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Action of bicyclic isoxazole GABA analogues on GABA transporters and its relation to anticonvulsant activity

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Abstract

The inhibitory action of bicyclic isoxazole γ-aminobutyric acid (GABA) analogues and their 4,4-diphenyl-3-butenyl (DPB) substituted derivatives has been investigated in cortical neurones and astrocytes as well as in human embryonic kidney (HEK 293) cells transiently expressing either mouse GABA transporter-1 (GAT-1), GAT-2, -3 or -4. It was found that 4,5,6,7-tetrahydroisoxazolo(4,5-*c*)pyridin-3-ol (THPO) and 5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*c*]azepin-3-ol (THAO) displayed some inhibitory activity on GAT-1 and GAT-2, where the compounds exhibited a slightly lower potency on GAT-2 compared to GAT-1. DPB substituted THPO displayed higher inhibitory potency than the parent compound regarding the ability to inhibit GABA uptake via GAT-1 and GAT-2. Concerning the inhibitory mechanism, THPO, THAO and DPB–THPO were competitive inhibitors on GAT-1 transfected HEK 293 cells and the same mechanism was observed for THPO in GAT-3 transfected cells. Regarding GABA uptake into neurones and astroglia cells THAO and DPB–THAO both displayed competitive inhibitory action. The observations that THPO, THAO as well as their DPB derivatives act as competitive inhibitors together with earlier findings such as potent anticonvulsant activity, lack of proconvulsant activity and the ability of THPO to increase extracellular GABA concentration, indicate that these bicyclic isoxazole GABA analogues and their DPB derivatives may be useful lead structures in future search for new antiepileptic drugs.

Keywords: GABA (γ-Aminobutyric acid) transporter; GABA uptake inhibitor; THPO (4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol); THAO (5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*c*]azepin-3-ol); DPB (4,4-diphenyl-3-butenyl)–THPO; DPB–THAO; Epilepsy; Anticonvulsant

1. Introduction

Termination of action of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in synapses of the central nervous system is mediated by high affinity transport either into presynaptic GABAergic nerve endings or astroglial cells surrounding the synapse (Schousboe, 1981, 1990; Krogsgaard-Larsen et al., 1987). Studies of the pharmacological characteristics of GABA uptake in brain tissue preparations, as well as primary cultures of neurones and astroglial cells, have suggested that neuronal and astroglial GABA uptake exhibit distinctly different pharmacological properties (for references, see Krogsgaard-Larsen et al., 1987; Schousboe and Westergaard, 1995). Additionally, the uptake systems in neurones and astroglial cells play different functional roles for termination of the inhibitory neurotransmission. Reuptake of GABA into the presynaptic endings of the GABAergic neurones allows recycling of neurotransmitter GABA while uptake into the surrounding astroglia cells results in a degradation which potentially would drain the relevant GABA pool and thereby reduce the optimal GABAergic inhibitory tonus (Schousboe, 1990). In recent years it has been established that inhibitors of GABA transport and in particular astroglial uptake such as 4,5,6,7-tetrahydroisoxazolo(4,5-*c*)pyridin-3-ol (THPO) and 5,6,7,8-tetrahydro-4*H*-isoxazolo(4,5-*c*)azepin-3-ol (THAO) can act as anticonvulsant agents (Krogsgaard-Larsen et al., 1987, 1994; Gonsalves et al., 1989a,b; Schousboe et al., 1991). Furthermore, it has

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been shown that L-2,4-diaminobutyric acid (L-DABA), which preferentially inhibits the neuronal carrier (Krogsgaard-Larsen et al., 1987) acts as a proconvulsant after intracerebroventricular administration, whereas THPO and THAO exhibit no proconvulsant activity (Gonsalves et al., 1989a,b). The observation that THPO is not transported by the carrier (Schousboe et al., 1990) may also be a factor of importance in this context as a similar observation has been made for the antiepileptic drug tiagabine ((*R*)-1-(4,4-bis(3-methyl-2-thienyl)-3-butenyl)-3-piperidencarboxylic acid) (Bræstrup et al., 1990; Nielsen et al., 1991) which is a lipophilic derivative of nipecotic acid, a substrate for the GABA carriers (Johnston et al., 1976; Larsson and Schousboe, 1981; Larsson et al., 1983). Thus, using the microdialysis technique it was shown that administration of THPO, but not nipecotic acid, led to an increase of the extracellular GABA level (Juhász et al., 1997) indicating that the non-transportable characteristic is of functional importance.

