisms of glutamate-induced neurotoxicity mediated by EAATs during brain ischemia are still elusive.

The role of EAAC1 for glutathione synthesis in the brain

EAATs can transport not only extracellular glutamate but also cysteine into the cells (Zerangue and Kavanaugh 1996a). In an in vitro study, the Km values of cysteine transport system were 1,830, 967 and 193 μM, and the ratios of maximum current induced by cysteine to that induced by glutamate were 0.79, 0.59, and 1.28 for GLAST, GLT-1, and EAAC1, respectively (Zerangue and Kavanaugh 1996a). An early study showed that oral cysteine administration induced brain damage in vivo (Olney and Ho 1970). Subsequent studies revealed that cysteine has excitotoxic properties similar to glutamate (Lehmann et al. 1993; Olney et al. 1972, 1990). Elevated extracellular cysteine levels induce non-vesicular glutamate release and inhibit glutamate reuptake in neurons, leading to excitotoxic activation of NMDA receptors (Zerangue and Kavanaugh 1996a). EAATs are the major route for cysteine transport in both neurons and astrocytes. EAAC1 in particular mediates 70–80 % of neuronal cysteine uptake (Shanker et al. 2001). These results indicate that EAAC1 might play a critical role as a cysteine transporter, rather than a glutamate transporter, in the brain.

Cysteine is an important substrate for glutathione (GSH) synthesis in the brain (Aoyama et al. 2008, 2012b). GSH has a variety of intracellular functions as an antioxidant, an enzyme co-factor, and a modulator of redox signaling, cell proliferation, and cell differentiation (Aoyama et al. 2008; Dringen 2000; Schafer and Buettner 2001). GSH plays particularly essential roles as an antioxidant for neuroprotection in the brain (Fig. 1). The brain GSH concentration is approximately 2–3 mM, which is higher than that in blood (~15 μM) or cerebrospinal fluid (~5 μM) and lower than that in the liver (7–8 mM) (Commandeur et al. 1995; Cooper and Kristal 1997). GSH is composed of glutamate, cysteine, and glycine with the reactions of two enzymatic steps. In neurons, the intracellular cysteine level is considered the rate-limiting precursor for GSH synthesis (Dringen et al. 1999). Knockdown of EAAC1 by antisense ODN reduced both neuronal cysteine uptake and intracellular GSH levels, while it increased hydrogen peroxide (H_2O_2) vulnerability in neurons (Himi et al. 2003). EAAC1-deficient mice showed brain atrophy, spatial learning/memory dysfunction, loss of dopaminergic neurons in the substantia nigra and movement disorder at senescence, but not at adolescence (Aoyama et al. 2006; Berman et al. 2011). In another study, even young EAAC1-deficient mice showed cognitive dysfunction in some learning/memory tasks, compared with age-matched control mice (Lee et al. 2012). Brain GSH levels were lower, and brain damage by oxidative stress was greater in EAAC1-deficient mice than in wild-type mice (Aoyama et al. 2006; Berman et al. 2011; Cao et al. 2012; Won et al. 2010). These aberrant behavioral and biochemical changes in EAAC1-deficient mice were attenuated by the treatment with *N*-acetylcysteine (NAC), which is a membrane permeable cysteine precursor for GSH synthesis (Aoyama et al. 2006; Berman et al. 2011; Cao et al. 2012; Jang et al. 2012). These results suggest a critical role of EAAC1 in maintaining neuronal GSH levels against neurodegeneration.

