0026-895X/92/040563-07$03.00/0

MOLECULAR PHARMACOLOGY, 42:563-569

Cloning and Expression of a High Affinity Taurine Transporter from Rat Brain

KELLI E. SMITH,[1] LAURENCE A. BORDEN,[1] CHING-HUA DAVID WANG, PAUL R. HARTIG, THERESA A. BRANCHEK, and RICHARD L. WEINSHANK

Synaptic Pharmaceutical Corporation,[2] Paramus, New Jersey 07652

Received March 26, 1992; Accepted June 30, 1992

SUMMARY

A cDNA clone (designated rB16a) encoding a taurine transporter has been isolated from rat brain, and its functional properties have been examined in mammalian cells. The nucleotide sequence of the clone predicts a 621-amino acid protein with significant homology to other neurotransmitter transporters. Hydropathy analysis reveals stretches of hydrophobic amino acids indicative of 12 transmembrane domains, similar to those proposed for other cloned neurotransmitter transporters. The transporter displays high affinity for taurine ($K_m \approx 40$ μM) and is dependent on external sodium and chloride for transport activity. Specific transport is sensitive to inhibition by β-alanine and γ-aminobutyric acid, similar to taurine transporters *in vivo*. Localization studies demonstrate that the transporter mRNA is located in both the brain and peripheral tissues. The structural similarity of the taurine transporter to neurotransmitter transporters is consistent with a neuromodulatory role for taurine in the nervous system.

Taurine (2-aminoethanesulfonic acid) is a sulfur-containing amino acid present in high concentrations in mammalian brain as well as various non-neural tissues. Many functions have been ascribed to taurine in both the nervous system and peripheral tissues. The best understood (and phylogenetically oldest) function of taurine is as an osmoregulator (1, 2). Osmoregulation is essential to normal brain function and may also play a critical role in various pathophysiological states, such as epilepsy, migraine, and ischemia. The primary mechanism by which neurons and glial cells regulate osmolarity is via the selective accumulation and release of taurine. Taurine influx is mediated via specific, high affinity transporters, which may contribute to efflux as well. Because taurine is degraded slowly, transport is an important means of regulating extracellular taurine levels.

Taurine is structurally related to the inhibitory amino acid GABA and exerts inhibitory effects on the brain, suggesting a role as a neurotransmitter or neuromodulator. Taurine can be released from both neurons and glial cells by receptor-mediated mechanisms, as well as in response to cell volume changes (3). Its effects in the central nervous system may be mediated by $GABA_A$ and $GABA_B$ receptors (4, 5) and by specific taurine receptors (6). Additionally, taurine can also regulate calcium homeostasis in excitable tissues, such as the brain and heart (7, 8), via an intracellular site of action. Together, the inhibitory and osmoregulatory properties of taurine suggest that it acts as a cytoprotective agent in the brain. Depletion of taurine results in retinal degeneration in cats (9), supporting a role in neuronal survival.

Although most animals possess the ability to synthesize taurine, many are unable to generate sufficient quantities and, therefore, rely on dietary sources. Taurine transport is thus critical to the maintenance of appropriate levels of taurine in the body. High affinity, sodium-dependent taurine uptake has been observed in brain and various peripheral tissues (1, 3), but little is known about the molecular properties of the taurine transporter(s). Cloning of the taurine transporter not only will help elucidate the function of this important neuroactive molecule but also may provide important insight into novel therapeutic approaches to treat neurological disorders.

Experimental Procedures

Materials. [^{3}H]Taurine (25.6 Ci/mmol) was from New England Nuclear (Boston, MA), taurine, GABA, hypotaurine, AEPA, AMSA, APSA, CSA, MEA, and β-alanine were from Sigma Chemical Co. (St.

[1] K.E.S. and L.A.B. contributed equally to this work.
[2] Formerly Neurogenetic Corporation.