Molecular cloning of the GABA transporters has revealed the existence of a family of four subtypes (Uhl and Harting, 1992). These subtypes, cloned from mouse brain, were termed GAT-1 through GAT-4 (Liu et al., 1992, 1993; Lopéz-Corcuera et al., 1992). Pharmacological characterization of these subtypes of GABA transporters has shown that nipecotic acid, guvacine and to a much lower extent L-DABA are capable of inhibiting GABA uptake via GAT-1. With regard to GAT-2 and GAT-3 only betaine and β-alanine showed GABA uptake inhibitory effects. β-Alanine, nipecotic acid and guvacine inhibit GABA uptake by GAT-4 (Liu et al., 1993). When comparing the pharmacological characteristics of GABA transport mediated by the cloned carriers with that of neuronal and glial GABA uptake (Schousboe and Westergaard, 1995; Borden, 1996) it is evident that no simple correlation seems to exist. Therefore, in order to elucidate the functional importance of the GABA transporters in the neuronal–glial GABA homeostasis and its role in seizure activity, new and improved pharmacological tools are needed.

The present study was undertaken in order to characterize the kinetics of THPO and THAO as well as their lipophilic 4,4-diphenyl-3-butenyl (DPB) derivatives in cultured neurones and astrocytes as well as at the cloned GABA transporters expressed in HEK 293 cells. Such studies are a prerequisite for the design and development of new GABA analogues of restricted conformation with the aim of producing novel anticonvulsant and antiepileptic drugs.

2. Materials and methods

2.1. Materials

Newborn mice and 15-day-old mouse embryos were obtained from the animal quarters in the Panum Institute (Copenhagen, Denmark). Plastic tissue culture dishes were purchased from NUNC Denmark, and fetal calf serum from Sera-Lab, Sussex, UK. Poly-D-lysine (molecular weight greater than 300.000), trypsin, soybean trypsin inhibitor, dibutyryl adenosine monophosphate (dBcAMP), DNAse, cytosine arabinoside and amino acids were obtained from Sigma (St. Louis, MO, USA), insulin from NOVO Nordisk, Denmark, and penicillin from Leo, Denmark. [^{3}H]GABA (79.0 Ci mmol^{-1}) was from Dupont-NEN (Frankfurt, Germany). All other chemicals were of the purest grade available from regular commercial sources. The accession numbers for mouse GAT-1–4 are M92378, M97632, L04663, and L04662, respectively.

2.2. Primary cell culture

Astrocytes were cultured essentially as described by Hertz et al. (1989a). Prefrontal cortex was taken from new-born mice and passed through Nitex nylon sieves (80 μm pore size) into a slightly modified Dulbecco's medium (DMEM) as defined by Hertz et al. (1982) containing 20% (v/v) fetal calf serum and subsequently plated onto NUNC 24-well multidishes. After 14 days in culture, the serum concentration in the culture medium was reduced to 10% (v/v). The cultures were grown for a total of three weeks, with change of the medium 2 days after inoculation and subsequently two or three times a week. When the cells were confluent after approximately 14 days in culture dBcAMP was added to the culture medium at a final concentration of 0.25 mM. This addition led to the formation of well-differentiated astrocytes (Hertz et al., 1982).

Cerebral cortical neurones were isolated and cultured from 15-day-old mouse embryos. After dissociation of the tissue by trypsinization and trituration in a DNAse solution containing soybean trypsin inhibitor as described by Hertz et al. (1989b), the cells were plated onto NUNC 24-well multidishes. After 48 h in culture, 20 μM cytosine arabinoside was added to the culture medium to prevent astrocytic proliferation (Larsson et al., 1985). Cells were cultured for 7–8 days at which time the neurones had become functionally differentiated (Schousboe and Hertz, 1987).

