Glutamate and GABA synthesis, release, transport and metabolism as targets for seizure control

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ABSTRACT

The synthesis, release, reuptake, and metabolism of the excitatory and inhibitory neurotransmitters glutamate and GABA, respectively, are tightly controlled. Given the role that these two neurotransmitters play in normal and abnormal neurotransmission, it is important to consider the processes whereby they are regulated. This brief review is focused entirely on the metabolic aspects of glutamate and GABA synthesis and neurotransmission. It describes in limited detail the synthesis, release, reuptake, metabolism, cellular compartmentation and pharmacology of the glutamatergic and GABAergic synapse. This review also provides a summary and brief description of the pathologic and phenotypic features of the various genetic animal models that have been developed in an effort to provide a greater understanding of the role that each of the aforementioned metabolic processes plays in controlling excitatory and inhibitory neurotransmission and how their use will hopefully facilitate the development of safer and more efficacious therapies for the treatment of epilepsy and other neurological disorders.

1. Introduction

The two most important neurotransmitters in the brain are glutamate and GABA. These two neurotransmitters control excitatory and inhibitory neurotransmission, respectively (Hassel and Dingledine, 2006; Olsen and Betz, 2006; Schousboe and Waagepetersen, 2008). Alteration of the balance in neurotransmission can contribute to increased or decreased seizure activity (Schousboe and White, 2009). Since the mainstay of seizure control resides largely with pharmacotherapy, it is not surprising that a number of available therapies either directly or indirectly shift the balance away from excitatory neurotransmission in favor of inhibitory neurotransmission. Neurotransmission typically involves biosynthesis, packaging, release, receptor interaction and inactivation of the neurotransmitter. The present review will focus on a discussion of the potential therapeutic targets associated with glutamate and GABA homeostasis, i.e. the pertinent metabolizing enzymes and the plasma membrane-associated high affinity transporters.

2. Enzymes involved in glutamatergic and GABAergic neurotransmission

Enzymes that catalyze reactions in which glutamate is either a substrate or a product include amino transferase, glutamate dehydrogenase, glutaminase, and glutamine synthetase. Amino transferases always utilize glutamate and α-ketoglutarate (αKG) as a pair of substrates together with another amino acid and its corresponding keto-acid (Fig. 1). Examples of pertinent aminotransferases are aspartate aminotransferase (AAT) and alanine aminotransferase (ALAT). Of these, AAT plays an important role in mitochondrial glutamate metabolism (McKenna et al., 2006).

2.1. Biosynthesis of glutamate

Glutamate dehydrogenase (GDH) catalyzes the reaction between glutamate, αKG, and ammonia using NAD⁺ or NADP⁺ as the co-enzyme (McKenna et al., 2006). In the brain, this reaction normally proceeds as an oxidative deamination, i.e. glutamate is oxidized to αKG and ammonia (Cooper et al., 1979; Schousboe and Waagepetersen, 2007). However, in glutamatergic neurons in which there may be a high content of ammonia in discrete microenvironments of the mitochondria (see below), the reaction may constitute reductive amination, i.e. formation of glutamate from αKG and ammonia (Bak et al., 2005; Waagepetersen et al., 2000).
Glutaminase, which is often referred to as phosphate activated glutaminase (PAG) since it is stimulated by phosphate, catalyzes the hydrolysis of the amide group of glutamine to form glutamate and ammonia (Kvamme et al., 2001). The activity and expression of PAG is higher in neurons than in astrocytes with the highest values in glutamatergic neurons (Hogstad et al., 1988; Zaganas et al., 2001). It should be noted that an immunohistochemical study of the cerebellum found PAG immunoreactivity only in neuronal elements (Laake et al., 1999).

Glutamine synthetase (GS) catalyzes the reverse reaction, which is an ATP-dependent reaction that is responsible for the formation of glutamine from glutamate and ammonia (Fig. 1). Importantly, this enzyme is exclusively expressed in astrocytes (Norenberg and Martinez-Hernandez, 1979), making both glutamatergic and GABAergic neurons dependent on astrocytic delivery of the precursor glutamine for transmitter biosynthesis (Bak et al., 2006).

2.2. Pharmacology of glutamate metabolizing enzymes

2.2.1. PAG

The glutamate synthesizing enzyme PAG is highly regulated. PAG is activated not only by phosphate but also by phosphorylated compounds such as trinucleotides (Well-Malherbe, 1972). Interestingly, PAG is also activated by the TCA cycle intermediates succinate and citrate, albeit to a lesser extent than by phosphate (Kvamme et al., 1988). It is also of physiological and functional importance that this enzyme is inhibited by its products, ammonia and glutamate. Interestingly, the inhibition by ammonia is much less pronounced for the astrocytic than the neuronal enzyme (Hogstad et al., 1988; Kvamme et al., 1982). It is of pharmacological interest that the compound 6-diazo-5-oxo-L-norleucine (DON) is an efficacious inhibitor of PAG (Shapiro et al., 1979) and can be used to investigate the functional importance of PAG in glutamatergic and GABAergic neurotransmission (Bradford et al., 1989; Conti and Minelli, 1994). In the present context it is of interest that DON has been shown to protect seizure-susceptible mice from convulsions (Chung and Johnson, 1984).

2.2.2. GDH

GDH is important in glutamatergic and GABAergic neurotransmission as it directly regulates the glutamate concentration and indirectly modulates GABA levels by altering the availability of precursors. GDH is potently inhibited by GTP and activated by ADP (Plaitakis and Zaganas, 2001). The amino acid leucine also activates GDH, an effect that is synergistic with ADP (Plaitakis and Zaganas, 2001). While it may not be pharmacologically relevant, it is of considerable interest that GDH is inhibited by antipsychotic drugs such as chlorpromazine and haloperidol (Shemisa and Fahien, 1971). It appears, however, that the potency of these drugs as inhibitors of GDH is quite low relative to blood levels observed in patients treated with chlorpromazine and haloperidol (Couee and Tipton, 1990). It must be kept in mind that, in addition to the ubiquitous GDH1 isozyme, humans express a unique GDH2 isoform (Plaitakis and Zaganas, 2001) that exhibits a higher sensitivity to haloperidol. In this example, haloperidol may affect GDH in humans at clinically relevant blood levels (Plaitakis et al., 2011).