ABBREVIATIONS: GABA, γ-aminobutyric acid; AEPA, 2-aminoethylphosphonic acid; AMSA, aminomethanesulfonic acid; APSA, 3-amino-1-propanesulfonic acid; CSA, cysteinesulfinic acid; GES, guanidinoethanesulfonic acid; MEA, 2-mercaptoethylamine; HBS, HEPES-buffered saline; SDS, sodium dodecyl sulfate; SSC, standard saline citrate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; kb, kilobases; PCR, polymerase chain reaction.

Louis, MO), and GES was a kind gift of Dr. J. Barry Lombardini (Department of Pharmacology, Texas Tech University).

Cloning and sequencing. A rat brain cDNA library in the LambdaZAP II vector (Stratagene, La Jolla, CA) was screened at low stringency with the complete coding region of the rat GABA transporter cDNA (GAT-1) (10). Exact primers were used to generate PCR products from randomly primed rat brain cDNA; the products were labeled and used to screen the library under reduced stringency (hybridization, 25% formamide, 40°; wash, 0.1× SSC, 40°), as described previously (11). λ phage hybridizing at low stringency with the GAT-1 sequence were plaque purified and rescreened with the same probes at high stringency (hybridization, 50% formamide, 40°; wash, 0.1× SSC, 50°) to eliminate clones identical to GAT-1. Clones hybridizing only at low stringency were converted to phagemids by *in vivo* excision with F1 helper phage. Nucleotide sequences of double-stranded cDNAs in pBluescript were analyzed by the Sanger dideoxy nucleotide chain-termination method (12), using Sequenase (United States Biochemical Corp., Cleveland, OH).

Expression. A cDNA (designated rB16a) containing the complete coding region of a putative transporter was cloned into the eukaryotic expression vector pEVJB (modified from pcEXV-3) (13) as a 2.5-kb *XbaI\SalI* fragment, using restriction enzyme sites within the vector. In addition to the coding region, 0.1 kb of 5′ untranslated sequence and 0.5 kb of 3′ untranslated sequence were included in the construct. Transient transfections of COS cells with the plasmid pEVJB-rB16a were carried out using DEAE-dextran with dimethylsulfoxide according to the method of Lopata *et al.* (14), with minor modifications. COS cells were grown (37°, 5% CO_2) in high glucose Dulbecco's modified Eagle medium supplemented with 10% bovine calf serum, 100 units/ml penicillin G, and 100 μg/ml streptomycin sulfate. Cells were routinely used for transport studies 2 days after transfection.

Transport studies. To measure transport, COS cells grown in six-well (well diameter, 35 mm) or 24-well (well diameter, 18 mm) plates were washed three times with HBS (in mM: NaCl, 150; HEPES, 20; $CaCl_2$, 1; glucose, 10; KCl, 5; $MgCl_2$, 1; pH 7.4) and allowed to equilibrate in a 37° water bath. After 10 min the medium was removed and a solution containing [^{3}H]taurine (specific activity, 25.6 Ci/mmol; New England Nuclear) and required drugs in HBS was added (1.5 ml/35-mm well; 0.5 ml/18-mm well). Nonspecific uptake was defined in parallel wells with 1 mM unlabeled taurine and was subtracted from total uptake (no competitor) to yield specific uptake; all data represent specific uptake. Plates were incubated at 37° for 10 min unless indicated otherwise and were then washed rapidly three times with ice-cold HBS. Cells were solubilized with 0.05% sodium deoxycholate/0.1 N NaOH, an aliquot was neutralized with 1 N HCl, and radioactivity was determined by scintillation counting. Protein was quantified in an aliquot of the solubilized cells using a Bio-Rad protein assay kit, according to the manufacturer's directions.