2.3. Subcloning and expression of GABA-transporters

The cDNAs encoding the four murine GABA transporters (Liu et al., 1992, 1993; Lopéz-Corcuera et al., 1992) were subcloned into the mammalian expression vector, pCis (Gorman et al., 1990). GAT-1pBSK was digested with *Xba*I which was then blunt-ended with Klenow enzyme and subsequently digested with *Nhe*I. The 2.2 kbp fragment containing GAT-1 was then ligated into the 5′-*Cla*I (blunt-ended with Klenow enzyme) and 3′-*Xba*I sites of pCis vector. The 1.9 kbp *Xba*I (5′)–*Nhe*I (3′) GAT-2 fragment from GAT-2pBSK was ligated into the *Xba*I site of pCis vector. GAT-3pGEM4Z was digested

with *Hin*DIII, blunt-ended with Klenow enzyme and then digested with *Nar*I. The 2.5 kbp fragment containing GAT-3 was then ligated into the 5′-*Cla*I and 3′-*Xho*I (blunt-ended with Klenow enzyme) sites of pCis vector. GAT-4pBSK was digested with *Xba*I and the 2.4 kbp *Xba*I–*Xba*I fragment containing GAT-4 ligated into the *Xba*I site of pCis vector. GATpCis cDNAs were transformed into XL1-Blues bacteria (Stratagene, La Jolla, CA) and plasmids prepared by Qiagen (Chatsworth, CA) column purification. Human embryonic kidney (HEK 293) cells were maintained in complete growth medium (MEM with Earle's salts, supplemented with 5% fetal calf serum, 1% Anti-PPLO and 1% Glutamax I, pH 7.3 under 5% CO_2). GATpCis transfections were carried out as described by Chen and Okayama (1987) and Pritchett et al. (1988).

2.4. [^{3}H]GABA uptake

The uptake of [^{3}H]GABA in cultured astrocytes and neurones as well as in recombinant cell systems was investigated essentially as described previously (Schousboe et al., 1977; Larsson et al., 1986b). The incubations were carried out at 37°C in phosphate buffered saline (PBS) and terminated after 3 min. Incubations were initiated by exchanging the culture medium with incubation medium leaving the cells attached to the bottom of the wells during the entire procedure. In the kinetic experiments, the GABA concentration varied over the range 1–1000 μM while in IC_{50} studies it was constant at 1 μM. The concentrations of uptake inhibitors present during incubations in the kinetic assays are stated in Tables 2 and 3. After incubation, the cells were dissolved in 0.4 M KOH

