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Review

Role of the betaine/GABA transporter (BGT-1/GAT2) for the control of epilepsy

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Abstract

Inactivation of γ-aminobutric acid (GABA) as a neurotransmitter is mediated by diffusion in the synaptic cleft followed by binding to transporter sites and translocation into the intracellular compartment. The GABA transporters of which four subtypes have been cloned (GAT1–4) are distributed at presynaptic nerve endings as well as extrasynaptically on astrocytic and neuronal elements. This anatomical arrangement of the transporters appears to be of critical functional importance for the maintenance of GABAergic neurotransmission. Pharmacological characterization of the GABA transporters using a large number of GABA analogs having restricted conformation and lipophilic character has been of instrumental importance for elucidation of the functional importance of the different transporters. One such analog EF1502 (*N*-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-hydroxy-4-methylamino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol) has been shown to selectively inhibit GAT1 (GABA transporter 1) and GAT2/BGT-1 (betaine/GABA transporter). Moreover, this GABA analog exhibits an unusually high efficiency as an anticonvulsant suggesting a novel role of the betaine/GABA transporter in epileptic seizure control. It is hypothesized that extrasynaptic actions of GABA may be involved in this phenomenon.

Keywords: GABA; Epilepsy; Transporter; Betaine transporter; Astrocyte; *exo*-THPO; Neuron; Astroglia; Anticonvulsant; Seizure

Contents

1. Introduction

GABAergic neurotransmission is to a large extent based on recycling of γ-aminobutric acid (GABA), which means that the high affinity transport processes in the presynaptic nerve endings and surrounding astrocytes play a key role (Schousboe et al., 2004). In a GABAergic synapse it is likely that approximately 80% of the released GABA is transported back into the GABAergic nerve ending, whereas the remaining GABA will be taken up into the astrocytes enwrapping the synapse (Hertz and Schousboe,

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1987). GABA taken up into the presynaptic nerve ending is available for the refilling of vesicles and subsequent exocytotic release (Gram et al., 1988). In the astrocytes, on the other hand, GABA can be metabolized via GABA-transaminase/succinic semialdehyde dehydrogenase to succinate, which enters the tricarboxylic acid (TCA) cycle. In astrocytes succinate may either be metabolized to CO_2 via pyruvate recycling or it may be converted to α-ketoglutarate and subsequently to glutamine which can be shuttled back into the GABAergic neuron. This is normally referred to as the GABA–glutamate–glutamine cycle (Waagepetersen et al., 2003). The prerequisite for this is the presence of pyruvate recycling, pyruvate carboxylase and glutamine synthetase in astrocytes (Norenberg and Martinez-Hernandez, 1979; Yu et al., 1983; Waagepetersen et al., 2002). In spite of this cycling between the neuronal and the astrocytic compartment, astrocytic uptake of GABA will inevitably lead to a loss of GABA from the neurotransmitter pool. This is likely to be the reason why selective inhibitors of astrocytic GABA uptake have been found to be more effective and better tolerated as anticonvulsants than inhibitors that act preferentially on neuronal GABA transporters (White et al., 2002).

Since the advent of the cloning technique, a number of GABA transporters have been identified. GAT1 was the first of four transporters to be isolated from rat brain (Radian et al., 1986; Guastella et al., 1990), which recognize GABA as a substrate (see Dalby, 2003; Sarup et al., 2003b). One of these also transports the osmolyte betaine and is referred to as the betaine transporter (Liu et al., 1993; Borden et al., 1995). The nomenclature of the cloned GABA transporters is somewhat confusing (Schousboe and Kanner, 2002; Dalby, 2003) due to the betaine/GABA transporter which in rats and humans is referred to as BGT-1 but in the mouse is called GAT2 (Dalby, 2003; Sarup et al., 2003b). Hence, the rat GAT2 and GAT3 correspond to the mouse GAT3 and GAT4, respectively (Dalby, 2003; Sarup et al., 2003b; Sarup et al., 2004). The present review shall discuss the possible functional role of BGT-1 or GAT2 in the light of the recent finding that inhibitors of this carrier appear to have an unusual pharmacological profile with regard to prevention of epileptic seizures in a number of animal models (White et al., in press). In this context it is, however, first necessary to briefly outline the pharmacological profiles of the cloned GABA transporters as well as discuss their anatomical and cellular localization in astrocytes and GABAergic neurons.