2.2.3. GS

As stated above, GS is a key enzyme in the glutamate-glutamine cycle and plays a major role in the brain homeostasis of glutamate, glutamine and ammonia. Hence, compounds that inhibit this enzyme are of potential importance for studies that aim to elucidate the functional role of GS in glutamatergic and GABAergic neurotransmission. The most widely used compound to selectively inhibit GS is 1-methionine sulfoximine (MSO), which at a concentration of 5 mM in the presence of ATP almost completely inhibits the
2.4. Biosynthesis of GABA

GABA metabolism is less complex than that of glutamate. As first demonstrated by Roberts and Frankel (Roberts and Frankel, 1950), GABA is synthesized by decarboxylation of glutamate by glutamic acid decarboxylase (GAD). GAD exists in two isoforms, GAD65 and GAD67, which have different molecular weights (65 and 67 KDa), catalytic and kinetic properties, and subcellular localization (see Walls et al., 2010, 2011). As discussed below, this is of importance for seizure control. Degradation of GABA requires GABA-transaminase (GABA-T) to convert GABA to succinic semialdehyde (SSA) by transamination with the co-substrates glutamate and α-KG. SSA is subsequently oxidized by SSA dehydrogenase (SSADH) to succinate, a constituent of the TCA cycle. Alternatively, SSA can be reduced by γ-OH-butyric acid dehydrogenase (GHBDH) to γ-OH-butyric acid which can activate GABA_β receptors (Kaupmann et al., 2003).

2.5. Pharmacology of GABA metabolizing enzymes

2.5.1. GAD

Inhibition of GAD, the GABA synthesizing enzyme, is known to produce convulsions (Tapia and Pasantes, 1971). Since GAD is dependent on pyridoxal phosphate as the co-enzyme, carbonyl trapping agents like derivatives of hydrazine are generally convulsant in nature (Tapia, 1975). Interestingly, aminooxyacetic acid will act as a convulsant at high doses while at lower doses it is an anticonvulsant (Tapia, 1975). This is best explained by the fact that it inhibits GABA-T more potently than it inhibits GAD (Schousboe et al., 1974; Wu and Roberts, 1974). In this context it should be noted that the convulsant 3-mercaptopropionic acid (Lamar, Jr., 1970) is a more potent inhibitor of GAD than of GABA-T (Schousboe et al., 1974; Wu and Roberts, 1974).

2.5.2. GABA-T

Like GAD, GABA-T is a pyridoxal phosphate-dependent enzyme (Schousboe et al., 1974). Thus, GABA-T is sensitive to carbonyl trapping agents. One of the most potent inhibitors of GABA-T is aminooxyacetic acid (K = 60 nM) (Schousboe et al., 1974). As such, it increases GABA levels and displays anticonvulsant activity in a variety of seizure models (Sarup et al., 2003; Tapia, 1975). The synthesis of the GABA analog, γ-vinyl GABA (Lippert et al., 1977), has been instrumental in the development of a novel antiepileptic drug that acts by elevating synaptic GABA concentrations (Gram et al., 1988). This compound is a GABA-T substrate that displays irreversible covalent binding to the active site of GABA-T upon transamination and thus acts as a suicide, catalytic site-directed inhibitor (Lippert et al., 1977). This action explains the remarkable specificity displayed by γ-vinyl GABA. Interestingly, its analog, γ-acetylenic-GABA, which also acts as a suicide inhibitor of GABA-T, inhibits GAD as well. It acts as an anticonvulsant drug against some but not all types of experimentally induced seizures despite the fact that it is generally more potent as an inhibitor of GABA-T than γ-vinyl-GABA (Schechter et al., 1977a, 1977b). Due to its higher selectivity towards GABA-T relative to GAD, γ-vinyl GABA was developed as a clinically active antiepileptic drug named vigabatrin (Gram, 1990). Vigabatrin currently represents the only antiepileptic agent whose mechanism of action specifically relies on inhibition of GABA-T. Vigabatrin is also of particular interest because it, like tiagabine (discussed in Section 4.5), emerged from a mechanism-based drug discovery program.

2.6. Transgenic animals of GABA metabolizing enzymes

Animals lacking expression of GAD65, GAD67, and SSADH provide insight into the role of each of these enzymes. GAD65 knockout mice display no morphological abnormalities. Based on their genetic background, GAD65 knockout mice may or may not possess altered basal GABA levels and/or display spontaneous seizures and increased susceptibility to induced seizures. GAD65 knockouts are also more susceptible to increased mortality compared to WT mice (Asada et al., 1996; Kash et al., 1997). Homozygous KO of GAD67 is lethal due to formation of a cleft palate, and drastically reduced GABA levels are observed. No increase in susceptibility to picrotoxin-induced seizures was observed in GAD67 heterozygous

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**Table 1**: Phenotypic features of transgenic animals that target enzymes involved in glutamate metabolism.

<table>
<thead>
<tr>
<th>Pathway component</th>
<th>Epilepsy-related phenotype</th>
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<tr>
<td><strong>Enzymes</strong></td>
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<tr>
<td>GS+/−</td>
<td>Homozygous KO lethal. Increased susceptibility to induced seizures in heterozygotes</td>
<td>van Gassen et al. (2009)</td>
</tr>
<tr>
<td>GDH Tg</td>
<td>Increased GDH levels, glutamate release, mEPSC frequency and amplitude. No seizure phenotype</td>
<td>Bao et al. (2009)</td>
</tr>
<tr>
<td>PAG+/−</td>
<td>Altered glutamatergic transmission. KO lethal within 36 h of birth</td>
<td>Masson et al. (2006)</td>
</tr>
</tbody>
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Abbreviations: GS, glutamine synthetase; GDH, glutamate dehydrogenase; Tg, transgenic; PAG, phosphate activated glutaminase.
animals (Asada et al., 1997). SSADH KO mice experience absence-like seizures during post-natal week two that progress to generalized convulsive seizures in late post-natal week three, and are associated with the onset of lethal status epilepticus (Cortez et al., 2004). No gross morphological deficits exist in the SSADH KO mice, although there is enhanced astrogliosis in the hippocampus (Hogema et al., 2001). SSADH KO mice exhibit an imbalance in several amino acids and their metabolism, including glutamate, GABA and glutamate (Chowdhury et al., 2007; Gupta et al., 2004). The phenotypic features of transgenic animals of GABAergic enzymes are summarized in Table 2.