PCR tissue localization studies. To identify tissues expressing mRNA for the taurine transporter, exact primers (25-mers) were designed such that a 707-base pair fragment of rB16a could be amplified from cDNA and detected by Southern blot analysis. The sequences of the sense and antisense primers were derived from amino acids 40–47 (5′-TCAGAGGGAGAAGTGGTCCAGCAAG) and 268–275 (5′-ATTTCATGCCTTCACCAGCACCTGG), respectively. Primers were alsodesigned to amplify the cDNA encoding cyclophilin (15), a constitutively expressed gene, as control (sense, 5′-ACGCTTCGACTTCCTCATGTCCTGT; antisense, 5′-TTAGAGTTGTCCACAGTCGGAGATG). To detect amplified sequences, an oligonucleotide probe was synthesized (corresponding to amino acids 249–271) that was specific for rB16a. Poly(A)$^+$ RNA (1 μg; Clontech, Palo Alto, CA) from each of seven rat tissues was converted to single-stranded cDNA by random priming using Superscript reverse transcriptase (BRL, Gaithersburg, MD). PCR reactions were carried out in a buffer containing 20 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 2 mM deoxynucleotide triphosphates, 1 μM levels of each primer, *Taq* polymerase, and either cDNA, RNA, water, or a control plasmid containing rB16a, for 30 cycles of 94° for 2 min, 68° for 2 min, and 72° for 3 min. PCR products were separated by electrophoresis in 1.2% agarose gels, blotted to nylon membranes (Genescreen Plus; New England Nuclear, Boston, MA), and hybridized at 40° overnight with specific ^{32}P-labeled oligonucleotides, in a solution containing 50% formamide, 10% dextran sulfate, 5× SSC, 1× Denhardt's (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), and 100 μg/ml sonicated salmon sperm DNA. Blots were washed at high stringency (0.1× SSC, 50°) and exposed to Kodak XAR film for 0.5–4 hr, with one intensifying screen, at −70°.

Northern blot analysis. Samples of poly(A)$^+$ RNA isolated from each of eight rat tissues (5 μg; Clontech) were separated in a 1.0% agarose gel containing 3.3% formaldehyde and were transferred to a nylon membrane (Genescreen Plus; New England Nuclear) by overnight capillary blotting in 10× SSC. Before hybridization, the Northern blot was incubated for 2 hr at 42° in a solution containing 50% formamide, 1 M NaCl, 10% dextran sulfate, and 1% SDS. The blot was hybridized overnight at 42° with ^{32}P-labeled DNA probe (randomly primed *HindIII/KpnI* fragment of rB16a representing amino acids 6–336) in the prehybridization solution containing 100 μg/ml sonicated salmon sperm DNA. The blot was washed successively in 2× SSC/2% SDS, 1× SSC/2% SDS, and 0.2× SSC/2% SDS at 65° and was exposed to Kodak XAR-5 film, with one intensifying screen, at −70° for 1–4 days. To confirm that equal amounts of RNA were present in each lane, the same blot was rehybridized with a probe encoding cyclophilin (15).

Results and Discussion

Cloning. We screened a rat brain cDNA library at low stringency with probes encoding the rat brain GABA transporter GAT-1 (10), in order to identify additional inhibitory amino acid transporter genes. Several clones were isolated that hybridized at low but not at high stringency with the GABA transporter probes. Characterization of the clones by DNA sequence analysis revealed that they represented a novel transporter sequence related to GAT-1. None of the clones contained the complete coding region of the putative transporter, and thus the library was rescreened at high stringency using oligonucleotides designed from the new sequence. A 2.5-kb cDNA clone (designated rB16a) was isolated that contained an open reading frame of 1863 base pairs, encoding a protein of 621 amino acids. Comparison of this sequence with the rat GABA transporter cDNA revealed 58% nucleotide identity within the coding region.[3] Comparison with sequences in GenBank and EMBL databases demonstrated that the sequence was novel and that the most closely related sequence was the rat GABA transporter (10), followed by the human norepinephrine transporter (16). Subsequent comparisons with recently cloned transporters indicate that the most homologous sequences are two novel GABA transporters designated GAT-2 and GAT-3[4] and the betaine transporter (17), which exhibit 62–64% nucleotide identity with rB16a.