2. Pharmacology of GABA transporters

The identification of nipecotic acid as a specific inhibitor of GABA transport in brain slices (Krogsgaard-Larsen and Johnston, 1975) has been instrumental for the development of a large number of structurally related analogs as inhibitors of GABA transport in a variety of preparations. Early on, the bicyclic isoxazole bioisostere of nipecotic acid, THPO (4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol) was thought to act preferentially as an inhibitor of astrocytic GABA uptake (Schousboe et al., 1981). However, subsequent studies using more appropriate preparations of astrocytes and neurons revealed a less pronounced selectivity (see Sarup et al., 2003b). In spite of this, THPO has been used as a lead compound (structure) to produce a second generation bicyclic isoxazoles (Falch et al., 1999) having the nitrogen atom of the tetrahydro-pyridine ring as a primary amine, i.e. *exo*-THPO (4-amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol). This group of compounds are analogs of *exo*-THPO (Fig. 1) and one of these, i.e. *N*-methyl-*exo*-THPO (methylamino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol), Fig. 1, has been identified as one of the most pronounced selective inhibitors of GABA transport reported so far (Falch et al., 1999; White et al., 2002; Sarup et al., 2003a). Interestingly, the methyl group on the amino-nitrogen in *N*-methyl-*exo*-THPO seems to be very important in this context since the corresponding *N*-ethyl or *N*-dimethyl analogs show no selectivity and only modest or no inhibitory activity on neuronal and astroglial GABA uptake. One would assume that this may be related to steric factors reflecting the molecular size of these substituents. However, the finding that the more bulky *N*-4-phenyl-butyl-*exo*-THPO also exhibits an eight-fold selectivity as an inhibitor of astroglial versus neuronal GABA transport (Sarup et al., 2003a) seems to contradict this notion. In this context it is also interesting that larger and much more lipophilic substituents on the amino group of *exo*-THPO such as the *N*-diphenyl-butenyl or *N*-[4,4-bis(3-methyl-2-thienyl)-3-butenyl entities and analogs hereof normally result in GABA transport inhibitors with much higher potency (Sarup et al., 2003a; Clausen et al., submitted; White et al., in press). This confirms the principle first reported for such analogs of

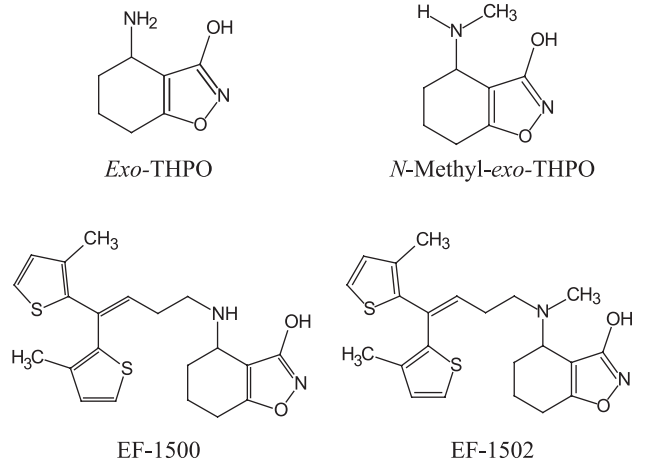

Fig. 1. Chemical structures of *exo*-THPO, *N*-methyl-*exo*-THPO and their lipiphilic *N*-[4,4-bis(3-methyl-2-thienyl)-3-butenyl] substituted analogs.

Table 1
IC_{50} values in cloned GABA transporters for GABA analogs exhibiting different degrees of selectivity between neuronal and astrocytic GABA transport

Inhibitor	IC_{50} (μM)					
	Neuron	Astrocyte	GAT1	GAT2	GAT3	GAT4
THPO	487	258	1300	3000	800	5000
exo-THPO	800	200	1000	3000	>3000	>3000
N-methyl-*exo*-THPO	405	48	450	>3000	>3000	>3000
N-acetyloxyethyl-*exo*-THPO	200	18	550	>1000	>1000	>1000
N-allyl-*exo*-THPO	220	73	190	>500	>500	>500
N-4-phenyl-butyl-*exo*-THPO	100	15	7	>500	>1000	>1000

Data from Sarup et al. (2003a,b).

nipecotic acid (Yunger et al., 1984; Ali et al., 1985). It may be noted that even though these lipophilic analogs are potent inhibitors of GABA transport, none are substrates for the transporter (see Sarup et al., 2004). Recently, lipophilic GABA transport inhibitors structurally different from GABA, nipecotic acid and THPO have been used to characterize the cloned GABA transporters (Thomsen et al., 1997) but these compounds have not yet been studied in neurons or astrocytes. The fact that they cannot strictly be considered GABA analogs may explain why these compounds have been reported to act on non-GABAergic transmitter systems; e.g. the adrenergic system (Dalby et al., 1997).