### 2.7. Neuron-glia interactions in the biosynthesis of glutamate and GABA

Neurons cannot perform the net synthesis of glutamate and GABA from glucose due to the fact that neurons lack both the glutamine synthesizing enzyme, GS, and the prevailing anaplerotic enzyme in the brain, pyruvate carboxylase (PC) (Bak et al., 2006; Norenberg and Martinez-Hernandez, 1979; Yu et al., 1983). Astrocytes, on the other hand, express both of these enzymes and are therefore obligatory for maintaining a supply of glutamate, the precursor for glutamate and GABA (Bak et al., 2006). The exchange of GABA, glutamate and glutamine is referred to as the glutamate-glutamine cycle (Bak et al., 2006). This was originally thought to represent a stoichiometric exchange of glutamate and glutamine among neurons and astrocytes (Catton et al., 1981), i.e. glutamate taken up by astrocytes would be quantitatively converted to glutamine. It is, however, clear that this is not the case as glutamate in astrocytes can be oxidatively metabolized (Sonnewald et al., 1997; Yu et al., 1982). The loss of glutamate is compensated for by de novo synthesis via pyruvate carboxylase which has been shown to represent about 30% of the glutamate exchange related to glutamatergic activity (Oz et al., 2004).

In GABAergic neurons, glutamine released from astrocytes appears to be an important precursor for GABA (Battaglioni and Martin, 1991; Sonnewald et al., 1993; Walls et al., 2011) via formation of glutamate that is subsequently decarboxylated. It may be of interest that it has been shown using $^{13}$C-labeled precursors combined with magnetic resonance spectroscopy that conversion of glutamine to GABA via glutamate may to a large extent involve the mitochondrial TCA cycle (Waagepetersen et al., 2001). Hence, at least 50% of GABA synthesized by this pathway originates from glutamate derived from αKG generated by the TCA cycle.

### 3. Release and termination of GABA and glutamate neurotransmitter activity

Release of intracellular GABA and glutamate to the extracellular space can be accomplished by several mechanisms. Two of these include: (1) A presynaptic action potential induces $\mathrm{Ca}^{2+}$-dependent exocytosis of vesicularly stored transmitter from the presynaptic neuron into the synaptic cleft (Nicholls, 1989); (2) Reversal of the membrane-bound neurotransmitter transporter located either on pre- or postsynaptic neurons or adjacent astrocytes. A third process that can lead to release of intracellular glutamate and GABA involves the reversal of the cysteine/glutamate exchangers (Warr et al., 1999), which can under certain conditions release non-vesicular GABA and glutamate to the extracellular space (Allen et al., 2004).

The release of neurotransmitters involves complex assembly and release machinery that is outside the scope of this review (see Rizo and Rosenmund, 2008, for review and pertinent references). Rather, particular exocytotic proteins known to alter excitability will be described and the lessons learned from animals lacking these proteins will be discussed. Fig. 2 provides a very simplified schematic of these proteins. Synapsins are a family of proteins involved in the release of vesicles, and control the number available for release. Synaptic vesicle proteins control the regulated secretion of synaptic vesicles. SV2A and SV2B are the major isoforms of synaptic vesicle protein 2. SV2A selectively enhances low-frequency neurotransmission and maintains the readily releasable pool of transmitters. Synaptotagmin-1 is localized to vesicle membranes and triggers calcium-dependent exocytosis. Munc proteins are important for vesicle priming prior to exocytotic release. The SNARE complex is a complex of proteins that mediate vesicle fusion. Three important proteins in the SNARE complex are SNAP-25, synaptobrevin, and syntaxin. All of the aforementioned proteins can alter neuronal excitability as discussed in section 3.1.

### 3.1. Transgenic animals of vesicular transporters

Knockout animals exist that lack the vesicular transporters for GABA and glutamate. The homozygous knockout of vGLUT1 may be lethal (Wojcik et al., 2004), or animals may exhibit a severe decline in excitatory neurotransmission (Fremeau, Jr. et al., 2004). While no behavioral data exists, vGLUT1 KO animals display altered electrophysiological characteristics (Fremeau, Jr. et al., 2004; Wojcik et al., 2004). The homozygous KO of vGLUT2 is lethal. Heterozygous vGLUT2 animals do not display any EEG abnormalities; however, they are more susceptible to i.v.PTZ-induced seizures (Schallier et al., 2009). Neurons from vGLUT2 homozygous KO mice display drastically reduced EPSC amplitudes in the thalamus but not hippocampus (Moechars et al., 2006). On average, vGLUT3 KO mice display one electrographic non-convulsive seizure per day. No morphological abnormalities have been described in vGLUT3 KO mice (Seal et al., 2008). The knockout of vGAT1 is lethal; these mice lack functional inhibitory synaptic transmission and display several morphological abnormalities (Wojcik et al., 2006). Several transgenic animals are available that display alterations in the trafficking, packaging, and release of vesically stored neurotransmitters. Different constructs for a given protein may yield differing results and a full discussion is outside the scope of this re-
view. However, a brief overview is given here and in Table 3. Synapsin KO mice exhibit various alterations in synaptic vesicle release while displaying no anatomical abnormalities (Bogen et al., 2006; Li et al., 1995; Rosahl et al., 1995). Knockout mice of various synapsins display spontaneous seizures and/or increased susceptibility to induced seizures (Gitler et al., 2004; Li et al., 1995; Rosahl et al., 1995). While SV2B KO mice display no phenotype, SV2A and SV2A/SV2B double KO mice display spontaneous seizures and die by two to three weeks of age. No differences were detected in morphology, synaptic formation, or EPSCs or IPSCs recorded from neurons cultured from SV2A, SV2B, or double KO mice. It is also important to note that KO of SV2A leads to a loss of function of the anticonvulsant drug levetiracetam (Lynch et al., 2004). Synaptotagmin 1 KO mice die within two days of birth while heterozygotes display normal survival. There are no morphological or synaptic formation differences between KO and WT animals. Synaptotagmin 1 is implicated in only synchronous release (Geppert et al., 1994). KO of Munc 13–1 yields a 90% reduction in excitatory post-synaptic responses and weak animals that fail to feed and die within a few hours of birth (Augustin et al., 1999). KO of Munc 13–2 results in animals that experience sporadic seizures after 12 months. Double KO of Munc 1 and 2 is lethal within one hour of birth. Embryonic neurons from these double KO animals display no evoked or spontaneous release in electrophysiological studies (Varoquaux et al., 2002). Munc 18–1 KO mice are born completely paralyzed and die immediately after birth. Neurons from embryonic KO mice reveal a loss of synaptic events despite functional post-synaptic receptors and ion channels (Verhage et al., 2000).