The amino acid sequence deduced from the nucleotide sequence of rB16a is shown in Fig. 1, with a membrane topology similar to that proposed for the rat GABA transporter (10) and other cloned neurotransmitter transporters (16, 18–21). The translation product of rB16a is predicted to have a relative molecular mass of ~70,000 Da. Hydropathy analysis indicates

[3] The sequence of the rat taurine transporter has been deposited in GenBank (accession number M96601).

[4] L. A. Borden, K. E. Smith, P. R. Hartig, T. A. Branchek, and R. L. Weinshank. Molecular heterogeneity of the GABA transport system: cloning of two novel high-affinity GABA transporters from rat brain. *J. Biol. Chem.*, in press.

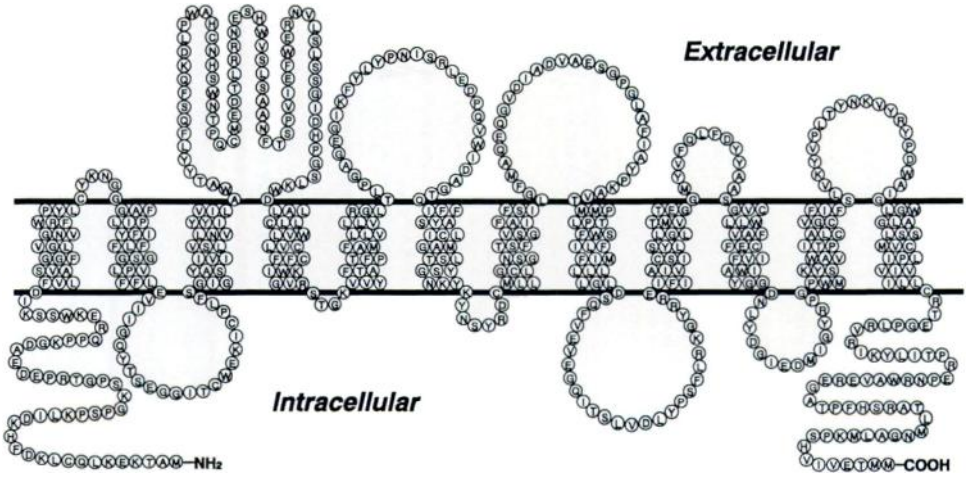

Fig. 1. Deduced amino acid sequence and putative membrane topology of the taurine transporter (rB16a). Deduced amino acid sequence by translation of a long open reading frame in rB16a is shown. Membrane topology is modeled after that proposed for GAT-1 (10).

the presence of 12 hydrophobic domains that may represent membrane-spanning segments. Three potential sites for asparagine-linked glycosylation are found in the extracellular loop between the third and fourth transmembrane domains. Alignment of the deduced amino acid sequence of rB16a with the rat GABA transporter (GAT-1) (10) and the dog betaine transporter (17) revealed 50% and 58% amino acid identities, respectively (Fig. 2). Comparison of rB16a with the glycine transporter (Fig. 2) (11) and the human norepinephrine transporter (16) also showed significant amino acid homology (41–45%), similar to that between GAT-1 and the norepinephrine transporter (46%). As predicted from nucleotide comparisons, the strongest amino acid homology (~61%) is with the GABA transporters GAT-2 and GAT-3 recently cloned from rat brain.[4] In contrast, the sodium/glucose co-transporter (22), which shows a low degree of homology with cloned neurotransmitter transporters, displays only 21% amino acid identity with rB16a. These data suggested that the new sequence might encode an inhibitory amino acid transporter expressed in the brain. To explore this possibility, rB16a was placed in a mammalian expression vector, transfected into COS cells, and screened for transport of a variety of radiolabeled neurotransmitters and amino acids.