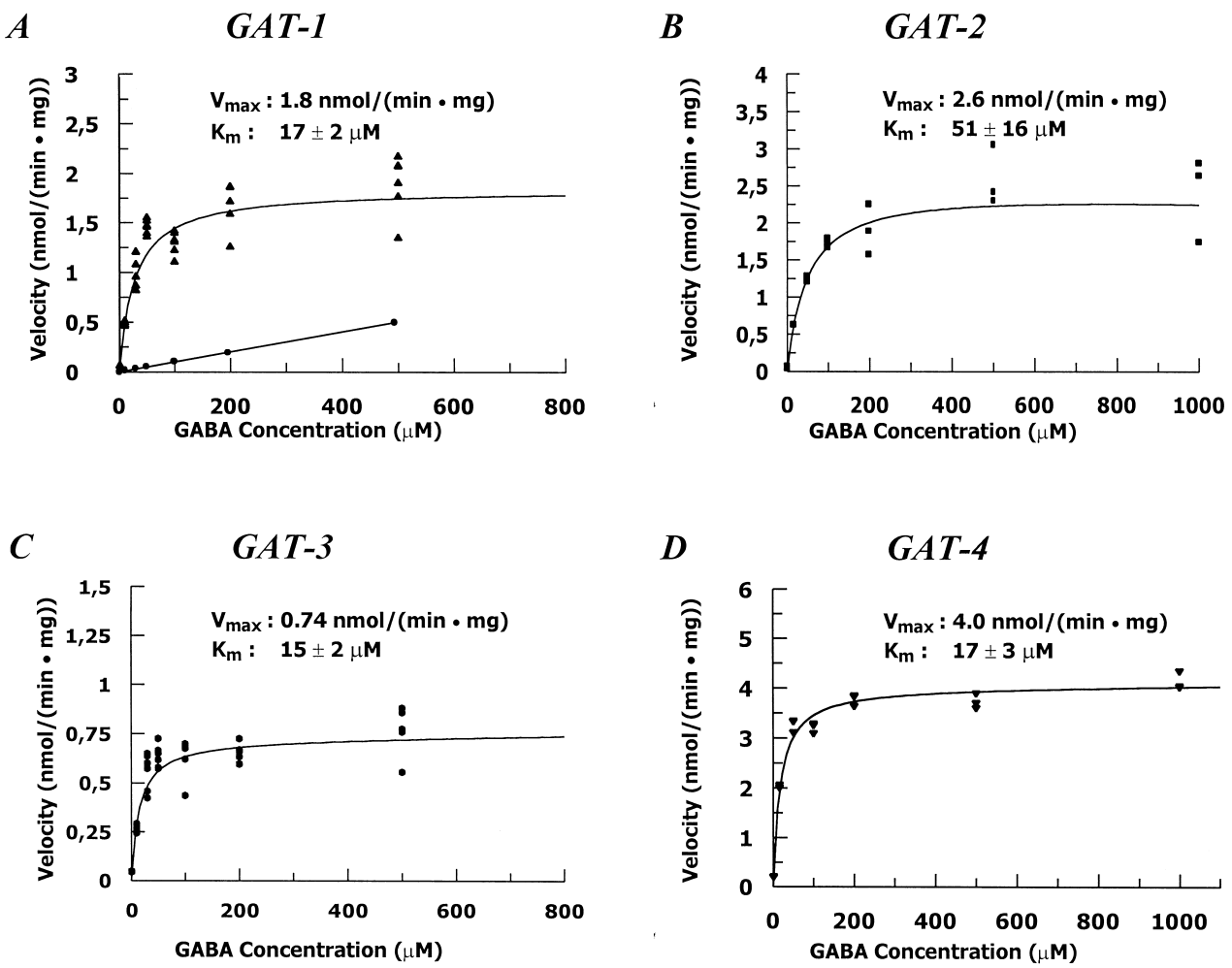

Fig. 1. GABA transport into transfected cells. GABA uptake was measured in untransfected (●) and in GAT-1 (▲) transiently transfected HEK 293 cells (A). GABA transport via GAT-2 (B), GAT-3 (C) and GAT-4 (D) transfected HEK 293 cells was measured as well. For each GABA concentration employed, GABA uptake was measured 3 to 6 times. The curves were fitted to the experimental points represented by the symbols and the V_{max} and K_m values were determined by means of Grafit v3.0 (see Section 2). The K_m value for GAT-2 was statistically significantly different from that of GAT-1, -3 and -4 (Student's *t*-test, $P < 0.05$).