There are three major components that form the SNARE complex, all of which have transgenic animals available: SNAP-25, VAMP/synaptobrevin, and syntaxin. The full KO of SNAP-25 abolishes evoked exocytotic release and mutants die at birth (Washbourne et al., 2002). Heterozygotes are indistinguishable from WT mice; therefore, several different SNAP-25 gene-targeted mouse mutants with distinct phenotypes have been developed (for review and references see Bark, 2009). SNAP-25 deficiency can lead to seizure activity, as well as pathological changes that progress with age (Bark, 2009; Tafoya et al., 2006; Washbourne et al., 2002). Mice completely lacking neuronal synaptobrevin-2 experience impaired neurotransmission and early death (Schoch et al., 2001) and the size and shape of vesicles is impaired while the number and docking are normal (Deak et al., 2004). In syntaxin 1a KO mice, release parameters are not distinguishable from WT animals, and KO mice survive and are viable (Fujiwara et al., 2006). In another study, mice lacking syntaxin 1a displayed no increased mortality or phenotype, while mice that exclusively expressed the open form of syntaxin 1b (1b open mutants) were ataxic and experienced lethal seizures at approximately two weeks of age. Mice lacking syntaxin 1a displayed no differences in EPSC characteristics, while 1b open mutants displayed a 40% increase in mEPSC frequency (Gerber et al., 2008). The interaction between Munc-18 and syntaxin 1a is regulated by cyclin dependent kinase 5 (CdK5) (Shuang et al., 1998). KO of CdK5 leads to increased seizure susceptibility (Hawasli et al., 2009). The phenotypes of transgenic animals involved in the vesicular transport, trafficking, packaging, and release of neurotransmitters are summarized in Table 3.

4.1. Glutamate transporters

Glutamate transporters are expressed in the plasma membrane as well as in mitochondria and synaptic vesicles in glutamatergic neurons (Gegelashvili and Schousboe, 1997; Ozkan and Ueda, 1998; Sluse, 1996). High affinity transporters for l-glutamate as well as L- and D-aspartate have been found on both neurons and astrocytes with $K_m$ values ranging from 20–90 μM (Drejer et al., 1982, 1983; Schousboe et al., 1977). Isolation of a high affinity sodium- and potassium-coupled glutamate transporter greatly aided the cloning of the glutamate transporters (Danbolt et al., 1990). To date five plasma membrane glutamate transporters have been cloned and named GLAST (EAAT1) (Storck et al., 1992; Tanaka, 1993), GLT-1 (EAAT2) (Pines et al., 1992), EAAC1 (EAAT3) (Kanai and Hediger, 1992), EAAT4 (Fairman et al., 1995) and EAAT5 (Arrizza et al., 1997). Due to this confusing nomenclature, the abbreviation excitatory amino acid transporter (EAAT) was introduced and will be used throughout the remainder of this review. It is widely believed that EAAT1 and 2 are primarily localized on astrocytes, whereas EAAT3 is primarily localized postsynaptically on neurons (Danbolt, 2001).

The sequence homology of glutamate transporters is highly conserved (>90%) amongst eukaryotes, with 38–65% sequence homology among the different subtypes (Gegelashvili and Schousboe, 1997; Robinson and Dowd, 1997). The molecular structure of the glutamate transporters EAAT1 (Seal et al., 2000) and EAAT2 (Grunevald and Kanner, 2000) has been proposed to be comprised of eight transmembrane domains (TMDs) with a large extracellular loop between TMD3 and TMD4 containing N-glycosylation sites. Furthermore, the crystal structure of the glutamate transporter from Pyrococcus horikoshii (GltPh) has shed further light on the structure of EAATs, confirming the eight TMD topology (Yennoo et al., 2004). However, the structure of GltPh does not exactly match that of the mammalian EAATs, and importantly the large extracellular loop between TMD3–4 is not present in GltPh (Yennoo et al., 2004). Furthermore, it has been proposed that EAAT1, EAAT2, and GltPh exist as homo-multimers (Haugeto et al., 1996; Yennoo et al., 2004). The glutamate transporters utilize the electrochemical gradient across the cell membrane to drive the uphill transport of substrate. The stoichiometry of a single translocation cycle differs amongst the EAATs, but there is an absolute requirement for extracellular sodium influx and intracellular potassium efflux (Kanner and Sharon, 1978). It has been suggested for EAAT2 and 3 and GltPh that three sodium ions and one proton are co-transported with glutamate into the cell while one potassium ion is transported out of the cell (Levy et al., 1998; Yennoo et al., 2004; Zerangue and Kavanaugh, 1996). For EAAT1 the co-transport of a proton was not demonstrated (Klockner et al., 1993).

4.2. Pharmacology of glutamate transporters

Dysfunction of glutamate transporters has been implicated in several diseases such as epilepsy (Tanaka et al., 1997), amyotrophic lateral sclerosis (ALS), Alzheimer’s disease (Guo et al., 2003), and neuropathic pain (Mao, 2005). A common feature amongst these diseases is neuronal cell death that can be caused by glutamate-induced excitotoxicity (Rothman and Olney, 1986). Neurodegeneration is implicated in the pathology of epilepsy and it is generally believed that the balance between inhibitory and excitatory neurotransmission is tipped in favor of the latter.

Pharmacological approaches such as inhibiting glutamate transport or causing down regulation have been used in identifying the functional significance of each glutamate transporter subtype in vitro and in vivo; however, this strategy is without therapeutic significance. Quite to the contrary, it might lead to over-excitation and excitotoxicity. On the other hand, genetic overexpression of
EAAT2 in a mouse model of ALS significantly increases the lifespan of the mice, suggesting that elevated expression of EAAT2 can be neuroprotective (Guo et al., 2003). The underlying hypothesis for EAAT2-mediated neuroprotection is that glutamate excitotoxicity is limited due to enhanced uptake. However, overexpression of EAAT2 is not always beneficial if the transporter reverses during pathological situations (Yamada et al., 2006).

Interestingly, β-lactam antibiotics increase the expression and function of EAAT2 both in in vivo and in vitro models due to an increase in promoter activation. The experimental paradigms used to elucidate the neuroprotective action of the β-lactam antibiotic, ceftriaxone (CEF), include ischemia preconditioning (IPC) oxygen-glucose deprivation (OGD) of cultured neurons, which models the neurodegeneration sustained during ischemic injury (Rothstein et al., 2005). It has been demonstrated that lethal OGD increases glutamate release, causing neuronal death; however, subjecting the neurons to a sublethal OGD insult 24 h prior to the lethal OGD insult induces resistance to neuronal cell death, partly by mediating an increase in EAAT2 function (Romera et al., 2004). CEF pretreatment 48 h prior to the lethal OGD insult prevented neuronal cell death. Moreover, it protected against THA- and TBOA-mediated motor neuron loss in an organotypic spinal cord model (Rothstein et al., 2005). The G93A SOD1 mouse is an animal model of ALS and shows reduced expression of EAAT2 near the onset of clinical signs (Howland et al., 2002). When CEF treatment model of ALS and shows reduced expression of EAAT2 near the on-

Fig. 2. Cartoon illustrating the proteins involved in the docking of synaptic vesicles. Synapsins and SV2A allow regulation of the readily releasable pool of vesicles. Synaptobrevins, SNAP-25, and syntaxin form the SNARE complex, which binds directly with Munc-18 to allow fusion with the membrane and release of neurotransmitter.