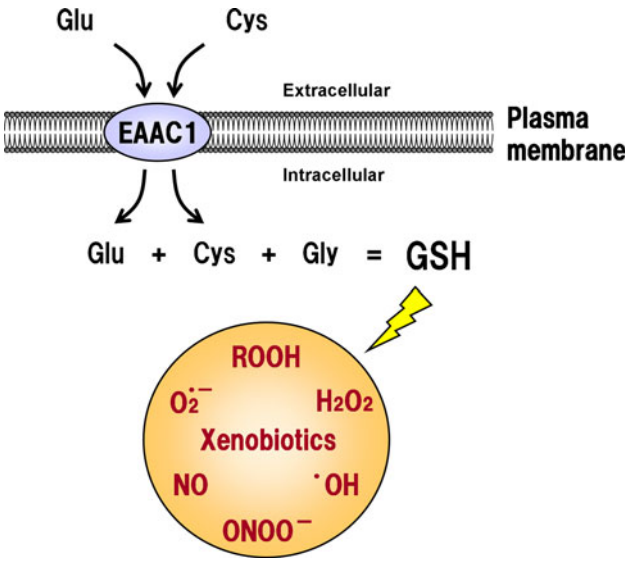

Fig. 1 Schematic representation of neuronal glutathione (GSH) synthesis. GSH consists of three amino acids, i.e., glutamate (Glu), cysteine (Cys), and glycine (Gly). Cys uptake through excitatory amino acid carrier 1 (EAAC1) is the rate-limiting step for neuronal GSH synthesis. GSH can non-enzymatically detoxify superoxide ($O_2^{\cdot-}$), nitric oxide (NO), hydroxyl radical ($^{\cdot}OH$), and peroxynitrite ($ONOO^-$), while it enzymatically degrade hydroperoxides (ROOH), hydrogen peroxide (H_2O_2), and xenobiotics

Glutathione as an important antioxidant in the brain

The brain, which accounts for only 2 % of body weight, requires 20 % of the total oxygen consumed by the body. Mitochondria generate superoxide from oxygen in the process of ATP production (Sas et al. 2007). Under normal physiological conditions, superoxide is catalyzed by superoxide dismutase (SOD) to produce H_2O_2, which is then degraded to O_2 and H_2O by GSH peroxidase (GPx). This intracellular antioxidant system maintains both superoxide and H_2O_2 levels as low as around 10^{-10} M and 10^{-9}–10^{-8} M, respectively (Cadenas and Davies 2000; Chance et al. 1979). However, under insulted conditions, mitochondrial dysfunction leads to increases in both superoxide and H_2O_2 production (Coyle and Puttfarcken 1993; Turrens 2003). Activation of NADPH oxidase also induces superoxide production, leading to neuronal death (Suh et al. 2007). H_2O_2 reacts with Fe^{2+} to form hydroxyl radicals, which are highly oxidizing radicals within cells (Halliwell 1992). Furthermore, the activation of NMDA receptors elicits elevation of intracellular Ca^{2+} levels, leading to nitric oxide (NO) synthase (NOS) activation and NO production. The brain NO concentration is below 10^{-8} M at baseline and increases at a 100-fold greater rate under insulted conditions (Cherian et al. 2000; Malinski et al. 1993). Neither superoxide nor NO is a potent oxidant in vivo; however, superoxide can react with NO to produce the toxic oxidant peroxynitrite (Pacher et al. 2007; Szabo et al. 2007). The rate of peroxynitrite formation by the reaction of superoxide and NO is elevated in a synergistic manner; i.e., a tenfold increase in superoxide, and NO production will increase the rate of peroxynitrite formation 100-fold (Pacher et al. 2007). Peroxynitrite has a variety of cytotoxic effects mediated by lipid peroxidation, protein nitration and oxidation, DNA damage, and enzyme inactivation (Pacher et al. 2007). In addition, the decomposition of peroxynitrite produces hydroxyl and nitrogen dioxide radicals (Szabo et al. 2007). Peroxynitrite can react with several amino acids, i.e., tyrosine, cysteine, tryptophan, methionine, and histidine, leading to modification of the protein structure and function (Pacher et al. 2007). In particular, tyrosine nitration or cysteine oxidation of certain critical residues in the proteins causes inactivation of enzymes or impaired signal transduction (Pacher et al. 2007). Indeed, peroxynitrite potently inhibits EAAT activity dose-dependently (50 % inhibition at 50 μM) (Trotti et al. 1996). GSH can non-enzymatically react with superoxide, NO, hydroxyl radical, and peroxynitrite, and can enzymatically react with H_2O_2 and other hydroperoxides via GPx to prevent oxidative damage in the brain (Aoyama et al. 2008, 2012b). GSH also removes endogenous xenobiotics from the cell to form GSH-*S*-conjugates by GSH-*S*-transferase (Commandeur et al. 1995). Considering the comprehensive capacity of GSH against these multiple targets, inducing oxidative stress, GSH has a fundamental role as a first-line antioxidant in the brain.

