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Na^+-dependent γ-aminobutyric acid (GABA) transport in the choroid plexus of rabbit

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Abstract

The goal of this study was to examine the mechanisms of transport of γ-aminobutyric acid (GABA) in the choroid plexus. Choroid plexus slices from the rabbit were depleted of ATP with 2,4-dinitrophenol. GABA accumulated in the choroid plexus slices in a concentrative manner in the presence of an inwardly-directed Na^+ gradient. Uptake occurred in the presence of Cl^-; replacement of Cl^- with gluconate abolished uptake. SCN^-, NO_3^- or Br^- were able to support uptake in the absence of Cl^- to a significant extent (80, 68 and 61% of control, respectively). GABA uptake was saturable (K_m of 37 ± 8.5 μM, V_{max} of 409 ± 43 nmol/g/min). Na^+-driven GABA uptake was inhibited by β-alanine ($IC_{50} = 22.9$ μM) and hypotaurine ($IC_{50} = 21.9$ μM) but less potently by nipecotic acid ($IC_{50} = 244$ μM) and hydroxy-nipecotic acid ($IC_{50} = 284$ μM). Betaine, L-(2,4)-diaminobutyric acid, guvacine and 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol were weak inhibitors ($IC_{50} > 500$ μM). GABA inhibited Na^+-driven uptake of taurine ($IC_{50} = 230$ μM); taurine, however, did not inhibit GABA uptake ($IC_{50} > 1$ mM). RT–PCR, using degenerate primers for cloned GABA transporters, did not result in the amplification of a band from rat choroid plexus RNA. The location of the choroid plexus in the ventricles of the brain, and its role in the secretion of the cerebrospinal fluid, suggest a role for the choroid plexus Na^+-GABA transporter in the disposition of GABA in the brain. © 1997 Elsevier Science B.V.

Keywords: γ-Aminobutyric acid; Taurine; Transport; Epithelium; Choroid plexus

Abbreviations: GABA, γ-aminobutyric acid; L-DABA, L-(2,4)-diaminobutyric acid; THPO, 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol; CSF, cerebrospinal fluid; DNP, 2,4-dinitrophenol; GAT, GABA transporter

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1. Introduction

γ-Aminobutyric acid (GABA) is a neurotransmitter amino acid which is found throughout the central nervous system of vertebrates, formed in neuronal cells by the decarboxylation of glutamic acid and further metabolized by GABA-α-keto-glutarate transaminase. Upon release into the synaptic cleft, GABA binds to post-synaptic $GABA_A$ receptors and elicits a neuroinhibitory response by activating Cl^- channels. Na^+ and Cl^--coupled GABA transporters terminate the actions of GABA by mediating its rapid removal from the synapse. Previously, it was proposed that there were two types of GABA transporters based on their different sensitivity to β-alanine [1,2]. The recent cloning and heterologous expression of GABA transporters has made it clear that multiple subtypes of this transporter exist, and that these can be differentiated on the basis of pharmacological sensitivities to GABA transport inhibitors [3–7].

Studies of GABA transport have focused on neuronal and glial cells [1,2,8,9]. In addition, GABA transport has been described in the epithelia of the retina [10], stomach [11], duodenum [12], kidney [13] and oviduct [14], although the significance of these processes is generally less clear. The choroid plexus epithelium, which secretes the cerebrospinal fluid (CSF), is a major site for transport mechanisms into and out of the brain via the ventricles of the brain [15–17]. The location of the choroid plexus within the ventricles of the brain and its production of the CSF suggest a physiological role for the choroid plexus in maintaining the protected milieu of the brain. A previous study, in vivo, suggested bi-directional transport of GABA between the CSF and blood; the transport of GABA from the CSF to blood was inhibited by probenecid (an inhibitor of the organic anion transporter) [18]. In addition, an early, preliminary in vitro study reported saturable uptake of GABA in the choroid plexus of the cat [19]; however, the mechanisms of the uptake were not characterized.