Pharmacological characterization. COS cells transiently transfected with rB16a (COS/rB16a) accumulated approximately 6-fold more [^{3}H]taurine than did control nontransfected cells (Fig. 3). Specific uptake represented >95% of total uptake in transfected cells. In contrast, the uptake of [^{3}H]glutamate, [^{3}H]glycine, [^{3}H]5-hydroxytryptamine, [^{3}H]dopamine, and [^{3}H] GABA was unaltered. Uptake of [^{3}H]taurine was not observed after mock transfection, indicating that the enhanced uptake was not the result of nonspecific perturbation of the membrane. The transport of [^{3}H]taurine by COS/rB16a was decreased >95% when Na^+ was replaced by Li^+ or when Cl^- was replaced by acetate (Fig. 3). In the absence of sodium or chloride, taurine transport in COS/rB21a decreased to levels below that of nontransfected controls, demonstrating that endogenous taurine transporter activity present in COS cells is also dependent on these ions. A similar ion dependence has been observed for taurine transport *in vivo* (1), as well as for the activity of other cloned neurotransmitter transporters such as those for GABA (10), glycine (11), and norepinephrine (16).

To determine the affinity of taurine for the cloned transporter, COS/rB16a was incubated with various concentrations of [^{3}H]taurine and the specific accumulation of radioactivity was determined. Accumulation of [^{3}H]taurine was dose dependent and reached saturation at higher concentrations (Fig. 4). Nonlinear regression analysis of the data yielded the following values: K_m = 43 ± 6 μM and V_{max} = 0.96 ± 0.27 nmol/mg of protein (mean ± standard error, four experiments). The affinity of the cloned transporter for taurine is similar to that of high affinity taurine transporters in both the central nervous system (23, 24) and peripheral tissues (25), which exhibit K_m values from 10 to 60 μM. Taken together, these data indicate that rB16a encodes a saturable, high affinity, sodium- and chloride-dependent taurine transporter.

To determine the pharmacological specificity of the cloned transporter, various agents were examined for their ability to inhibit the transport of [^{3}H]taurine by COS/rB16a (Table 1). Because the endogenous taurine transporter in COS cells accounted for, on average, 16% of the total transport activity observed in transfected cells, we were concerned that this could influence results. Accordingly, we also examined the sensitivity of the endogenous taurine transporter present in nontransfected cells. As shown in Table 1, the pharmacological properties of the cloned taurine transporter closely matched those of the endogenous transporter and thus did not lead to erroneous results.

The most potent inhibitors were taurine and hypotaurine, each of which inhibited specific [^{3}H]taurine uptake approximately 30–40% at 10 μM, 90% at 100 μM, and 100% at 1 mM. β-Alanine was slightly less potent, inhibiting specific uptake 15%, 51%, and 96% at 10 μM, 100 μM, and 1 mM, respectively; the high potency of β-alanine as an inhibitor of taurine uptake is consistent with the finding that COS/rB16a showed a 6-fold increase in the specific uptake of β-[^{3}H]alanine (data not shown), essentially identical to the fold increase observed with [^{3}H]taurine. The taurine analogue GES was also quite potent, inhibiting specific uptake of [^{3}H]taurine 11%, 45%, and 92% at 10 μM, 100 μM, and 1 mM, respectively. APSA and GABA both inhibited uptake approximately 10% and 40% at 100 μM and 1 mM, respectively. The observations that GABA is a poor inhibitor of taurine uptake and that transfection with rB16a did not result in enhanced uptake of [^{3}H]GABA (see above) are