Neurodegenerative diseases induced by EAAC1 dysfunction

Some neurodegenerative diseases showed GSH depletion and increased levels of oxidative stress in the CNS. GSH depletion would precede neurodegeneration via oxidative stress (Jenner 1994, 2003). In an in vitro study, decreased GSH levels with elevated ROS levels were found in primary neurons prepared from a mouse model of HD ($HD^{140Q/140Q}$) in which a human *huntingtin* gene with 140 *CAG* repeats was inserted into the mouse genome (Li et al. 2010). This study also demonstrated that these results were attributable to EAAC1 dysfunction, which led to impaired cysteine uptake into the neurons. In a more recent study, a transgenic mouse model of AD showed decreased EAAC1 content in the hippocampus (Cassano et al. 2012). Similarly, a study on AD patients found that degenerating hippocampal neurons exhibited aberrant EAAC1 accumulation in the cell bodies (Duerson et al. 2009). These findings support the notion of EAAC1 dysfunction in AD brains. Indeed, a recent study using magnetic resonance spectroscopy demonstrated that brain GSH levels were depleted in AD patients as compared to healthy subjects

(Mandal et al. 2012). Human dopaminergic (DA) neurons in the substantia nigra have also been shown to express EAAC1 (Berman et al. 2011; Plaitakis and Shashidharan 2000). DA neurons are more vulnerable to EAAC1 dysfunction than non-DA neurons (Nafia et al. 2008). Finally, in PD patients, the brain GSH level was shown to be depleted in the substantia nigra, but not in the other regions, as compared to that of age-matched controls (Sian et al. 1994). These results suggest an involvement of EAAC1 dysfunction leading to GSH depletion in neurodegenerative diseases, although further clinical evidences will be required.