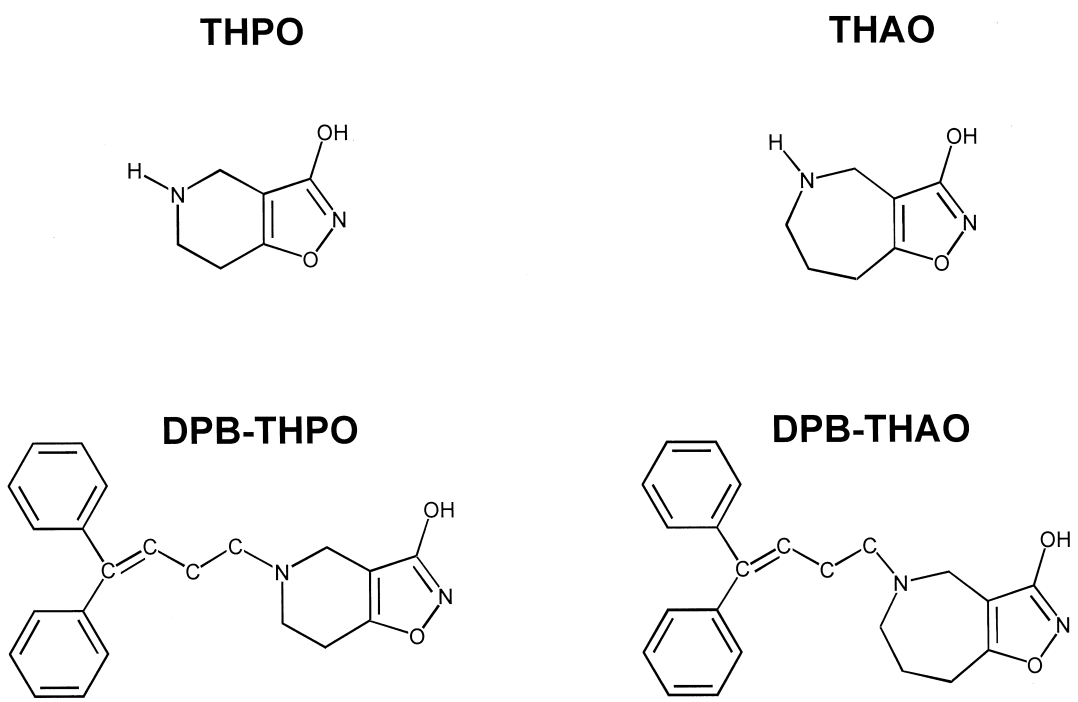

Fig. 2. Chemical structures of (γ-aminobutyric acid) GABA analogues. THPO, 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol. THAO, 5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*c*]azepin-3-ol. DPB–THPO, 5-(4,4-diphenyl-3-butenyl)-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol. DPB–THAO, 5-(4,4-diphenyl-3-butenyl)-5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*c*]azepin-3-ol.

and radioactivity (Schousboe et al., 1977) and protein concentration (Lowry et al., 1951) were determined.

2.5. Data and statistical analysis

The IC_{50} values were determined by use of SigmaPlot for Windows v3.02 (Jandel). The percent GABA uptake as a function of the inhibitor concentration in probit-log scale was plotted. The kinetic parameters, V_{max} and K_m, of the carrier-mediated high-affinity glial and neuronal uptake were calculated (Larsson et al., 1986a,b) by means of a computer program for unconstrained minimization (Stewart, 1967; Dixon, 1972). In the experiments with recombinant GABA transporters, V_{max}, K_m and k were determined via the computer program Grafit v3.0 (Erithacus Software, UK), fitting to the following equation; $[(V_{max}S)/(K_m + S)] + kS$. In neurones, astroglial cells and HEK 293 cells expressing the GAT-subtype, the non-saturable influx component (kS) varied somewhat between batches but was in the range of 10^{-3}–10^{-2} ml min^{-1} mg^{-1}. There was no systematic variation of this component depending on the presence or absence of inhibitors. In the case of simple competitive inhibition, the K_i values were calculated from the determined K_m values of the control- and test-situation using the following equation; $K_i = I/[(K_m/K'_m) - 1]$; where I is inhibitor concentration, K_m is the control value and K'_m is the value determined in the presence of inhibitor.

3. Results

The uptake of GABA into untransfected HEK 293 cells (Fig. 1A) can be fitted to a first order equation with a calculated slope of 1×10^{-3} ml min^{-1} mg^{-1}. The values varied but were in the range of $(0.7–2.1) \times 10^{-3}$ ml min^{-1} mg^{-1}. This indicates uptake of GABA into these cells by diffusion processes rather than uptake by high affinity uptake systems. In contrast, uptake of GABA into HEK 293 cells transiently transfected with GAT-1, -2, -3 or -4 (Fig. 1A, B, C and D) is clearly mediated by saturable, high-affinity uptake systems indicating that the four subtypes of GABA transporter have been expressed by the HEK 293 cells. Depending on the transfection

Table 1
The inhibitory effect of THPO, THAO and DPB–THPO on GABA uptake into GAT-1, -2, -3 or -4 transfected HEK 293 cells

Inhibitor/system	IC_{50} (μM)			
	GAT-1	GAT-2	GAT-3	GAT-4
THPO	1000	3000	800	5000
THAO	1000	4500	> 3000	> 10.000
DPB–THPO	30	200	> 300	> 1000

Transfected HEK 293 cells were incubated for 3 min at 37°C in incubation medium containing different concentrations of inhibitor together with GABA and [^{3}H]GABA in concentrations of 1 μM and 1.0 μCi ml^{-1}, respectively. For each employed inhibitor concentration, the GABA uptake was measured in quadruplicate. The IC_{50} values were determined as described in Section 2.