The demonstration that neuronal and astroglial GABA transport exhibit differences with regard to their pharmacological properties would lead to the assumption that this may be related to differences in the expression of the different subtypes of transporters, GAT1, GAT2 (BGT-1), GAT3 and GAT4 (Dalby, 2003; Sarup et al., 2003b). A large number of GABA analogs have been investigated with regard to inhibition of GABA uptake in neurons, astrocytes and the cloned GABA transporters expressed in cell lines (Sarup et al., 2003a, Sarup et al., 2003b; Clausen et al., submitted). IC_{50} values for the analogs exhibiting selectivity with regard to inhibition of GABA uptake in neurons and astrocytes are presented in Table 1. It is clear that all of these analogs inhibit only GAT1 and hence, no clues as to a pharmacological explanation for the cellular selectivity can presently be provided. This clearly represents a challenge for future studies (see Sarup et al., 2003b; Sarup et al., 2004).

In a recent study (Clausen et al., submitted), a large number of *exo*-THPO analogs were synthesized and investigated with regard to inhibition of GABA transport in Human Embryonic Kidney (HEK 293) cells expressing one of the cloned mouse GABA transporters GAT1–4. Among these lipophilic analogs of *exo*-THPO, one structure, *N*-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-hydroxy-4-methylamino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol (EF1502; Fig. 1) turned out to inhibit equipotently GABA uptake by GAT1 and GAT2 (BGT-1). Interestingly, the structurally very closely related compound *N*-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-hydroxy-4-amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol (EF1500; Fig. 1) only inhibited GAT1. Likewise, all other lipophilic analogs of *exo*-THPO selectively inhibited GAT1. The difference in structure between EF1502 and EF1500 is the methyl group at the amino group (Fig. 1) again underlining the importance of this group for the interaction with the binding site of the transporters.

3. Anticonvulsant activity of GAT inhibitors

It has been known for more than 20 years that inhibitors of GABA transport can exert anticonvulsant activity in different animal models of epilepsy (see Schousboe et al., 1983; Schousboe, 1990; Dalby, 2003). In agreement with the notion that GABA neurotransmission is dependent upon recycling of GABA, i.e. transport into the presynaptic nerve ending of released GABA, it was proposed that preferential inhibition of the astrocytic GABA transport could be functionally very important (Schousboe et al., 1983). Indeed, it has been repeatedly demonstrated that GABA transport inhibitors having a preferential inhibitory action on astrocytic GABA transport are efficient anticonvulsants (Wood et al., 1983; Gonsalves et al., 1989a; Gonsalves et al., 1989b; White et al., 1993; White et al., 2002). In this context it has recently been shown that a better correlation exists between anticonvulsant potency and astrocytic GABA uptake than between anticonvulsant potency and potency for inhibition of neuronal GABA uptake (White et al., 2002). It should be kept in mind that up to a 10-fold difference exists in astroglial GABA transport activity in primary cultures of astrocytes derived from different brain regions (Hansson et al., 1985). Accordingly, the high correlation coefficient reported between inhibition of astroglial GABA transport in vitro and efficacy in audiogenic seizure-susceptible Fring's mice (White et al., 2002) may reflect a complex modulatory action on glial GABA transport regionally in vivo. However, it should be noted that the majority of, if not all, anticonvulsant GABA transport inhibitors known so far are inhibitors of GAT1 (Sarup et al., 2003b). This may appear somewhat enigmatic in light of the repeated finding that GAT1 is predominantly expressed in neurons (see below). It should, however, be pointed out that recently inhibitors of GABA transporters other than GAT1

have been reported (Thomsen et al., 1997; White et al., in press; Clausen et al., submitted) some of which have also anticonvulsant activity that can be correlated to inhibition of GABA transport by non-GAT1 transporters (Dalby et al., 1997; Dalby, 2003; White et al., in press). It may be particularly intriguing that EF1502 which equipotently inhibits GABA transport mediated by GAT1 and GAT2/BGT-1 has been shown to exert a synergistic anticonvulsant activity when administered together with either one of the selective GAT1 inhibitors tiagabine or *N*-[4,4-bis(4-fluorophenyl)-butyl]-3-hydroxy-4-amino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol (White et al., in press). This was revealed by the construction of isobolograms of ED_{50} values of co-administration of EF1502 with either one of the other drugs to audiogenic seizure-susceptible Fring's mice. Similarly, a synergistic interaction between tiagabine and EF1502 was also observed in the mouse pentylenetetrazole seizure threshold test (White et al., in press). On the contrary, no synergistic action was observed in the rotarod performance test carried out on mice receiving tiagabine and EF1502 either alone or in combination. Moreover, EF1502 was much less toxic than tiagabine in this test. Based on these findings it may be concluded that emphasis should be placed on GAT2/BGT-1 as an interesting target for development of anticonvulsant drugs. As discussed further below in relation to the anatomical localization of the GABA transporters, this notion contrasts with previous conclusions in which this transporter was discounted relative to GAT3 and GAT4 (Dalby, 2003). This latter conclusion was based on the anticonvulsant profile of inhibitors of GABA uptake mediated by GAT2, -3 and -4 (Thomsen et al., 1997; Dalby et al., 1997).