The use of β-lactam antibiotics as neuroprotective drugs might not be ideal when one considers the increasing problem of multi-drug resistant bacteria, which will have a more obvious and severe effect in already compromised patients. Moreover, the dose needed to treat ALS is 5–6 times higher than the dose used for meningitis, and severe side effects often cause ALS patients to discontinue the CEF treatment (Rawls et al., 2010). Inhibitors of the enzyme β-lactamase such as clavuacan acid and Tazobactam, as well as CEF, have been shown to inhibit planar seizure-like activity induced by 3 mM of either cocaine or glutamate. Due to the greater oral bioavailability of the β-lactamases versus β-lactam antibiotics and their negligible antibacterial effect, this class of drugs is of potential interest for managing CNS disorders (Rawls et al., 2010).

Further studies have revealed that activation of the nuclear receptor peroxisome proliferator-activated receptor (PPAR) γ using the agonist rosiglitazone is involved in the upregulation of EAAT2. Pretreatment with rosiglitazone 24 h prior to lethal OGD prevented neuronal cell death. This effect is reversed by dihydrokainate, demonstrating that the neuroprotective effect is mediated by EAAT2 (Romera et al., 2007). Several other compounds have been shown to enhance EAAT2 function including estrogens, glucocorticoids, the novel neuroprotective compound MS-153, and riluzole, which is a registered drug for the treatment of ALS (Rilutek® from Sanofi-Avensis)(Azbill et al., 2000; Pawlak et al., 2005; Shimada et al., 1999; Zschocke et al., 2005).

Interestingly, CEF induced upregulation of EAAT2 at doses of 100 and 200 mg/kg but not 50 mg/kg, and was shown to significantly reduce cue-induced reinstatement of cocaine-seeking, but not food-seeking, behavior in rats. Increased expression of EAAT2 was found in both the prefrontal cortex and nucleus accumbens which is in agreement with the proposed hypothesis that relapse to cocaine-seeking behavior requires the release of glutamate from the prefrontal cortex projections to the nucleus accumbens. This places EAATs as potential key targets for the treatment of addiction (Sari et al., 2009).

4.3. Transgenic animals of glutamate transporters

As summarized in Table 4, the generation of several knockout animals have helped to elucidate the function of the various EAATs. EAAT1 genetic KO in mice yields no overt seizure phenotype (Stoffel et al., 2004) and no difference in kindling acquisition, but KO
were decreased 67% by antisense infusion and, although a motor response in WT animals (Tanaka et al., 1997). In rats, EAAT2 levels bursts in response to a subthreshold PTZ dose that produces no re-age. EEG performed on KO mice reveals high-voltage sharp wave that can be lethal, leading to a 50% survival rate at six weeks of age. Kosstein et al., 1996). EAAT2 KO mice display spontaneous seizures where EAAT1 levels were decreased by antisense oligonucleotide infusion, progressive motor impairment culminating in hindlimb paralysis was observed, but no seizure phenotype emerged (Rothstein et al., 1996). EAAT2 KO mice display spontaneous seizures that can be lethal, leading to a 50% survival rate at six weeks of age. EEG performed on KO mice reveals high-voltage sharp wave bursts in response to a subthreshold PTZ dose that produces no response in WT animals (Tanaka et al., 1997). In rats, EAAT2 levels were decreased 67% by antisense infusion and, although a motor syndrome emerged, no seizure phenotype was observed (Rothstein et al., 1996). Hippocampal slices from EAAT1 and EAAT2 KO mice reveal that these transporters restrict the activation of mGlRs, while EAAT3 does not (Huang et al., 2004b). An EAAT1/EAAT2 double KO mouse was created, which displayed no overt seizure phenotype (Stoffel et al., 2004). In rats, EAAT1 levels were decreased in two different studies by antisense knockdown. In one study, a motor syndrome was observed in half of the animals and 85% of all animals displayed tonic forepaw extension and clonic seizures (Rothstein et al., 1996). In another study, an absence seizure-like phenotype and EEG pattern, a 50% decrease in hippocampal GABA levels, and excessive excitability were found (Seppuku et al., 2002). In contrast, genetic KO of EAAT3 in mice revealed no seizure phenotype and no morpho-

Table 3

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<tr>
<th>Pathway component</th>
<th>Epilepsy-related phenotype</th>
<th>Reference(s)</th>
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<tr>
<td><strong>Vesicular transporters</strong></td>
<td>Reduced evoked and field EPSP amplitudes, reduced glu release. Homozygous KO may be lethal depending on construct</td>
<td>Freneau et al. (2004) and Wojcik et al. (2006)</td>
</tr>
<tr>
<td>vGlut1 (GLAST)</td>
<td>Homozygous KO lethal. In hets, no EEG phenotype but increased susceptibility to induced seizures. Reduced EPSP amplitude in thalamus</td>
<td>Moechars et al. (2006) and Schallier et al. (2009)</td>
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<tr>
<td>vGlut2 (GLT)</td>
<td>Increased spike-wave activity with no motor seizures</td>
<td>Seal et al. (2008)</td>
</tr>
<tr>
<td>vGAT (VGLUT1)</td>
<td>Absence of vesicular release of GABA and glycine. KO lethal</td>
<td>Wojcik et al. (2006)</td>
</tr>
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**Proteins involved in trafficking, packaging, and release of vesicles**

| Synaptotagmin 1/- | Early lethality in homozygous KO. Decreased post-synaptic responses. Normal mEPSCs and calcium-independent release | Geppert et al. (1994) |
| Munc 13/- | Reduced excitatory post-synaptic responses, normal IPSCs. Early death in Munc 13-1 KOs, sporadic spontaneous seizures in older (1 + years) Munc 13-2 KOs | Augustin et al. (1999) and Varoqueaux et al. (2002) |
| Munc 18/- | Loss of synaptic events. Paralyzed and die immediately at birth | Verhage et al. (2000) |
| SNAP 25 1/- | Early death in homozygous KO. Alterations in release | Bark (2009) and Washbourne et al. (2002) |
| VAMP/Synaptobrevin 1/- | Early death in homozygous KO. Alterations in release | Deak et al. (2004) and Schoch et al. (2001) |
| Syntaxin 1/- | Spontaneous lethal seizures and increased mEPSC frequency only in 1b open mutants | Fujisawa et al. (2006) and Gerber et al. (2008) |

**Abbreviations:** vGlut, vesicular glutamate transporter; vGAT, vesicular GABA transporter, SV2, synaptic vesicle protein 2; Munc, mammalian uncoordinated; SNAP, synaptosomal-associated protein; VAMP, vesicle-associated membrane protein.