Regulation of EAAC1

EAAC1 is constitutively expressed on and off the plasma membrane with a half-life of approximately 5–7 min for residence at the plasma membrane (Fournier et al. 2004). Under a steady state, approximately 20 % of the total EAAC1 proteins are expressed on the plasma membrane in vitro (Fournier et al. 2004). EAAC1 works as a transporter on the plasma membrane, and thus its transport activity seems to depend on the cell surface expression, rather than the total protein amount in the cell (Davis et al. 1998). Phorbol 12-myristate 13-acetate (PMA), a protein kinase C (PKC) activator, induces translocation of EAAC1 to the plasma membrane (Fournier et al. 2004). PKCα activation induces phosphorylation of serine 465 in EAAC1 to increase the transport activity and the redistribution to the cell surface, while PKCε activation mediates the increase of glutamate transport activity but not the translocation to the plasma membrane (Gonzalez et al. 2002; Huang et al. 2006) (Fig. 2). PKCδ, another PMA-sensitive PKC subtype, was found not to be involved in the regulation of EAAC1 (Gonzalez et al. 2002). Inhibition of phosphatidylinositol 3-kinase (PI3K) blocks PMA-stimulated glutamate uptake and EAAC1 translocation to the plasma membrane (Davis et al. 1998), although PKC and PI3K independently regulate the cell surface expression of EAAC1. Platelet-derived growth factor (PDGF) also increases both the activity and cell surface expression of EAAC1 through the PI3K pathway, but not the PKC pathway (Sims et al. 2000). Regulation of EAAC1 trafficking by PDGF requires the C-terminal domain ^{502}YVN^{504} of EAAC1 (Sheldon et al. 2006) and is also mediated by Akt, known as protein kinase B (PKB), which is a downstream target of PI3K (Krizman-Genda et al. 2005). PI3K also activates serum- and glucocorticoid-inducible kinase 1 (SGK1) and Akt/PKB through phosphoinositide-dependent kinase 1 (PDK1) activation. The constitutively active SGK1 and Akt/PKB stimulate glutamate uptake by EAAC1 in vitro (Schniepp et al. 2004). *PDK1*-knockdown mice showed a decreased expression of EAAC1 in the kidney (Rexhepaj et al. 2006). EAAC1 is also up-regulated by the serine/threonine kinase mammalian target of rapamycin (mTOR) (Almilaji et al. 2012), or Janus-activated tyrosine kinase-2 (JAK-2) (Hosseinzadeh et al. 2011), both of which play a critical role in cell growth and proliferation (Hay and Sonenberg 2004; Imada and Leonard 2000). In *Xenopus* oocytes expressing EAAC1, injection with cRNA encoding mTOR or JAK-2 enhanced the glutamate-induced current concomitant with the increased expression of EAAC1 on the plasma membrane (Almilaji et al. 2012; Hosseinzadeh et al. 2011). Conversely, both the glutamate-induced current and the EAAC1 expression on the plasma membrane were reduced by expression of the constitutively active AMP-activated protein kinase (AMPK) in *Xenopus* oocytes expressing EAAC1 (Sopjani et al. 2010). Although the precise regulatory mechanism of AMPK is still unclear, EAAC1 has the typical AMPK consensus sequences as the targets for phosphorylation (Sopjani et al. 2010; Towler and Hardie 2007). These results indicate that EAAC1 is regulated by various signal transduction pathways mediated by protein phosphorylation.

EAAC1 is also regulated by protein–protein interactions (Fig. 3). RTN2B, a member of the reticulon family, is generally located in the endoplasmic reticulum (ER) (Liu et al. 2008). The NH_2-terminal domain of RTN2B interacts with EAAC1 to facilitate the trafficking of EAAC1 from ER to the cell surface and increase the glutamate uptake activity (Liu et al. 2008). EAAC1 is recycled on and off the

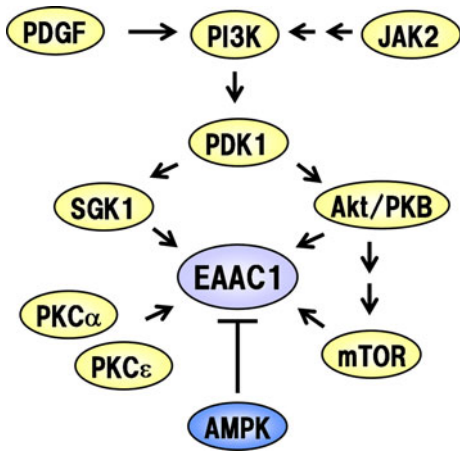

Fig. 2 Signaling cascades with stimulatory (*right arrow*) and inhibitory (⊣) modifications for EAAC1 activity. The abbreviations are as follows: platelet-derived growth factor (PDGF), phosphatidylinositol 3-kinase (PI3K), Janus-activated tyrosine kinase-2 (JAK-2), phosphoinositide-dependent kinase 1 (PDK1), serum- and glucocorticoid-inducible kinase 1 (SGK1), Akt/protein kinase B (Akt/PKB), protein kinase C (PKC), mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK), excitatory amino acid carrier 1 (EAAC1)