4. Anatomical localization of GABA transporters

A number of studies have addressed the localization and distribution of GAT1 in the mature mammalian central nervous system (Gadea and Lopez-Colome, 2001). GAT1 mRNA is located mainly in neurons throughout the brain (Liu et al., 1993; Swan et al., 1994; Durkin et al., 1995; Minelli et al., 1995, Yasumi et al., 1997; Frahm et al., 2000) and in retina (Brecha and Weigmann, 1994; Ruiz et al., 1994). The expression pattern of GAT1 mRNA is supported by observations obtained using light microscopy of the immunocytochemical localization of GAT1 (Ikegaki et al., 1994; Minelli et al., 1995, Ribak et al., 1996a; Johnson et al., 1996; Conti et al., 1998). Ultrastructural analysis of the GAT1 localization demonstrates that it is confined to axon terminals that form symmetrical synapses (i.e. GABAergic) in the rat cerebral cortex and that it is also to some extent expressed in proximal astrocytic processes (Minelli et al., 1995). A similar expression pattern is seen in the primate cerebral cortex (Conti et al., 1998), although others have found the astroglial expression to be absent or at least below the detection level in this brain area (Ong et al., 1998). It should be noted that in the thalamus GAT1 may be restricted to distal processes of astrocytes non-discriminatingly positioned at both symmetrical and asymmetrical synapses (De Biasi et al., 1998). GAT1 is also expressed in astrocytes in other brain areas (Morara et al., 1996; Minelli et al., 1995,Minelli et al., 1996; Johnson et al., 1996; Ribak et al., 1996b; Biedermann et al., 2002). This obviously is in keeping with the finding that GAT1 inhibitors always act on both neuronal and astrocytic GABA transport (see above). Further studies using quantitative immunogold electron microscopy to address the number and subcellular localization of GAT1 may elucidate the differential roles GAT1 in neurons and astrocytes may exhibit. A recent study has complemented this method by counting the number of GAT1 proteins in GAT1-GFP knock-in mice (Chiu et al., 2002). On average surface densitity of 800–1300 GAT1 molecules/μm^2 was found in presynaptic boutons in hippocampus, while in similar structures in cerebellum the number may be several orders higher (Chiu et al., 2002). The relative number, distribution and activity of the different GAT subtypes in neurons and astrocytes will be instrumental for our understanding of how to pharmacologically interact with GABA transport in the CNS.

The betaine/GABA transporter, GAT2, is mainly localized in tissues other than the brain but it is also present in several brain areas (Lopéz-Corcuera et al., 1992; Borden et al., 1995). It appears to have a mainly astrocytic expression (Borden et al., 1995), although it remains to be validated whether the subcellular localization and predominant astrocytic expression of GAT2 in vitro is preserved in vivo. It may be of interest that when expressed in polarized epithelial cells such as the Madin–Darby canine kidney cell line (MDCK cells), GAT1 and GAT2 exhibit a differential location at the apical and the basolateral cell surface, respectively (Ahn et al., 1996). In addition, GAT2 cDNA microinjected into cultured hippocampal neurons is sorted to extrasynaptic loci as indicated by the absence of co-localization with synapsin (Ahn et al., 1996). It seems therefore likely that GAT1 and GAT2 would have different expression patterns also in neural cells, not only at the multicellular level but also at the cellular level. This may be important for the understanding of the mechanism underlying the synergistic anticonvulsant action of EF1502 in combination with tiagabine mentioned above (White et al., in press). While GAT1 may be located in close proximity to the synaptic area in neuronal as well as astrocytic membranes it is less likely that GAT2 (BGT-1) is present at this location. It is therefore possible that the efficacy of EF1502 likely owing to its action on GAT2 (White et al., in press) could best be explained by modulation of extracellular GABA concentrations at extrasynaptic sites. This would be compatible with an action of GABA on extrasynaptic receptors resulting in tonic inhibition (Mody, 2001). It