The goal of this study was to determine the mechanisms of GABA transport in the choroid plexus. Our data provide the first evidence for a Na^+-dependent GABA transporter in the choroid plexus. The choroid plexus is increasingly being recognized as a target tissue for hormones [20] and neurotransmitters [21]. The GABA transporter in the choroid plexus may play a role in CSF production or in maintaining homeostasis of GABA in the CSF and ultimately in the extracellular fluids of the brain.

2. Methods

2.1. Preparation of ATP-depleted choroid plexus

Choroid plexus was obtained from brains of New Zealand White rabbits and depleted of ATP using a modification of the method of Carter-Su and Kimmich [22] that has been described previously [23–25]. The animals were anesthetized with ketamine (5 mg/kg) and decapitated. The choroid plexus was quickly removed from the lateral ventricles and placed in one of the following buffers at room temperature (mM): KCl (120), mannitol (40), and HEPES (25) or NaCl (120), mannitol (40), and HEPES (25); the pH of each buffer was adjusted to 7.4 with 1 M Tris. Each choroid plexus was cut into 2–3-mm pieces and depleted of ATP by placing them in appropriate pre-loading buffers containing 250 μM 2,4-dinitrophenol (DNP), for 20 min at 37°C. Following ATP-depletion, all tissue pieces in the DNP-containing buffer were stored on ice until the start of the uptake studies. The tissue pieces were removed from the pre-loading buffer and blotted gently on laboratory tissue. Uptake commenced with placing the pieces in the appropriate uptake medium.

2.2. Uptake studies

Uptake of GABA into the choroid plexus was studied by incubating the tissue in 140 μl of uptake medium (37°C), which contained [^{3}H]GABA (0.01 μM), [^{14}C]mannitol (17.9 μM) and unlabeled GABA (1 μM), in appropriate buffer (indicated in the figure legends). The uptake medium contained DNP (250 μM) to ensure continued depletion of ATP. Uptake in the presence of Na^+ increased linearly and reproducibly with time, until 2.5 min (Fig. 1). Therefore, uptake was determined at 1 min in all subsequent studies.

For Michaelis–Menten studies, the uptake medium contained [^{3}H]GABA and [^{14}C]mannitol and different amounts of unlabeled GABA (to obtain the indicated

GABA concentrations). For IC_{50} studies, the uptake medium consisted of [^{3}H]GABA, [^{14}C]mannitol, unlabeled GABA (1 μM) and different amounts of unlabeled compounds. Data for both Michaelis–Menten and inhibition studies were generated by measuring GABA uptake into the tissue at 1 min.

Uptake was terminated in each choroid plexus tissue piece by removing it from the uptake medium and blotting it gently on laboratory tissue. Preliminary experiments showed that rinsing tissue in ice cold buffer versus blotting on laboratory tissue had equal effect in stopping the uptake reaction. The blotted tissue piece was placed on a pre-weighed piece of aluminum foil, dried under an IR-lamp heater (approximately 15 cm away from tissue) for 1 h, and then weighed to calculate the net dry tissue weight. The tissue was carefully detached from the foil using forceps and digested in 50 μl of 3 M KOH solution in a liquid scintillation counting vial. After dissolution was complete, 50 μl of 3 M HCl solution was added to neutralize the KOH. The corresponding aluminum foil was then added to the vials and the tissue-associated radioactivity was determined by liquid scintillation. In addition, the uptake medium (50 μl) was sampled and added to separate vials for liquid scintillation counting. Quantitation of ^{14}C and ^{3}H was done by dual isotope liquid scintillation counting on a Beckman Model 1801 liquid scintillation counter (Beckman Instruments, Fullerton, CA). Counting efficiency of ^{3}H ranged from 45% to 47% and that of ^{14}C ranged from 92% to 94%.