plasma membrane in a small GTP-binding protein Rab11-dependent manner (Gonzalez et al. 2007b). Rab11 activation was shown to induce the cell surface expression of EAAC1 together with both increases in cysteine uptake and GSH levels, leading to neuroprotection against oxidative stress (Li et al. 2010). Neuronal plasma membrane transporters could be regulated by lipid rafts, which are detergent-resistant, low-density regions as important locales for vesicle fusions on the plasma membrane (Head and Insel 2007; Ikonen 2001). The soluble *N*-ethylmaleimide-sensitive attachment protein receptor (SNARE) family, a component of lipid rafts, facilitates the fusion of transport vesicles to the plasma membrane (Chen and Scheller 2001). Synaptosomal-associated protein of 23 kDa (SNAP-23), a member of the SNARE complex, is required for constitutive recycling of EAAC1 (Fournier and Robinson 2006). Caveolin-1, another structural component of lipid rafts, also regulates EAAC1 trafficking (Gonzalez et al. 2007a). Caveolin-1 interacts with EAAC1 to facilitate both the delivery and endocytosis of EAAC1 to and from the plasma membrane, although further studies are required to clarify precise mechanisms of interaction between caveolin-1 and EAAC1 (Gonzalez et al. 2007a). Protein interaction also negatively regulates EAAC1 translocation to the cell surface. Glutamate transport associated protein 3-18 (GTRAP3-18) is also an ER protein, which was isolated from the rat brain by a yeast two-hybrid screen system as an EAAC1-interacting protein (Lin et al. 2001). GTRAP3-18 is a member of the prenylated Rab acceptor (PRA) family (Abdul-Ghani et al. 2001) and retains EAAC1 in the ER to impede EAAC1 trafficking to the plasma membrane (Ruggiero et al. 2008). The C-terminal domain of GTRAP3-18 has a weak coiled-coil formation for protein–protein interaction (Abdul-Ghani et al. 2001), which causes GTRAP3-18 to bind to the C-terminal domain of EAAC1 (Lin et al. 2001). GTRAP3-18 also directly binds to RTN2B to inhibit the effects of RTN2B on EAAC1 (Liu et al. 2008). Furthermore, as other PRA members (Abdul-Ghani et al. 2001), GTRAP3-18 can interact with Rab1, which promotes the ER-Golgi transport of cargo proteins, and then inhibits Rab1-controlled trafficking of EAAC1 (Maier et al. 2009). Our recent study demonstrated that GTRAP3-18-deficient mice showed an increased expression of EAAC1 on the plasma membrane and GSH contents in neurons (Aoyama et al. 2012a). Brain slices from GTRAP3-18-deficient mice were tolerant to oxidative stress. Negative regulation of GTRAP3-18 may potentiate EAAC1 function to increase cysteine uptake, leading to GSH synthesis in neurons. Notably, GTRAP3-18-deficient mice showed better performances at forced motor/spatial learning and memory tests compared to age-matched wild-type mice (Aoyama et al. 2012a). GTRAP3-18 is reported to interact not only with EAAC1 but also with other transporters and receptors for various neurotransmitters (Ruggiero et al. 2008). GTRAP3-18-deficient mice might have facilitated learning/memory functions not mediated by EAAC1 but by other unknown mechanisms. Conversely, GTRAP3-18 is negatively regulated by the direct interaction with ADP-ribosylation factor-like 6 interacting protein 1 (Arl6ip1), leading to reduced interaction between GTRAP3-18 and EAAC1 in neurons (Akiduki and Ikemoto 2008). The δ-opioid receptor (DOR) is a G-protein-coupled receptor and directly modulates EAAC1 functions (Xia et al. 2006). DOR can directly interact with EAAC1 to reduce the glutamate uptake activity without changing EAAC1 expression on the plasma membrane, while DOR activation releases the interaction to facilitate EAAC1 activity (Xia et al. 2006). The endogenous modulation of protein–protein interactions might be a critical strategy for enhancing EAAC1 functions leading to neuronal GSH synthesis.