should be mentioned that GAT1-deficient mice per se display an increased tonic postsynaptic conductance due to overactivation of $GABA_A$ receptors and in part also by presynaptic signalling via $GABA_B$ receptors (Jensen et al., 2003). Whether such mechanisms comply with neurotransmitter overspill and the neurophysiology of wild-type mice and pharmacological inhibition of GAT1 alone or in combination with GAT2 (BGT-1) by e.g. EF1502 is not known. An analogous mechanism of action has been proposed for the GABA transport inhibitors reported by Thomsen et al. (1997) and which inhibit GAT2, -3 and -4. The anticonvulsant activity of these inhibitors has been associated primarily with inhibitory activity on GAT3 and GAT4 (Dalby et al., 1997). The main reason for this conclusion was that GAT3 and GAT4 have higher affinity for GABA than does GAT2 (Bolvig et al., 1999; Dalby, 2003) and that the anatomical distribution of GAT3 and GAT4 favors such a mechanism (Dalby, 2003). In particular, GAT4 (GAT3 in the rat) is located primarily in astrocytic processes (Morara et al., 1996; Ribak et al., 1996a, Ribak et al., 1996b; De Biasi et al., 1998; Yan and Ribak, 1998a,Yan and Ribak, 1998b) making it highly suitable for mediating an extrasynaptic modulatory action of GABA. Development of more selective inhibitors of GAT2 and GAT4 appears a prerequisite for elucidation of the role of this mechanism in the fine tuning of GABAergic activity and its role in control of epileptic seizure activity. Efforts are currently being made to develop GABA transport inhibitors with this pharmacological profile.

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References

Ahn, J., Mundigl, O., Muth, T.R., Rudnick, G., Caplan, M.J., 1996. Polarized expression of GABA transporters in Madin–Darby canine kidney cells and cultured hippocampal neurons. J. Biol. Chem. 271, 6917–6924.

Ali, F.E., Bondinell, W.E., Dandridge, P.A., Frazee, J.S., Garvey, E., Girard, G.R., Kaiser, C., Ku, T.W., Lafferty, J.J., Moonsammy, G.I., Oh, H.-J., Rush, J.A., Setler, P.E., Stringer, O.D., Venslavsky, J.W., Volpe, B.W., Yunger, L.M., Zircle, C.L., 1985. Orally active and potent inhibitors of gamma-aminobutyric acid uptake. J. Med. Chem. 28, 653–660.

Biedermann, B., Bringmann, A., Reichenbach, A., 2002. High-affinity GABA uptake in retinal glial (Müller) cells of the guinea pig: electrophysiological characterization, immunohistochemical localization, and modeling of efficiency. Glia 39, 217–228.

Brecha, N.C., Weigmann, C., 1994. Expression of GAT-1, a high-affinity gamma-aminobutyric acid plasma membrane transporter in the rat ratina. J. Comp. Neurol. 345, 602–611.

Bolvig, T., Larsson, O.M., Pickering, D.S., Nelson, N., Falch, E., Krogsgaard-Larsen, P., Schousboe, A., 1999. Action of bicyclic isoxazole GABA analogues on GABA transporters and its relation to anticonvulsant activity. Eur. J. Pharmacol. 375, 367–374.

Borden, L.A., Smith, K.E., Gustafson, E.L., Branchek, T.A., Weinshank, R.L., 1995. Cloning and expression of a betaine/GABA transporter from human brain. J. Neurochem. 64, 977–984.

Chiu, C.S., Jensen, K., Sokolova, I., Wang, D., Li, M., Deshpande, P., Davidson, N., Mody, I., Quick, M.W., Quake, S.R., Lester, H.A., 2002. Number, density, and surface/cytoplasmic distribution of GABA transporters at presynaptic structures of knock-in mice carrying GABA transporter subtype 1-green fluorescent protein fusions. J. Neurosci. 22, 10251–10266.

Conti, F., Melone, M., De Biasi, S., Minelli, A., Brecha, N.C., Ducati, A., 1998. Neuronal and glial localization of GAT-1, a high-affinity gamma-aminobutyric acid plasma membrane transporter, in human cerebral cortex: with a note on its distribution in monkey cortex. J. Comp. Neurol. 396, 51–63.

Dalby, N.O., 2003. Inhibition of γ-aminobutyric acid uptake: anatomy, physiology and effects against epileptic seizures. Eur. J. Pharmacol. 479, 127–137.