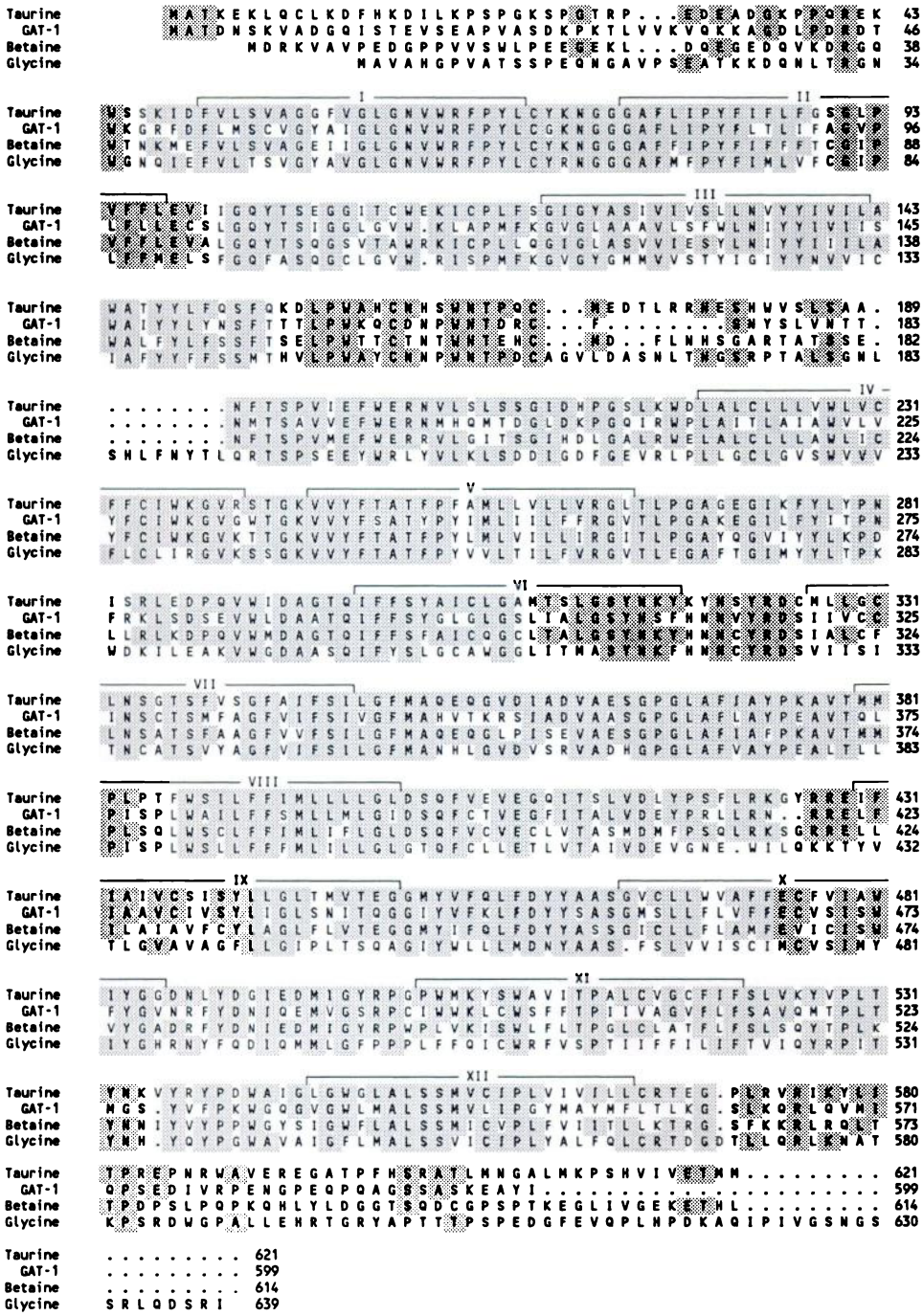

Fig. 2. Alignment of the taurine transporter with the GABA transporter GAT-1, the betaine transporter, and the glycine transporter. The 12 putative α-helical membrane-spanning domains (I–XII) are *bracketed*. Residues identical to those of the taurine transporter are *shaded*. *Taurine*, taurine transporter encoded by rB16a; *GAT-1*, rat brain GABA transporter (10); *Betaine*, dog betaine transporter (17); *Glycine*, rat glycine transporter (11).

consistent with the previous report (26) that GABA is a weak noncompetitive inhibitor of taurine uptake. Less than 10% inhibition of [^{3}H]taurine uptake was observed for the following compounds (each tested at 1 mM): the structural analogues AEPA and MEA, the sulfur-containing amino acids cysteine and methionine (Table 1), and norepinephrine, dopamine, glutamate, glycine, serine, betaine, L-methionine, and α-methylaminoisobutyric acid [a substrate for the amino acid transporter designated system A (27)] (data not shown). Taken together, these results indicate that the taurine transporter encoded by rB16a is similar to the endogenous taurine transporter in COS cells (Table 1), as well as the endogenous taurine transporter(s) present in neural tissue (28) (also see Ref. 1 and references therein).