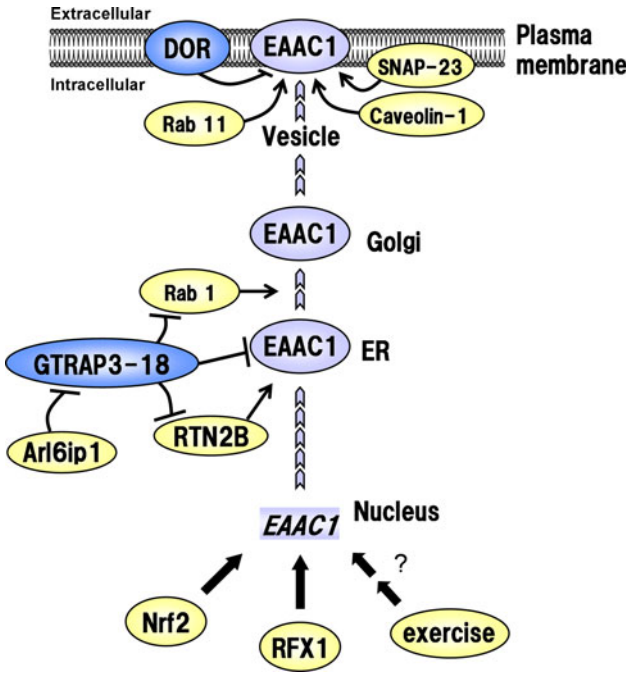

Fig. 3 Regulatory mechanisms of excitatory amino acid carrier 1 (EAAC1). The gene transcriptional expression of EAAC1 is stimulatingly (*thick arrow*) regulated by nuclear factor erythroid 2-related factor 2 (Nrf2), regulatory factor X1 (RFX1), and exercise. EAAC1 translocation to the plasma membrane through the endoplasmic reticulum (ER) and Golgi body is induced by Reticulon 2B (RTN2B), Rab1, Rab11, and the synaptosomal-associated protein of 23 kDa (SNAP-23). Caveolin-1 interacts with EAAC1 to facilitate both the delivery and endocytosis of EAAC1 to and from the plasma membrane. Arl6ip5 also induces the EAAC1 translocation, but does so indirectly through the inhibitory (⊣) modification for glutamate transport associated protein 3-18 (GTRAP3-18), which negatively regulates EAAC1 trafficking to the plasma membrane. The δ-opioid receptor (DOR) directly interacts with EAAC1 to reduce the glutamate uptake activity without changing EAAC1 expression on the plasma membrane

The gene transcription and protein expression of *GLT-1*, but not *GLAST* or *EAAC1*, in the CNS are induced by ceftriaxone, a β-lactam antibiotic, through the nuclear factor-κB signaling pathway (Lee et al. 2008; Rothstein et al. 2005), while the gene transcription of *EAAC1*, but not that of *GLAST* or *GLT-1*, is up-regulated by exercise (Molteni et al. 2002). *EAAC1* gene transcription is also up-regulated by activation of the nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant responsive element (ARE) pathway under oxidative stress conditions (Escartin et al. 2011). The regulatory factor X1 (RFX1), a transcriptional factor that exists in the promoter region of *EAAC1*, but not *GLAST* or *GLT-1*, increases EAAC1 protein expression and glutamate uptake in vitro (Ma et al. 2006). These findings suggest that EAAC1 is subject to certain gene transcription regulations that do not exist for other types of EAATs.

Conclusions

EAAC1 is an important neuronal transporter for both glutamate and cysteine in the brain. Neuronal cysteine uptake is the rate-limiting step for GSH synthesis, and dysfunction of EAAC1 induces neurodegeneration by neuronal GSH depletion rather than by glutamate neurotoxicity. Considering the neuroprotective functions of GSH in the brain, regulatory mechanisms facilitating EAAC1 functions would be promising for the treatment of neurodegenerative diseases.

Conflict of interest The authors declare that they have no conflict of interest.

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