Uptake of taurine into the choroid plexus pieces was examined in a manner identical to GABA except that, in this case, the uptake medium contained [^{3}H]taurine (0.0391 μM), [^{14}C]mannitol (17.9 μM) and different amounts of GABA. Taurine uptake into the tissue was measured at 15 min [24].

2.3. Reverse transcriptase–polymerase chain reaction (RT–PCR)

Specific oligonucleotide primers corresponding to nucleotides 242–276 and 1108–1139 of GAT-3/GAT-B were synthesized (UCSF Biomolecular Resource Center); the sequences were: sense, ACAAGAACGGCGGAGGGGCATTCCTGATTCCTTA and antisense, ATGAAAGCCAGTCCAGGACCTGATTCTGCCA. Degenerate oligonucleotide primers, previously described [5], corresponding to transmembrane domains II and VI of GAT-1 protein, were synthesized (UCSF Biomolecular Resource Center); the sequences were: sense, CCGCTCGAGAAGAACGG(C/T)GG(C/T)GG(C/T)GC(C/T)TTC-(C/T)T(G/A)AT(C/T)CC(A/G)TA and antisense, GCTCTAGAAA(G/A)AAGATCTG(G/A)GT(G/T)GC(G/A)GC(G/A)TC-(G/A/C)A(G/T)CCA. RNA was isolated from rat choroid plexus and rat brain using Trizol® reagent, according to the manufacturer's protocol. cDNA was generated from the RNA using a SuperScript™ preamplification kit (GIBCO-BRL). Polymerase chain reaction was performed with the cDNAs and oligonucleotide primers, using Taq DNA-polymerase (Boehringer Mannheim). Briefly, denaturation was performed for 3 min at 94°C; followed by 40 cycles each of 30 s at 94°C, 30 s at 55°C and 30 s at 72°C. Finally, 2 cycles were performed at 72°C for 7 min.

2.4. Data analysis

The uptake of GABA into the choroid plexus is expressed as a volume of distribution (V_d, ml/g dry tissue), calculated from the following equation [23,24]:

$$V_d = \frac{\text{dpm[}^3\text{H]GABA in choroid plexus/g of choroid plexus}}{\text{dpm[}^3\text{H]GABA in uptake medium/ml of uptake medium}} - \frac{\text{dpm[}^{14}\text{C]mannitol in choroid plexus/g of choroid plexus}}{\text{dpm[}^{14}\text{C]mannitol choroid plexus/ml of uptake medium}} \quad (1)$$

For Michaelis–Menten studies, the initial rate of GABA uptake is expressed as nmol/g dry choroid plexus tissue per minute and plotted against the concentration of GABA. The data were fit to the following equation:

$$\text{Rate} = \frac{V_{max} \cdot C}{K_m + C} \quad (2)$$

where V_{max} is the maximal rate of uptake, K_m is concentration of GABA at which the rate of uptake is 50% of the maximal rate and C is the concentration of GABA in the uptake medium. The parameters were estimated using a non-linear regression program on Minim 3.0.8® (obtained from Dr. R.D. Purves, University of Otago, New Zealand).

Inhibition of Na^+-GABA uptake by various test compounds was evaluated by estimation of IC_{50} values: the data were fit to the following four-parameter logistic function using a non-linear fitting routine on Minim 3.0.8:

$$V_d = \frac{(V_{d,0} - V_{d,ns})}{1 + (C/IC_{50})^n} + V_{d,ns} \quad (3)$$

where V_d is the volume of distribution of GABA in the choroid plexus; $V_{d,0}$ is the volume of distribution of GABA in the absence of inhibitor and in the presence of an inwardly directed Na^+-gradient; $V_{d,ns}$, the non-specific diffusional component, is the volume of distribution of GABA in the absence of a Na^+ gradient; C is the concentration of inhibitor in the uptake mixture; and n is the Hill coefficient. The IC_{50} of GABA in inhibiting Na^+-stimulated taurine uptake was computed in a manner similar to that described for GABA.