Table 4

<table>
<thead>
<tr>
<th>Pathway component</th>
<th>Epilepsy-related phenotype</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Membrane transporters</strong></td>
<td>No overt seizure phenotype. Alterations in susceptibility to induced seizures. No alterations in EPSC amplitude or rise-time kinetics in cerebellar parallel fiber synapses</td>
<td>Nagatomo et al. (2007), Nikkuni et al. (2007), Stoffel et al. (2004), Tsuru et al. (2002), Watanabe et al. (1999) and Watase et al. (1998)</td>
</tr>
<tr>
<td>EAAT1 (GLAST)</td>
<td>Excess glutamate and neurodegeneration leading to paralysis, but no seizure phenotype</td>
<td>Rothstein et al. (1996)</td>
</tr>
<tr>
<td>EAAT2 (GLT)</td>
<td>Spontaneous seizures</td>
<td>Tanaka et al. (1997)</td>
</tr>
<tr>
<td>EAAT3 (EAAC1)</td>
<td>No seizure phenotype. No alterations in synaptic currents, EPSC amplitude, or rise-time kinetics in cerebellar parallel fiber synapses</td>
<td>Huang et al. (2004a), Nikkuni et al. (2007), Peghini et al. (1997)</td>
</tr>
<tr>
<td>EAAT3 (EAAC1)</td>
<td>Some spontaneous clonic and absence-like seizures. Decrease in mIPSCs</td>
<td>Rothstein et al. (1996) and Sepkuty et al. (2002)</td>
</tr>
<tr>
<td>EAAT4</td>
<td>Increased or no alteration in amplitude and rise-time kinetics of EPSCs evoked by cerebellar parallel fiber stimulation</td>
<td>Huang et al. (2004a), Nikkuni et al. (2007) and Takayasu et al. (2005)</td>
</tr>
</tbody>
</table>

**Abbreviations:** EAAT, excitatory amino acid transporter; GLAST, glutamate aspartate transporter; GLT, glutamate transporter, EAAC, excitatory amino acid carrier; Munc, mammalian uncoordinated; SNAP, synaptosomal-associated protein; VAMP, vesicle-associated membrane protein.
logical abnormalities (Huang et al., 2004a; Peghini et al., 1997). No differences were observed in several electrophysiological parameters (Huang et al., 2004a; Nikkuni et al., 2007). In EAAT4 KO mice, mGlurS are more strongly activated than in WT mice (Nikkuni et al., 2007). In contrast, EAAT4 KO mice experience no difference in evoked EPSCs, although a long-lasting tail current did appear in an age-dependent manner in KO mice (Takayasu et al., 2005). EAAT4 KO mice possess no glutamate transporter current in Purkinje cell climbing fibers and no morphological abnormalities (Huang et al., 2004a). The phenotypes of transgenic animals of glutamine-glu- 
kinje cell climbing fibers and no morphological abnormalities (Kish et al., 1989). The isolation of an 80 kDa 
brahnes of neurons astrocytes (Iversen and Neal, 1968) and on syn-
aptic vesicles (Kish et al., 1989). The isolation of an 80 kDa 
4.4. GABA transporters 
GABA transporters (GATs) are expressed on the plasma membranes of neurons astrocytes (Iversen and Neal, 1968) and on syn-
aptic vesicles (Kish et al., 1989). The isolation of an 80 kDa 
glycoprotein that showed a dependency for sodium and chloride for transport and an estimated $K_m$ of 3 μM preceded the cloning of the GATs (Radian et al., 1986). Shortly following the findings of Radian and colleagues, four GATs were cloned from several spe-
cies. Unfortunately, this led to a confusing nomenclature that has been listed in Table 5 along with the solute carrier (SLC) gene names for simplicity.

The mouse GATs, mGAT1–4, correspond to GAT-1, BGT-1, GAT-
2, and GAT-3, respectively, in humans and rats. The standardized nomenclature proposed by the HUGO Gene Nomenclature Com-
mittee will be used in this review unless otherwise stated. mGAT1–4 have an apparent $K_m$ values for GABA of 7, 79, 18, and 0.7 μM (Liu et al., 1993), respectively, whereas the apparent $K_m$ for the human GATs have been reported as 11, 18, 8.1, and 0.56 μM, respectively (Krist et al., 2009). The only major difference between the two species is the apparent fourfold higher $K_m$ in mice for the betaine-GABA transporter, BGT1. BGT1 also transports beta-
ine, although with a lower $K_m$ (398 μM for mGAT2). GAT2 and GAT3 also have the capacity to transport β-alanine and taurine (Liu et al., 1993). The GATs move GABA against its concentration gradient by utilizing the inward sodium gradient. The stoichiome-
try for the transport process is two to three sodium ions, one chlo-
ride ion, and one GABA molecule (Kanner, 1994).

The genes for GAT1, BGT1, GAT2, and GAT3 encode proteins of 598, 614, 602, and 627 amino acids, respectively (Liu et al., 1992, 1993). GAT1 seems to be somewhat more distantly related to the other GATs, sharing only 50% sequence homology, whereas the sequence identity is 65–70% amongst BGT1, GAT2, and GAT3 (Miller et al., 2002). Crystallization of LeuT, a sodium-dependent bacterial Leucline transporter, has given great insight into the protein dynamics and translocation process of the SLC6 gene family, including the substrate binding pocket (Yamashita et al., 2005). The GATs are composed of 12 TMDs with the N- and C- termini located intra-
cellularly (Chen et al., 2004) for review please see (Madsen et al., 2007).