It is of interest that sensitivity to β-alanine is shared by the two high affinity GABA transporters recently cloned from rat brain (GAT-2 and GAT-3),[4] which are even more closely related to the taurine transporter (62% amino acid identity) than to the neuronal-type GABA transporter GAT-1 (51%). β-Alanine has been shown to activate an inward chloride current in spinal neurons (29, 30) and is released in a calcium-dependent manner from several brain areas (31, 32), suggesting a role as an inhibitory neurotransmitter in the central nervous system. The similar sensitivities of the newly cloned GABA transporters[4] and the taurine transporter to β-alanine, combined with their sequence homologies, suggest that they represent a subfamily of inhibitory neurotransmitter transporters. Despite these similarities, these transporters unexpectedly exhibit widely diver-

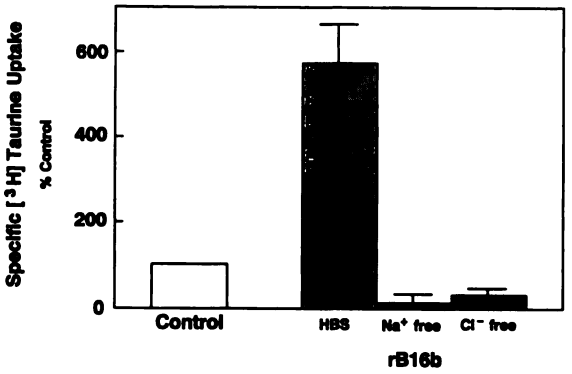

Fig. 3. Taurine transport by COS cells transfected with clone rB16a. Nontransfected COS cells (control) or COS cells transfected with rB16a were incubated for 10 min (37°) with 50 nM [^{3}H]taurine either in HBS (150 mM NaCl) or in a similar solution in which Na^+ was replaced by equimolar Li^+ (Na^+-free) or Cl^- was replaced by acetate (Cl^--free). Data show the specific uptake of taurine, expressed as percentage of control cells. Each *bar* represents the mean ± standard error of three to seven experiments.

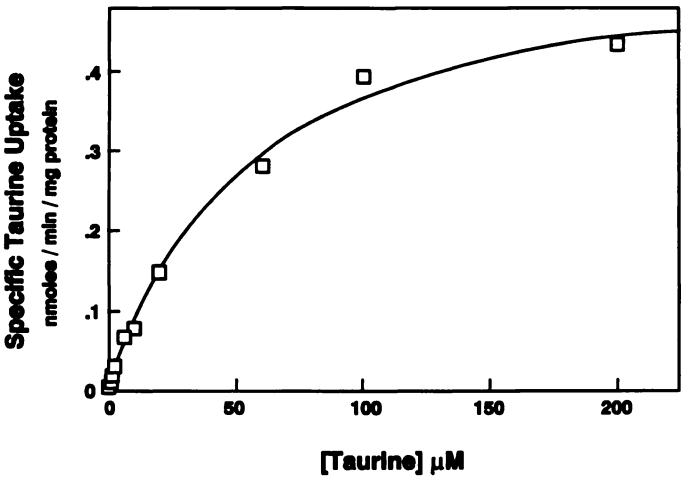

Fig. 4. Concentration dependence of taurine transport. COS cells transfected with rB16a were incubated with the indicated concentrations of [^{3}H]taurine for 30 sec, and the accumulated radioactivity was determined. The specific activity of [^{3}H]taurine was reduced with unlabeled taurine. Data represent specific transport expressed as nmol/min/mg of protein and are from a single experiment that was repeated with similar results (see text).