Dalby, N.O., Thomsen, C., Fink Jensen, A., Lundbeck, J., Sokilde, B., Man, C.M., Sorensen, P.O., Meldrum, B., 1997. Anticonvulsant properties of two GABA uptake inhibitors NNC 05-2045 and NNC 05-2090, not acting preferentially on GAT-1. Epilepsy Res. 28, 51–61.

De Biasi, S., Vitellaro-Zuccarello, L., Brecha, N.C., 1998. Immunoreactivity for the GABA transporter-1 and GABA transporter-3 is restricted to astrocytes in the rat thalamus. A light and electron-microscopic immunolocalization. Neuroscience 83, 815–828.

Durkin, M.M., Smith, K.E., Borden, L.A., Weinshank, R.L., Branchek, T.A., Gustafson, E.L., 1995. Localization of messenger RNAs encoding three GABA transporters in rat brain: an in situ hybridization study. Brain Res. Mol. Brain Res. 33, 7–21.

Falch, E., Perregaard, J., Frølund, B., Søkilde, B., Buur, A., Hansen, L.M., Frydenvang, K., Brehm, L., Bolvig, T., Larsson, O.M., Sanchez, C., White, H.S., Schousboe, A., Krogsgaard-Larsen, P., 1999. Selective inhibitors of glial GABA uptake: synthesis, absolute stereochemistry and pharmacology of the enantiomers of 3-hydroxy-4-amino-4,5,6,7-tetrahydro-1,2-benzisoxazole (*exo*-THPO) and analogues. J. Med. Chem. 42, 5402–5414.

Frahm, C., Engel, D., Piechotta, A., Heinemann, U., Draguhn, A., 2000. Presence of gamma-aminobutyric acid transporter mRNA in interneurons and principal cells of rat hippocampus. Neurosci. Lett. 288, 175–178.

Gadea, A., Lopez-Colome, A.M., 2001. Glial transporters for glutamate, glycine, and GABA: II. GABA transporters. J. Neurosci. Res. 63, 461–468.

Gonsalves, S.F., Twitchell, B., Harbaugh, R.E., Krogsgaard-Larsen, P., Schousboe, A., 1989a. Anticonvulsant activity of intracerebroventricularly administered glial GABA uptake inhibitors and other GABA-mimetics in chemical seizure models. Epilepsy Res. 4, 34–41.

Gonsalves, S.F., Twitchell, B., Harbaugh, R.E., Krogsgaard-Larsen, P., Schousboe, A., 1989b. Anticonvulsant activity of the glial GABA uptake inhibitor, THAO, in chemical seizures. Eur. J. Pharmacol. 168, 265–268.

Gram, L., Larsson, O.M., Johnsen, A.H., Schousboe, A., 1988. Effects of valproate, vigabatrin and aminooxyacetic acid on release of endogenous and exogenous GABA from cultured neurons. Epilepsy Res. 2, 87–95.

Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S., Miedel, M.C., Davidson, N., Lester, H.A., Kanner, B.I., 1990. Cloning and expression of a rat brain GABA transporter. Science 249, 1303–1306.

Hansson, E., Eriksson, P., Nilsson, M., 1985. Amino acid and monoamine transport in primary astroglial cultures from defined brain regions. Neurochem. Res. 10, 1335–1341.

Hertz, L., Schousboe, A., 1987. Primary cultures of GABAergic and glutamatergic neurons as model systems to study neurotransmitter functions: I. Differentiated cells. In: Vernadakis, A., Privat, A., Lauder, J.M., Timiras, P.S., Giacobini, E. (Eds.), Model Systems of Development and Aging of the Nervous System. M. Nijhoff Publ., Boston, pp. 19–31.

Ikegaki, N., Saito, N., Hashima, M., Tanaka, C., 1994. Production of specific antibodies against GABA transporter subtypes (GAT1, GAT2, GAT3) and their application to immunocytochemistry. Mol. Brain Res. 26, 47–54.

Jensen, K., Chiu, C.S., Sokolova, I., Lester, H.A., Mody, I., 2003. GABA transporter-1 (GAT1)-deficient mice: differential tonic activation of GABAA versus GABAB receptors in the hippocampus. J. Neurophysiol. 90, 2690–2701.

Johnson, J., Chen, T.K., Rickman, D.W., Evans, C., Brecha, N.C., 1996. Multiple gamma-aminobutyric acid plasma membrane transporters (GAT-1, GAT-2, GAT-3) in the rat retina. J. Comp. Neurol. 375, 212–224.