For each data point, 3–5 measurements were made per experiment; between one and five experiments were performed (see figure legends). Data are presented as mean ± SE of all determinations. All parameter estimates (Michaelis–Menten and IC_{50} studies), which were generated using non-linear regression (Minim 3.0.8), are expressed as mean ± SD of the estimates. Means were compared using unpaired Student's *t*-test or one-way ANOVA and a probability, P, of less than 0.05 was considered significant.

2.5. Materials

[^{3}H]Taurine (specific activity: 21.9 Ci/mmol) and [^{3}H]GABA (specific activity: 88.4 Ci/mmol) were obtained from Du Pont–New England Nuclear (Boston, MA). [^{14}C]Mannitol was obtained from Moravek Biochemicals (Brea, CA). Guvacine, nipecotic acid, OH-nipecotic acid (hydroxy-nipecotic acid) and 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol (THPO) were from RBI (Natick, MA). All other chemicals were purchased from Sigma (St. Louis, MO). Cytoscint ES scintillation fluid was obtained from ICN (Irvine, CA). New Zealand white rabbits (2–3 kg) were purchased from Nitabell Rabbitry (Hayward, CA).

3. Results

3.1. Time dependence

Uptake of GABA into ATP-depleted tissue pieces was examined in the presence and absence of an inwardly-directed Na^+ gradient (Fig. 1). A distinct ''overshoot'' phenomenon, typical of Na^+-driven uptake in ATP-depleted tissue preparations, was observed in the presence of an initial Na^+ gradient. Uptake in the presence of the Na^+ gradient increased linearly as a function of time, until 2.5 min. It reached a maximum at 15 min ($V_d = 27.7 \pm 2.9$ ml/g) and then declined at 30 min ($V_d = 8.58 \pm 2.01$ ml/g). Uptake at 30 min was not significantly different from that at 60 min, suggesting that an equilibrium value of uptake was reached by 30 min.

GABA uptake in the presence of an inwardly-directed Na^+ gradient was significantly higher than that when Na^+ was present in equal concentration inside and outside the tissue (120 mM), at all time points studied (Fig. 1). In addition, absence of Na^+

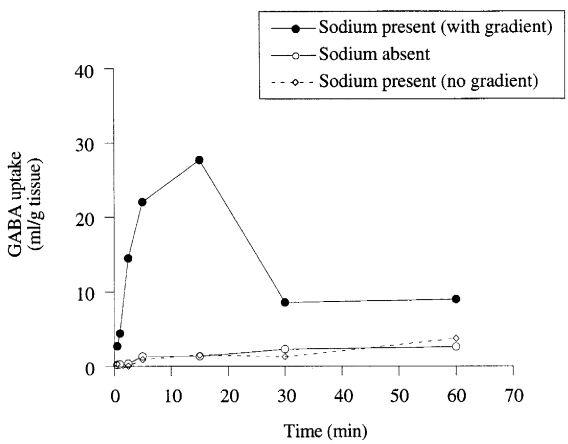

Fig. 1. Temporal profile of GABA accumulation in ATP-depleted rabbit choroid plexus. Uptake (expressed as V_d) of GABA was determined at 37°C. Points represent means ± SE of data obtained from two separate experiments, each performed in quadruplicate. Uptake at all time points in the presence of a Na^+-gradient was significantly greater than that in the absence of Na^+ and the absence of a Na^+ gradient ($P < 0.001$). Key: 120 mM inwardly-directed Na^+ gradient (filled circles); 120 mM equal K^+ inside and outside of the tissue (open circles); 120 mM equal Na^+ inside and outside of the tissue (open diamonds).

from the reaction mixture (equal K^+ inside and outside, 120 mM) resulted in an uptake that was not significantly different from that when equal Na^+ was present inside and outside the tissue, at all time points studied. These data suggest that a Na^+-gradient can drive the uptake of GABA in the choroid plexus.