The regional and cellular distribution of the GATs differs dra-
matically within the brain. GAT1 and GAT3 are restricted to the brain, whereas BGT1 and GAT2 are found in multiple other organs. GAT1, the most studied of the GATs, is expressed throughout the brain (Durkin et al., 1995) along GABAergic pathways (Radian et al., 1990). GAT1 is primarily found on presynaptic GABAergic neurons and to a minor degree on distal processes in close proximity to axon terminals forming symmetric synapses (Borden, 1996; Conti et al., 1998). The expression of GAT3 is much more restricted compared to the widespread expression of GAT1. GAT3 is primarily located on distal astrocytic processes that are in direct contact with GABAergic terminals and to a lesser degree on neuronal elements (Durkin et al., 1995; Minelli et al., 1996). The expression of BGT1 and GAT2 are very limited and they partition to the basolateral side in polarized MDCK cells, which is strikingly opposite to that of GAT1 and GAT3 which localize to the apical side (Ahn et al., 1996). GAT2 seems to be highly abundant in the mouse neonatal brain compared to the adult brain (Liu et al., 1993). In the adult rat brain, GAT2 shows strong expression in the leptomeninges and ependyma and weak expression in cortical neurons and astro-
cytes. The opposed localization of GAT2 compared to GAT1 and GAT3 in MDCK cells is to some extent also paralleled in brain cells. GAT2 is found on dendrites and in axon terminals forming both symmetric and asymmetric synapses but away from areas with synaptic specialization, contrary to GAT1. The localization of GAT2 in distal astrocytic processes is similar to that of GAT1 and GAT3, whereas the localization of GAT2 to astrocytic cell bodies and proximal processes is not (Conti et al., 1999). Although some-
what controversial due to the unavailability of highly specific anti-
bodies, BGT1 has been found throughout the adult brain (Borden et al., 1995) and in the leptomeninges (Evans et al., 1996; Takanaga et al., 2001). BGT1 has also been found on pyramidal neurons in the cerebral cortex and hippocampus. Furthermore, BGT1 was found on dendritic spines or small diameter dendrites in contact with asymmetric synapses (Zhu and Ong, 2004). Collectively, the local-
ization of BGT1 and GAT2 suggest that their involvement in direct synaptic GABAergic neurotransmission is limited. Rather, they may play a more important role in the regulation of extrasynaptic GABA levels.

4.5. Pharmacology of GABA transporters 
As alluded to in section 2.5, a mechanism-based drug discovery program established tiagabine as an anticonvulsant that selectively inhibits GAT1 (Nielsen et al., 1991). At the same time as Novo Nor-disk developed tiagabine, other companies like Parke-Davis/War-
er-Lambert and SKF were developing the GAT inhibitors CI-9 and SK&F 89976A, respectively (Ebert and Krmjevic, 1990; Yunker et al., 1984); however, the clinical trials were terminated due to considerable adverse effects (Radulovic et al., 1993). The use of tiagabine as an anticonvulsant is limited to add-on therapy for partial epilepsy.

As described above, the heterogenous localization of GATs in the CNS places them strategically within and around the synapse on both neurons and astrocytes (GAT1 and GAT3), and to the extrasyn-
aptic region (GAT2 and BGT1). Approximately 20% of released GABA is taken up by astrocytes (Schousboe, 2000). As described in sections 2.5 and 2.7, GABA is subsequently converted to succinate via GABA-T and SSADH, and replenishment of GABA precursor in neurons relies on the glutamine-glutamate cycle. In seizure-com-
promised neuronal circuits where GABAergic neurotransmission is attenuated, reduction of neuronal GABA stores is unwanted and will lead to a further exacerbation of seizures. It has therefore been pro-
posed that selective inhibition of astrocytic GABA transport would increase extracellular levels of GABA, thus increasing presynaptic uptake and recycling of GABA thereby avoiding transmitter drainage and lowering seizure susceptibility (Schousboe et al., 1983).

GAT3 would be the obvious pharmacological target for selective astrocytic transport because it is located almost exclusively on

<table>
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<th>Table 5 Nomenclature of GATs.</th>
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<tr>
<td>SLC gene</td>
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<tr>
<td>Mouse</td>
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<tr>
<td>Human/rat</td>
</tr>
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</table>

Abbreviations: SLC, solute carrier; GAT, GABA transporter; BGT, betaine-GABA transporter.
astrocytes and only to a minor degree on neurons, contrary to GAT1, which is the prevalent neuronal transporter with minor expression on astrocytes. Unfortunately, the development of selective GAT3 inhibitors has proven difficult. SNAP-5114, which also inhibits GAT2 and BGT1 but has little activity for GAT1, is the most commonly used GAT3 inhibitor (Borden et al., 1994).

A series of astrocyte-selective inhibitors have been developed. The most potent of these is N-methyl-exo-THPO, which displays an almost 10-fold selectivity for astrocytes compared to neurons (White et al., 2002). Interestingly, these compounds were all found to be GAT1 selective (White et al., 2002). Due to the poor blood brain barrier penetration of N-methyl-exo-THPO, it was substituted with a lipophilic aromatic side chain resulting in the interesting compound EF1502, which is selective for GAT1 and BGT1 but equipotent at neurons and astrocytes (Claussen et al., 2005).

EF1502 was the first compound synthesized that inhibits BGT1 and it has been extensively studied to elucidate the utility of BGT1 as a pharmacological target for the treatment of seizures. EF1502 was shown to possess a broad anticonvulsant profile in rodent seizure models. Moreover, EF1502 was tested in combination with the GAT1 selective inhibitors, tiagabine and LU-32–176B, using the isobologram experimental design (Loewe, 1953) in the audiogenic seizure-susceptible Frings mouse. A synergistic anticonvulsant effect was observed when EF1502 was combined with either of the GAT1 selective compounds, whereas the combination of the two GAT1 selective compounds tiagabine and LU-32–176B resulted in an additive anticonvulsant effect. It was concluded from this study that inhibition of BGT1, rather than inhibition of GAT1, was likely responsible for the observed synergistic effects of EF1502 with GAT1 inhibitors (White et al., 2005). Similarly, EF1502 was combined with the moderately selective GAT3 inhibitor, SNAP-5114, resulting in a synergistic anticonvulsant effect at some dose mixtures, whereas tiagabine combined with SNAP-5114 resulted in an additive anticonvulsant interaction (Madsen et al., 2009). The cellular localization of the GATs was proposed to explain the results obtained by combining GAT inhibitors with different subtype selectivity. Inhibition of GAT1, almost exclusively expressed on presynaptic neurons within the synapse (Borden, 1996; Conti et al., 1998), and GAT3, expressed on distal processes of astrocytes in close proximity to the synapse (Durkin et al., 1995; Minelli et al., 1996), results in increased synaptic GABA levels which activate synaptic GABA receptors that mediate inhibitory neurotransmission. Combined inhibition of GAT1 and GAT3 therefore only activates the synaptic GABA receptors. BGT1 is located outside the synapse, probably on glutamatergic neurons (Zhu and Ong, 2004), where there exists the potential of extrasynaptic receptor activation through locally elevated GABA levels. Therefore, the combined inhibitory effect of GAT1 and BGT1 or GAT3 and BGT1 may result in the activation of two very different populations of GABA receptors, namely synaptic benzodiazepine-sensitive and extrasynaptic benzodiazepine-insensitive GABA receptors, respectively. These have also been referred to as phasic and tonic GABA receptors, respectively. Taken together, these results highlight the mechanistic differences between tiagabine and EF1502 and support a functional role for BGT1 and extrasynaptic GABA receptors in mediating GABAergic neurotransmission.