Table 2
Kinetic parameters for GABA uptake into GAT-1 or GAT-3 transfected HEK 293 cells inhibited by THPO, THAO and DPB–THPO

Inhibitor	Concentration (μM)		Apparent K_m (μM)		V_{max} (% of control)		K_i (μM)	
	GAT-1	GAT-3	GAT-1	GAT-3	GAT-1	GAT-3	GAT-1	GAT-3
Control			17 ± 2	15 ± 2	100 ± 7	100 ± 12		
THPO	1500	750	35 ± 3***	28 ± 2***	102 ± 8	116 ± 5	2000	1000
	2000	1000	30 ± 6**	28 ± 3**	116 ± 15	125 ± 8		
THAO	300		25 ± 1***		112 ± 6		750	
	500		27 ± 2***		108 ± 4			
DPB–THPO	50		33 ± 3***		88 ± 6		50	

The transfected GAT-1 or GAT-3 cells were incubated in phosphate-buffered saline at 37°C as described in Section 2. For each employed GABA concentration, the velocities of GABA uptake were measured 3 to 6 times. Kinetic constants were obtained by computer-assisted nonlinear regression analysis of the experimental data as outlined in Section 2 and are given as means ± S.E.M. In the control experiments, V_{max} values (nmol min^{-1} mg^{-1}) were 1.40 ± 0.08 ($n = 3$) and 0.59 ± 0.07 ($n = 2$) for GAT-1 and GAT-3, respectively. Asterisks indicate statistically significant differences (Student's *t*-test); ** $P < 0.025$; *** $P < 0.01$. The K_i values are calculated as described in Section 2.

efficiency, V_{max} values varied between individual experiments and are given in Fig. 1. K_m values are different for the four subtypes of GABA transporters. For GAT-1, -3 and -4 transfected cells, K_m was 17 ± 2 μM, 15 ± 2 μM and 17 ± 3 μM, respectively. In the experiments with GAT-2 transfected HEK 293 cells, K_m was 51 ± 16 μM. The difference between the K_m value for GAT-2 and the K_m values for GAT-1, -3 and -4 is statistically significant (Fig. 1) indicating that GAT-2 has a lower affinity for GABA compared to GAT-1, -3 and -4. A previous study has shown K_m values of 12 μM, 95 μM, 8 μM and 13 μM, respectively, for GAT-1, -2, -3 and -4 stably expressed in baby hamster kidney (BHK) cells (Thomsen et al., 1997). Thus, the kinetics of saturable GABA uptake into GAT-1, -2, -3 or -4 transfected HEK 293 cells in the present study largely agree with the findings of Thomsen et al. (1997).

The chemical structures of the compounds under study are given in Fig. 2. The results from studies of THPO, THAO and DPB–THPO as inhibitors of GABA uptake via the 4 mouse subtypes of GABA transporters are shown in Table 1. THPO and THAO were equipotent as inhibitors of GABA uptake via GAT-1 and GAT-2 with IC_{50} values in the millimolar range. With regard to GAT-3, only THPO was able to prevent GABA transport with the same potency as seen for GABA uptake into GAT-1 transfected cells. The IC_{50} values for THPO are 1000 μM and 800 μM for GAT-1 and GAT-3 transfected cells, respectively. DPB–THPO displayed a 30- and 15-fold, respectively, higher inhibitory activity than THPO with regard to GABA transport by GAT-1 and GAT-2 expressed in HEK 293 cells. This is in concordance with earlier findings which show that DPB substituted nipecotic acid and guvacine are more potent than the parent compounds as inhibitors of GABA uptake (Yunger et al., 1984; Ali et al., 1985). None of the GABA analogues showed any inhibitory effect on GAT-4 (Table 1). Studies of the mechanism by which THPO, THAO and DPB–THPO inhibit GABA uptake

Table 3
Kinetic parameters for GABA uptake into cultured astrocytes and neurons inhibited by THPO, THAO and their DPB derivatives