3.2. Ion dependence

Replacement of Na^+ with choline in the uptake mixture abolished GABA uptake (5.08 ± 0.58 vs. 0.17 ± 0.05 ml/g, mean $\pm$ SE, $P < 0.001$) indicating a strong Na^+-dependence. Replacement of Cl^- with SCN^-, NO_3^- or Br^- resulted in a partial attenuation of transport which was, however, insignificant (80, 68 and 61% of control uptake, respectively) (Table 1). When gluconate was used as the anion in place of Cl^-, there was a complete loss of Na^+-dependent GABA uptake (5.08 ± 0.58 vs -0.04 ± 0.04 ml/g, mean $\pm$ SE, $P < 0.001$). These results suggest a moderately selective anion requirement for Na^+–GABA transport in the rabbit choroid plexus.

3.3. Concentration dependence

The initial rate of uptake of GABA (at 1 min) was measured as a function of concentration in the presence of an inwardly-directed Na^+ gradient. The rate of uptake increased initially with increasing substrate concentration and then achieved a plateau in a manner consistent with saturable kinetics (Fig. 2). The data were fitted to the Michaelis–Menten equation describing a single saturable component. The estimated V_{max} and K_m (mean $\pm$ SD) were 409 ± 43 nmol/g per min and 37 ± 9 μM, respectively. The uptake values at the 4 highest GABA concentrations were not significantly different from each other (ANOVA, $P = 0.741$), indicating that a non-saturable component was not a major component of GABA transport into the tissue. Hence, a linear term was not included in the equation used to fit the data.

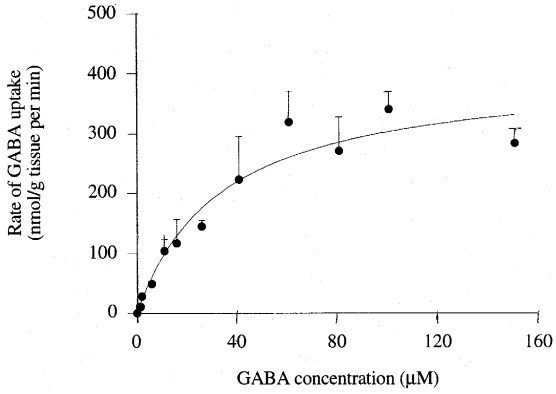

Fig. 2. Concentration dependency of Na^+-driven GABA uptake in ATP-depleted choroid plexus. Uptake at 1 min in nmol/g tissue was measured at 37°C in the presence of a Na^+ gradient (120 mM). Points represent the mean $\pm$ SE of between 1 to 3 experiments, each performed in triplicate or quadruplicate. GABA uptakes at the four highest substrate concentrations were not significantly different from each other ($P = 0.741$, ANOVA). The estimated V_{max} and K_m were 409 ± 43 nmol/g per min and 37 ± 9 μM, respectively (mean $\pm$ SD).

3.4. Inhibition

Various compounds previously shown to inhibit GABA transport in other tissue were tested for their ability to inhibit GABA transport in choroid plexus

Table 1
Ion-dependence of GABA uptake

Condition	V_d of GABA (ml/g)	% of control	P value compared to control
Na^+, Cl^- (control)	5.08 ± 0.58 (2)	100	–
Na^+, SCN^-	4.06 ± 0.28 (2)	80	n.s.
Na^+, NO_3^-	3.49 ± 0.35 (2)	68	n.s.
Na^+, Br^-	3.13 ± 0.67 (1)	61	n.s.
Na^+, Gluconate$^-$	-0.04 ± 0.04 (2)	0	< 0.001
Choline$^+$, Cl^-	0.17 ± 0.05 (2)	3	< 0.001

ATP-depleted choroid plexus pieces were pre-incubated in choline Cl (120 mM), KCl (40 mM) and HEPES (15 mM) pH 7.4. For uptake studies, tissue was incubated with [^{3}H]GABA in either buffer containing NaCl (120 mM), KCl (40 mM) and HEPES (15 mM) pH 7.4, or in buffers which were identical except for equimolar replacement of Cl^- with SCN^-, NO_3^-, Br^- or gluconate$^-$, or in which Na^+ was replaced with choline$^+$. Data represent mean $\pm$ SE of uptake (determined at 1 min); the number of experiments are indicated in parentheses. Each experiment was performed in replicates of five; n.s. denotes not significant.