Recently, a study was conducted to highlight the mechanistic difference between EF1502 and tiagabine in their ability to interact with the selective extrasynaptic GABA receptor agonist, gaboxadol (Madsen et al., 2011). Gaboxadol at low concentrations (1–3 μM) will selectively activate extrasynaptic GABA receptors (Wafford and Ebert, 2006). Concentrations in this range are achieved by clinically relevant doses of gaboxadol, which has a higher efficacy but lower potency than GABA at α4β3-containing GABA receptors (Cremers and Ebert, 2007; Storustovu and Ebert, 2006; Wafford and Ebert, 2006). Gaboxadol was shown to protect against sound-induced seizures in the Frings mouse and induce ataxia in C57 mice. Combining gaboxadol with tiagabine or EF1502 resulted in an additive or antagonistic anticonvulsant effect, respectively. Also, EF1502 was able to reverse the gaboxadol-induced ataxia while tiagabine was not. This clearly demonstrates the difference in mechanism of action of tiagabine and EF1502. In order to antagonize the anticonvulsant effect of gaboxadol and reverse the gaboxadol-induced ataxia, EF1502 is likely able to elevate the extrasynaptic GABA concentration, presumably by inhibiting BGT1 (Madsen et al., 2011). A recent study that produced a BGT1 knockout mouse didn’t reveal a BGT1-dependent seizure phenotype, and showed that BGT1 is present in very low amounts in the brain compared to GAT1 as analyzed by mRNA levels (Lehre et al., 2011). However, this data doesn’t necessarily exclude BGT1 as an important pharmacological target, as the global knockout of a transporter may involve complicated developmental compensation, and low expression levels do not necessarily preclude a function. Furthermore, brain BGT1 mRNA expression has been shown to be altered in a model of epilepsy (Rowley et al., 2011). Hence, the determination of a functional association between BGT1 and GABAergic neurotransmission remains under investigation.

### 4.6. Transgenic animals of GABA transporters

Table 6 summarizes the results obtained from transgenic animals developed to evaluate the role of various GABA transporters. GAT1 KO mice display a continuous tremor in the limbs and tail and are slightly more sensitive than WT mice to PTZ-, but not bicuculline-induced, seizures (Chiu et al., 2005). GAT3, vGAT, and GAD<sub>α</sub>, expression, as well as GABA<sub>α</sub> and GABA<sub>β</sub> receptor density, are not altered in GAT1 KO mice (Chiu et al., 2005; Jensen et al., 2003). GAD<sub>α/β</sub> was reported to be increased and tonic and phasic inhibitory conductances are significantly enhanced in GAT1 KO mice (Bragina et al., 2008; Jensen et al., 2003). GAT1 overexpressing transgenic (GAT1 Tg) mice display increased GABA uptake and increased seizure susceptibility to induced seizures (Ma et al., 2001a; Zhao et al., 2003). GAT1 Tg mice experience up-regulation of GABA<sub>α</sub> receptors and GLT1, and a reduction in asymmetric synapses (Ma et al., 2001a, 2001b). BGT1 KO mice display no seizure phenotype (Lehre et al., 2011). The phenotypes of transgenic animals of GABA transporters are summarized in Table 6.

<table>
<thead>
<tr>
<th>Pathway component</th>
<th>Epilepsy-related phenotype</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Membrane transporters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAT1 Tg</td>
<td>Increased susceptibility to induced seizures</td>
<td>Ma et al. (2001a,b) and Zhao et al. (2003)</td>
</tr>
<tr>
<td>GAT1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Continuous tremor and slightly increased susceptibility to induced seizures. Increased tonic but decreased phasic inhibitory conductances</td>
<td>Bragina et al. (2008), Chiu et al. (2005) and Jensen et al. (2003)</td>
</tr>
<tr>
<td>BGT1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>No spontaneous seizure phenotype or increased susceptibility to induced seizures</td>
<td>Lehre et al. (2011)</td>
</tr>
</tbody>
</table>

Abbreviations: GAT, GABA transporter; Tg, transgenic; BGT, betaine-GABA transporter.
5. Conclusion/Perspectives

Excitatory and inhibitory tone within the CNS is set by the neurotransmitters glutamate and GABA, respectively. The processes by which each of these neurotransmitters is synthesized, released, removed from the synaptic and extrasynaptic cleft and metabolized are all tightly regulated and have for many years been targets for drug discovery. For the GABAergic synapse, knowledge of the pathways whereby GABA is removed from the synaptic cleft (e.g., GAT1) and metabolized (e.g., GABA-T) has led to the synthesis and development of two commercially available antiepileptic drugs; i.e., tiagabine and vigabatrin, respectively. Furthermore, the recognition that GABA uptake is not limited to GAT1 but is likely controlled by at least three other GATs has fostered research to understand the role of these transporters and whether they too represent viable targets for therapy development. Unfortunately, similar efforts to exploit the mechanisms through which glutamate is synthesized, released, and removed from the synaptic cleft have failed to lead to the development of any commercially available neuroprotective or antiepileptic therapies. However, it is important to note that attempts to increase the expression of glutamate transporters has shown promise as a neuroprotective strategy for ALS and other neurodegenerative disorders. The use of genetically altered mice wherein the regulatory pathways controlling glutamate and GABA synthesis, release, reuptake, and metabolism have been altered are providing new and useful tools that could foster the development of new therapies for a number of neurological disorders. The myriad of emerging animal models will also provide greater insight into the pathophysiology of CNS disorders at the molecular and genetic levels. Each new advance will open more avenues for the early discovery and development of safer and more efficacious therapies in the years to come.

Acknowledgments

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References


Madsen KK, Ebert B, Clausen RP, Krogsgaard-Larsen P, Schousboe A and White HS (2009) GAT-1 and GAT-3 inhibitors target glutamate transporters N-4-[4-bis-(3-methyl-2-thienyl)-3-butyl-3]-hydroxy-4-(methylimino)-4,5,6,7-tetrahydrobenzo[d]isoaxazo-3-ol (EF1502) exhibit mechanistic differences in their ability to modulate the ataxia and convulsant action of the extrasynaptic GABA transporter against gaboxadol Neuropharm Exp Ther.