Inhibitor	Concentration (μM)		Apparent K_m (μM)		V_{max} (% of control)		K_i (μM)	
	Astrocytes	Neurons	Astrocytes	Neurons	Astrocytes	Neurons	Astrocytes	Neurons
Control			46 ± 12[a]	23 ± 2[a]	100 ± 3[a]	100 ± 4[a]		
THPO	200	500	86 ± 4*	49 ± 5*	99 ± 4	101 ± 3	262[a]	501[a]
	400	1000	109 ± 10**	65 ± 14*	98 ± 3	102 ± 6		
THAO	400	500	108 ± 12*	52 ± 8*	93 ± 3	110 ± 5	258	487
	600	1000	172 ± 13***	64 ± 10**	107 ± 3	95 ± 5		
DPB–THPO	20[a]	80[a]	96 ± 6*	79 ± 8***	100 ± 4	103 ± 6	26[a]	38[a]
	80[a]	200[a]	158 ± 12***	132 ± 15***	100 ± 5	102 ± 8		
DPB–THAO	10	10	205 ± 25***	49 ± 6**	99 ± 4	93 ± 3	3	9
	25	25	315 ± 35***	100 ± 10***	100 ± 4	95 ± 4		
		50		148 ± 19***		92 ± 5		

The cultured astrocytes and neurons were incubated in phosphate buffered saline at 37°C as described in Section 2. Depending upon the concentration of unlabelled GABA, the content of [^{3}H]GABA in the incubation medium varied from 0.4 μCi ml^{-1} to 1.0 μCi ml^{-1}. For each employed GABA concentration, the velocities of GABA uptake were measured in triplicate. Kinetic constants were obtained by computer-assisted nonlinear regression analysis of the experimental data as outlined in Section 2 and are given as means ± S.E.M. In the control experiments, the V_{max} values (nmol min^{-1} mg^{-1}) were 0.768 ± 0.031 ($n = 3$) and 2.425 ± 0.096 ($n = 3$) for astrocytes and neurons, respectively. Asterisks indicate statistically significant differences (Student's *t*-test); *** $P < 0.01$; ** $P < 0.02$; * $P < 0.05$.
[a] Data from Larsson et al. (1991).

mediated by the subtypes of GABA transporters revealed that THPO, THAO and DPB–THPO competitively inhibited GABA uptake into GAT-1 transfected HEK 293 cells (Table 2). The same inhibitory mechanism was found for THPO investigated at GAT-3 expressed in HEK 293 cells (Table 2). The calculated K_i values (Table 2) and the determined IC_{50} values (Table 1) for THPO, THAO and DPB–THPO at GAT-1 and THPO at GAT-3 expressed in HEK 293 cells showed similar inhibitory potency. Previous studies have shown that THPO and DPB–THPO show the characteristics of competitive inhibitors of GABA uptake into cortical neurones and astroglial cells (Larsson et al., 1991). THAO and DPB–THAO both displayed competitive inhibition of GABA uptake into neurones as well as astroglial cells (Table 3). As observed for THPO, both THAO and DPB–THAO exhibited the same slight preference for inhibition of GABA uptake into glia cells compared to neurones. Further, it was shown that DPB substitution of THPO or THAO led to more potent inhibitors of GABA uptake into astroglia cells and neurones. This was also the case for the THPO derivatives with regard to inhibition of the recombinantly expressed transporters (Table 2).