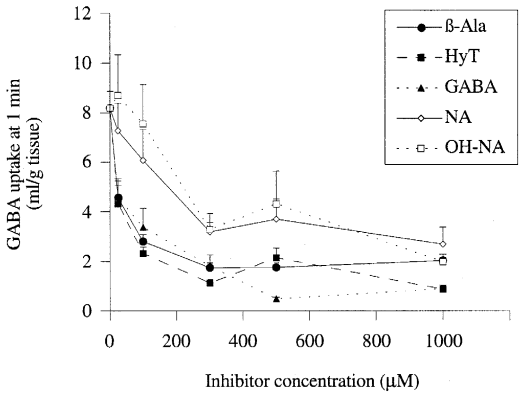

Fig. 3. Effect of various inhibitors on Na^+-driven GABA uptake in ATP-depleted choroid plexus tissue. Uptake (ml/g) of GABA (1 μM) was measured at 37°C in the presence of various concentrations (0, 25, 100, 300, 500 and 1000 μM) of unlabeled compounds. Key: β-Ala, β-Alanine; HyT, hypotaurine; NA, nipecotic acid; OH-NA, hydroxy-nipecotic acid. Points represent mean ± SE of between 1 and 4 experiments, each performed in quadruplicate. Where error bars are not visible, they are encompassed within the point. Points are connected by a simple linear spline. See Table 1 for a summary of these and other results.

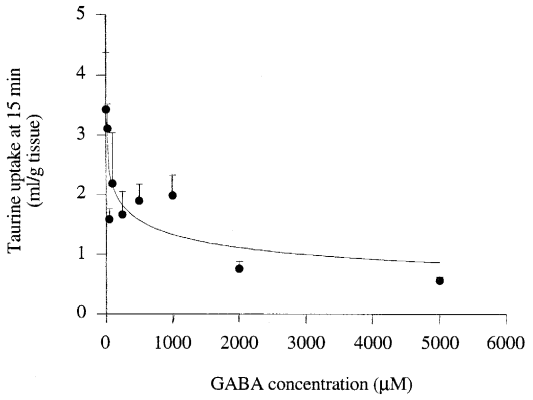

Fig. 4. Effect of GABA on Na^+-driven taurine uptake in ATP-depleted choroid plexus tissue. Uptake (ml/g) of taurine 25 μM) was measured at 37°C in the presence of various concentrations (25, 50, 100, 250, 500, 1000, 2000 and 5000 μM) of unlabeled GABA. Points represent mean ± SE of 1 experiment, performed in quadruplicate. The curve represents the best fit to the data and was generated using the equation for IC_{50} (see Section 2). The IC_{50} was estimated to be 230 μM.

tissue pieces. All potential compounds were tested at various concentrations (0, 25, 100, 300, 500 and 1000 μM), and IC_{50} values (mean ± SD) were obtained (Fig. 3, Table 2). Hypotaurine (IC_{50} = 21.9 ± 17.3 μM) and β-alanine (IC_{50} = 22.9 ± 18.1 μM) were potent inhibitors of Na^+-driven GABA uptake in choroid plexus tissue pieces. Nipecotic Acid (IC_{50} = 244 ± 113 μM) and OH-nipecotic acid (IC_{50} = 284 ± 128 μM) were moderately potent in their ability to inhibit Na^+-driven GABA uptake. Betaine, L-(2,4)-diaminobutyric acid (L-DABA), guvacine and THPO showed weak inhibitory activity, with IC_{50}values of > 1 mM. Neither taurine nor L-alanine, an isomer of β-alanine, significantly inhibited GABA uptake (IC_{50} > 1 mM).