Krogsgaard-Larsen, P., Johnston, G.A.R., 1975. Inhibition of GABA uptake in rat brain slices by nipecotic acid, various isoxazoles and related compounds. J. Neurochem. 25, 797–802.

Liu, Q.R., Lopez-Corcuera, B., Mandiyan, S., Nelson, H., Nelson, N., 1993. Molecular characterization of four pharmacologically distinct γ-aminobutyric acid transporters in mouse brain. J. Biol. Chem. 268, 2104–2112.

Lopéz-Corcuera, B., Liu, Q.R., Mandiyan, S., Nelson, H., Nelson, N., 1992. Expression of a mouse brain cDNA encoding novel γ-aminobutyric acid transporter. J. Biol. Chem. 267, 17491–17493.

Minelli, A., Brecha, N.C., Karschin, C., De Biasi, S., Conti, F., 1995. GAT-1, a high-affinity GABA plasma membrane transporter, is localized to neurons and astroglia in the cerebral cortex. J. Neurosci. 15, 7734–7746.

Minelli, A., De Biasi, S., Brecha, N.C., Zuccarello, L.V., Conti, F., 1996. GAT-3, a high-affinity GABA plasma membrane transporter, is localized to astrocytic processes, and is not confined to the vicinity of GABAergic synapses in the cerebral cortex. J. Neurosci. 16, 6255–6264.

Mody, I., 2001. Distinguishing between GABA(A) receptors responsible for tonic and phasic conductances. Neurochem. Res. 26, 907–913.

Morara, S., Brecha, N.C., Marcotti, W., Provini, L., Rosina, A., 1996. Neuronal and glial localization of the GABA transporter GAT-1 in the cerebellar cortex. NeuroReport 7, 2993–2996.

Norenberg, M.D., Martinez-Hernandez, A., 1979. Fine structural localization of glutamine synthetase in astrocytes of rat brain. Brain Res. 161, 303–310.

Radian, R., Bendahan, A., Kanner, B.I., 1986. Purification and identification of the functional sodium- and chloride-coupled gamma-aminobutyric acid transport glycoprotein from rat brain. J. Biol. Chem. 261, 15437–15441.

Ribak, C.E., Tong, W.M., Brecha, N.C., 1996a. GABA plasma membrane transporters, GAT-1 and GAT-3, display different distributions in the rat hippocampus. J. Comp. Neurol. 367, 595–606.

Ribak, C.E., Tong, W.M., Brecha, N.C., 1996b. Astrocytic processes compensate for the apparent lack of GABA transporters in the axon terminals of cerebellar Purkinje cells. Anat. Embryol. (Berl.) 194, 379–390.

Ruiz, M., Egal, H., Sarthy, V., Qian, X., Sarkar, H.K., 1994. Cloning, expression, and localization of a mouse retinal gamma-aminobutyric acid transporter. Investig. Ophthalmol. Vis. Sci. 35, 4039–4048.

Sarup, A., Larsson, O.M., Bolvig, T., Frølund, B., Krogsgaard-Larsen, P., Schousboe, A., 2003a. Effects of 3-hydroxy-4-amino-4,5,6,7-tetrahydro-1,2-benzisoxazol (*exo*-THPO) and its N-substituted analogs on GABA transport in cultured neurons and astrocytes and by the four cloned mouse GABA transporters. Neurochem. Int. 43, 445–451.

Sarup, A., Larsson, O.M., Schousboe, A., 2003b. GABA transporters and GABA-transaminase as drug targets. Curr. Drug Targets CNS Neurol. Dis. 2, 269–277.

Sarup, A., Larsson, O.M., Schousboe, A., 2004. Characterization of the substrate binding site in GABA transporters. In: Bräuner-Osborne, H., Schousboe, A. (Eds.), Molecular Neuropharmacology: Strategies and Methods. Humana Press, Totowa, NJ, pp. 175–190.

Schousboe, A., 1990. Neurochemical alterations associated with epilepsy or seizure activity. In: Dam, M., Gram, L. (Eds.), Comprehensive Epileptology. Raven Press, NY, pp. 1–16.

Schousboe, A., Kanner, B., 2002. GABA transporters: functional and pharmacological properties. In: Egebjerg, J., Schousboe, A., Krogsgaard-Larsen, P. (Eds.), Glutamate and GABA Receptors and Transporters. Taylor and Francis Publ., London, UK, pp. 337–349.

Schousboe, A., Larsson, O.M., Hertz, L., Krogsgaard-Larsen, P., 1981. Heterocyclic GABA analogues as new selective inhibitors of astroglial GABA transport. Drug Dev. Res. 1, 115–127.