4. Discussion

A summary of the anti-seizure effects of THPO, THAO, DPB–THPO and DPB–THAO is given in Table 4. None of the compounds displayed proconvulsant activity, which is of critical importance when searching for a new antiepileptic drug. Examination of the anti-seizure characteristics of THPO and THAO shows that these drugs are weak with regard to protection against isonicotinic acid hydrazide (INH) seizures, whereas they are both effective inhibitors of sound- and pentylenetetrazole (PTZ)-induced seizures. Regarding the DPB substituted derivatives, DPB–THPO and DPB–THAO are equally effective against sound-induced seizures, as observed for the parent compounds. These anticonvulsant properties of THPO, THAO as well as their DPB substituted analogues indicate that bicyclic isoxazole GABA analogues appear to be promising lead structures in future search for new antiepileptic drugs. The investigated GABA analogues were all selected for testing as anticonvulsants on the basis of initial studies of the kinetics of inhibition of neuronal and glial GABA transport. As shown above, THPO and THAO competitively inhibit GABA uptake into neurones and astroglial cells, as well as in HEK 293 cells expressing subtypes of GABA transporters. This inhibitory mechanism may be desirable due to a less persistent block of GABA transport as might be expected for a non-competitive inhibitor. It is also of functional importance that these GABA analogues are not transported (see Section 1). Previous observations show that THPO increases the extracellular concentration of GABA probably due to its non-transportable character since the transportable inhibitor, nipecotic acid did not exhibit this action (Juhász et al., 1997). These findings make THPO and similar types of compounds interesting with regard to the development of new anticonvulsant and antiepileptic drugs. However, THPO does not easily penetrate the blood–brain barrier (Schousboe et al., 1986). In order to overcome this problem, Yunger et al. (1984) and Ali et al. (1985) examined the effect of adding a lipophilic residue to the nitrogen atom of known GABA transport inhibitors. It was found that inhibitors substituted with DPB, such as SKF 89976-A (*N*-(4,4-diphenyl-3-butenyl)-3-piperidinecarboxylic acid), which is a DPB substituted nipecotic acid, not only crossed the blood–brain barrier but also increased inhibitory potency. Analogously, DPB–THPO and DPB–THAO exhibited an increased inhibitory potency on GABA uptake in primary cell cultures as well as in HEK 293 cells expressing GAT-1 and GAT-2.

With regard to investigations of subtypes of GABA transporters, a large number of GABA analogues inhibit GAT-1 but only a few GAT-2, -3 and -4 subtype selective inhibitors have been identified so far (Borden, 1996; Thomsen et al., 1997). Nipecotic acid is a non-selective inhibitor of subtypes of GABA transporters (Liu et al., 1993). In contrast to this, tiagabine, SKF 89976-A, CI-966 ((1-(2-(bis-4-(trifluoromethyl)phenyl)methoxy)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid) and NNC-711 (1-(2-(((diphenylmethylene) amino)oxy)ethyl)-1,2,5,6-tetrahydro-3-pyridine carboxylic acid), have been found to selectively inhibit GABA uptake by GAT-1 (Borden et al., 1994). All of these compounds contain either nipecotic acid or guvacine in the chemical structures. Another compound containing nipecotic acid, 1-(2-(tris(4-methoxyphenyl)methoxy)ethyl-3-piperidinecarboxylic acid (SNAP-5114) has been shown to selectively inhibit GABA transport through the human cloned GAT-3 which is homologous to mouse GAT-4 (Dhar et al., 1994). These differences in selectivity indicate that a change of the lipophilic side chain may represent a way to produce

Table 4
Semiquantitative summary of anti-seizure effects of bicylic isoxazole GABA uptake inhibitors

Treatment	Seizure model			Proconvulsant activity
	Sound induced	Maximum PTZ	INH	
THPO	++[b]	+++[a]	+[a]	No[a]
THAO	++[d]	+++[a]	+[a]	No[a]
DPB–THPO	++[c]	ND	ND	No[c]
DPB–THAO	++[d]	ND	ND	No[d]

Degree of anticonvulsant activity is indicated as follows: + = ≤25% increase over CSF control; ++ = >50%, ≤100% increase; +++ = >100% increase. Proconvulsant activity was recorded as present or absent. PTZ, pentylenetetrazole induced seizure. INH, isonicotinic acid hydrazide-induced seizure. ND, not determined.

[a] From Gonsalves et al. (1989a,b).

[b] From Croucher et al. (1983).

[c] From White et al. (1993).

[d] H.S. White and A. Schousboe (unpublished).

subtype selective GABA transport inhibitors. A new GABA transport inhibitor, 1-(3-(9*H*-carbazol-9-yl)-1-propyl)-4-(2-methoxyphenyl)-4-piperidinol (NNC 05-2090) was shown to selectively inhibit GAT-2 although it also affected the other subtypes (Thomsen et al., 1997). However, this compound contains no GABA-like structure, and may thus interact with GABA-unrelated transmitter systems operating in the central nervous system.

In order to elucidate the functional importance of the GABA transporters in neuronal–glial GABA homeostasis and their role in seizure activity, more selective pharmacological tools are needed. The design of novel types of GABA uptake inhibitors is in progress, and preliminary studies have disclosed that such compounds show new pharmacological profiles. The results of these studies, which are under completion, will be reported in the near future.

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