Table 2
Inhibitor sensitivity of GABA uptake in rabbit choroid plexus

Inhibitor	IC_{50} (μM)	K_i (μM)
β-Alanine	22.9 ± 18.1	22.2 ± 17.5
Hypotaurine	21.9 ± 17.3	21.2 ± 16.7
Nipecotic acid	244 ± 113	236 ± 109
OH-Nipecotic acid	284 ± 128	275 ± 124
Guvacine	565 ± 208	547 ± 201
THPO	> 1 mM	> 1 mM
L-DABA	> 1 mM	> 1 mM
Taurine	> 1 mM	> 1 mM
Betaine	> 1 mM	> 1 mM
L-Alanine	> 1 mM	> 1 mM

For IC_{50} estimates, the Na^+-driven uptake (1 min) of [^{3}H]GABA was measured in the presence of various inhibitors. Each inhibitor was tested at 0, 25, 100, 300, 500 μM and 1 mM. Uptake values, which were corrected for non-specific Na^+-independent uptake, were pooled from between 1–5 experiments and fitted to the equation as described in Section 2. Each experiment was performed in quadruplicate. IC_{50} and K_i values are the mean ± SD of the estimate; Fig. 3 depicts some of the data. To allow comparison with other studies, K_i values were generated from IC_{50} determinations using the Cheng and Prusoff relationship [41], which assumes competitive inhibition: $K_i = IC_{50}/(1 + S)$, where S = concentration of GABA/K_m of GABA.

The effect of GABA on the Na^+-dependent uptake of taurine (25 μM) into the tissue pieces was determined at 15 min. GABA inhibited taurine uptake with an IC_{50} value of 230 μM (Fig. 4). Since an inhibitor may not necessarily be a substrate of the transporter, further studies are required to determine if GABA is a substrate of the Na^+-taurine transporter in choroid plexus.

3.5. RT–PCR

RT–PCR with primers specific to GAT-3, resulted in the amplification of a band of predicted size from the rat brain but not from the choroid plexus (data not shown). Identical results were obtained using primers degenerate for GAT-1, which were designed to amplify both GAT-1 and GAT-3 (data not shown).

4. Discussion

GABA is the dominant inhibitory neurotransmitter in the brain and is present throughout the central nervous system. The neurotransmitter actions of GABA in the synaptic cleft are terminated by its reuptake via Na^+ and Cl^- coupled transporters; both neuronal and glial cells express one or more members of this closely related family of transporters [3–7]. Imbalances in GABA function in the brain have been associated with psychiatric [26] and neurological diseases [27,28]. Studies with post-mortem human brains suggest that an increased level of expression of GABA transporters may be involved in schizophrenia [29]. In various species, including humans [30], GABA transporters have been implicated in epilepsy and are an active target of drug design efforts for the disease [31–35]. Inhibitors of GABA transporters have demonstrated usefulness in animal models of seizures [36–38] presumably by sustaining the concentration of GABA in the synaptic cleft. Our finding of a GABA transporter in the choroid plexus of the brain suggests an additional mechanism that may govern the disposition of GABA in the brain.