Schousboe, A., Larsson, O.M., Wood, J.D., Krogsgaard-Larsen, P., 1983. Transport and metabolism of GABA in neurons and glia: implications for epilepsy. Epilepsia 24, 531–538.

Schousboe, A., Sarup, A., Bak, L.K., Waagepetersen, H.S., Larsson, O.M., 2004. Role of astrocytic transport processes in glutamatergic and GABAergic neurotransmission. Neurochem. Int. 45, 521–527.

Swan, M., Najlerahim, A., Watson, R.E., Bennett, J.P., 1994. Distribution of mRNA for the GABA transporter GAT-1 in the rat brain: evidence that GABA uptake is not limited to presynaptic neurons. J. Anat. 185, 315–323.

Thomsen, C., Sørensen, P.O., Egebjerg, J., 1997. 1-(3-(9H-carbazol-9-yl)-1-propyl)-4-(2-methoxyphenyl)-4-piperidinol, a novel subtype selective inhibitor of the mouse type II GABA-transporter. Br. J. Pharmacol. 120, 983–985.

Waagepetersen, H.S., Qu, H., Hertz, L., Sonnewald, U., Schousboe, A., 2002. Demonstration of pyruvate recycling in primary cultures of neocortical astrocytes but not in neurons. Neurochem. Res. 27, 1431–1437.

Waagepetersen, H.S., Sonnewald, U., Schousboe, A., 2003. Compartmentation of glutamine, glutamate and GABA metabolism in neurons and astrocytes: functional implications. Neuroscientist 9, 398–403.

White, H.S., Hunt, J., Wolf, H.H., Swinyard, E.A., Falch, E., Krogsgaard-Larsen, P., Schousboe, A., 1993. Anticonvulsant activity of the γ-aminobutyric acid uptake inhibitor *N*-4,4-diphenyl-3-butenyl-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol. Eur. J. Pharmacol. 236, 147–149.

White, H.S., Sarup, A., Bolvig, T., Kristensen, A.S., Petersen, G., Nelson, N., Pickering, D.S., Larsson, O.M., Frû¡lund, B., Krogsgaard-Larsen, P., Schousboe, A., 2002. Correlation between anticonvulsant activity and inhibitory action on glial GABA uptake of the highly selective mouse GAT1 inhibitor 3-hydroxy-4-amino-4,5,6,7-tetrahydro-1,2-benzisoxazole (*exo*-THPO) and its *N*-alkylated analogs. J. Pharmacol. Exp. Ther. 302, 636–644.

White, H.S., Watson, W.P., Hansen, S., Slough, S., Perregaard, J., Sarup, A., Bolvig, T., Petersen, G., Larsson, O.M., Clausen, R.P., Frølund, B., Falch, E., Krogsgaard-Larsen, P., Schousboe, A., 2004. First demonstration of a functional role for CNS betaine/GABA transporter (mGAT2) based on synergistic anticonvulsant action among inhibitors of mGAT1 and mGAT2. J. Pharmacol. Exp. Ther., in press.

Wood, J.D., Johnson, D.D., Krogsgaard-Larsen, P., Schousboe, A., 1983. Anticonvulsant activity of the glial-selective GABA uptake inhibitor, THPO. Neuropharmacology 22, 139–142.

Yan, X.X., Ribak, C.E., 1998. Developmental expression of gamma-aminobutyric acid transporters (GAT-1 and GAT-3) in the rat cerebellum: evidence for a transient presence of GAT-1 in Purkinje cells. Brain Res. Dev. Brain Res. 111, 253–269.

Yan, X.X., Ribak, C.E., 1998. Increased expression of GABA transporters, GAT-1 and GAT-3, in the deafferented suporior colliculus of the rat. Brain Res. 783, 63–76.

Yasumi, M., Sato, K., Shimada, S., Nishimura, M., Tohyama, M., 1997. Regional distribution of GABA transporter 1 (GAT1) mRNA in the rat brain: comparison with glutamic acid decarboxylase67 (GAD67) mRNA localization. Brain Res. Mol. Brain Res. 44, 205–218.

Yu, A.C.H., Drejer, J., Hertz, L., Schousboe, A., 1983. Pyruvate carboxylase activity in primary cultures of astrocytes and neurons. J. Neurochem. 41, 1484–1487.

Yunger, L.M., Fowler, P.J., Zarevics, P., Setler, P.E., 1984. Novel inhibitors of gamma-aminobutyric acid (GABA) uptake: anticonvulsant actions in rats and mice. J. Pharmacol. Exp. Ther. 228, 109–115.