In vivo studies suggest that the transfer of GABA from blood to the CSF and the disposition of GABA in the CSF is governed by a carrier-mediated process [18]. An early, preliminary study reported saturable uptake of GABA in the choroid plexus of the cat [19]. However, neither the driving force for GABA transport in the choroid plexus nor the specificity of the observed transport process was determined. Here, we provide evidence for a Na^+-dependent GABA transporter (CP–GABA transporter) in the choroid plexus of the rabbit brain. Following ATP-depletion of the tissue, we determined that GABA uptake was driven by an initial inwardly-directed Na^+-gradient (Fig. 1). This Na^+-gradient-dependent uptake is characteristic of concentrative accumulation of a substrate by the tissue, and it is similar to that seen for GABA uptake in other tissue. As with other GABA transporters, Cl^- supported GABA transport optimally; replacement of Cl^- with gluconate completely abolished transport. However, SCN^-, NO_3^- and Br^- were also able to sustain GABA transport, suggesting that Cl^- is not absolutely necessary for GABA transport in the choroid plexus. Similar results have been obtained for Na^+-GABA transport in the retina [10]. In contrast, while Cl^- replacement has been less well studied for the Na^+-GABA transporters in the brain, they generally show a strong requirement for Cl^- and in some cases Br^- [3,4,6]. These results suggest that the CP–GABA transporter may be similar to the GABA transporter in the retina. In this study, the estimated K_m (37 ± 8 μM) indicates that the affinity of the GABA transporter in the choroid plexus for GABA is similar to that of other GABA transporters in the brain [3,4,6,7] and other tissues [10,13,14]. We note that the K_m for GABA reported by Lorenzo (K_m = 230 μM) in the cat choroid plexus is considerably higher than the one we obtained in this study [19]. The reason for this discrepancy is not clear; it is possible that it results from methodological or species differences.

To determine the selectivity of the CP–GABA transporter, we examined the potency of various compounds known to inhibit GABA transport in other tissues. Of the compounds tested, β-alanine (IC_{50} = 22.9 μM) and hypotaurine (IC_{50} = 21.9 μM) were the most potent, whereas L-DABA and betaine were not able to effectively inhibit Na^+-driven GABA transport in choroid plexus. These data suggest that the CP–GABA transporter is most similar to GAT-3/GAT-B, which is sensitive to β-alanine but insensitive to L-DABA (GAT-1 is insensitive to β-alanine and GAT-2 is sensitive to L-DABA). However, the anion requirements (Table 1) suggest that there may be functional differences in the two transporters. Finally, the choroid plexus transporter is also different from the GABA-transporting renal epithelial transporter, BGT, by virtue of its refractoriness to betaine.

Because hypotaurine and β-alanine, both potent inhibitors of Na^+-GABA uptake in the rabbit choroid plexus, also inhibit taurine uptake via the Na^+-taurine transporter in the choroid plexus, we asked if both uptake processes were mediated via the same transporter. The finding that GABA inhibits the uptake of taurine (Fig. 4, IC_{50} = 230 μM), but taurine does not inhibit GABA uptake significantly (Table 2), suggests that the Na^+-GABA transporter, which we have characterized in the choroid plexus, is distinct from the Na^+-taurine transporter.

RT–PCR of rat brain RNA using oligonucleotide probes degenerate for the rat GAT-1 [5] resulted in amplification of a band of predicted size (data not

shown). Similarly probes specific for GAT-3/GAT-B resulted in the amplification of the predicted band (data not shown). We did not observe amplification of corresponding bands, with either set of probes, when RNA from rat choroid plexus was used. These data suggest that the rat choroid plexus may not express a GABA transporter similar to GAT-1 or GAT-3/GAT-B. However, alternative RT–PCR conditions may be necessary to identify GAT-1 or GAT-3 mRNA transcripts in the choroid plexus.

The presence of a $GABA_A$ receptor in the choroid plexus epithelium of the rat has been documented [39]; in vivo studies indicate that benzodiazepine ligands reduce the secretion rate of the CSF by up to 50% [40]. The concomitant presence of a GABA transporter is consistent with the suggested involvement of GABA-ergic mechanisms in modulation of CSF production. Furthermore, the transporter for GABA in the choroid plexus may play a role in maintaining homeostasis of GABA in the CSF, and ultimately the brain. Such a transporter may also be important in the targeting of GABA analogs to the central nervous system.

Acknowledgements

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