gent affinities for GABA; GAT-2 and GAT-3 show the highest affinity (K_m = 10 μM),[4] whereas the affinity of the taurine transporter is extremely low (~1 mM; Table 1). Interestingly, the dog betaine transporter (17), which displays a similar degree of homology to the members of this subfamily (~60%), exhibits an intermediate affinity for GABA (~100 μM). The finding that four structurally related transporters display overlapping substrate specificities for the neuroactive amino acids GABA and β-alanine suggests that multiple transporters may regulate the synaptic levels of these substances. This cross-reactivity underscores the importance of understanding the action of therapeutic agents at both GABA and taurine transporters.

Tissue localization studies. To define the tissue distribution patterns of the taurine transporter, PCR was used to detect the rB16a sequence in cDNA representing mRNA from seven different rat tissues. As a control, the distribution of the constitutively expressed protein cyclophilin was also examined. Radiolabeled oligonucleotides specific for rB16a were used to

TABLE 1

Pharmacological specificity of [^{3}H]taurine uptake

Nontransfected COS-7 cells (control) or COS-7 cells transfected with rB16a were incubated for 10 min (37°) with 50 nM [^{3}H]taurine and the indicated compounds. Data show percentage of displacement of specific [^{3}H]taurine uptake (mean ± standard error; values in parentheses indicate number of experiments).

Inhibitor	Concentration	Inhibition	
		Control	rB16a
		%	
AEPA	1 mM	0 ± 0 (4)	3 ± 3 (5)
AMSA	1 mM	1 ± 1 (4)	7 ± 3 (4)
APSA	100 μM	7 ± 3 (4)	8 ± 4 (4)
	1 mM	45 ± 3 (5)	36 ± 4 (5)
β-Alanine	10 μM	9 ± 2 (6)	15 ± 6 (6)
	100 μM	63 ± 3 (6)	51 ± 4 (10)
	1 mM	97 ± 1 (4)	96 ± 1 (8)
CSA	1 mM	2 ± 1 (4)	7 ± 5 (3)
Cysteine	1 mM	4 ± 3 (3)	2 ± 2 (3)
GABA	10 μM	1 ± 1 (4)	9 ± 6 (4)
	100 μM	9 ± 4 (6)	10 ± 4 (10)
	1 mM	49 ± 2 (5)	44 ± 6 (8)
GES	10 μM	6 ± 3 (4)	11 ± 4 (4)
	100 μM	47 ± 3 (5)	45 ± 5 (5)
	1 mM	89 ± 1 (5)	92 ± 1 (6)
Hypotaurine	10 μM	41 ± 3 (7)	26 ± 7 (7)
	100 μM	91 ± 1 (4)	84 ± 3 (4)
	1 mM	99 ± 1 (4)	100 ± 1 (4)
MEA	1 mM	1 ± 0 (3)	3 ± 3 (4)
Methionine	1 mM	1 ± 1 (3)	1 ± 1 (3)
Taurine	10 μM	38 ± 5 (7)	29 ± 8 (5)
	100 μM	89 ± 2 (4)	83 ± 2 (5)
	1 mM	100[a]	100[a]

[a] Nonspecific uptake was defined with 1 mM taurine.

detect PCR products by hybridization. As shown in Fig. 5A, the taurine transporter was detectable in all tissues examined, including brain, retina, liver, kidney, heart, spleen, and pancreas, after 30 cycles of PCR. Cyclophilin was amplified to a similar extent from all the tissues (data not shown), demonstrating that adequate cDNA was present in each sample.

To evaluate both the abundance and the size of the mRNA encoding the taurine transporter, Northern blot analysis was carried out on poly(A)$^+$ RNA isolated from the same rat tissues used for PCR analysis, as well as lung. As shown in Fig. 5B, a single ~6.2-kb transcript that hybridized with the taurine transporter cDNA probe was detected in brain, kidney, heart, spleen, and lung after an overnight exposure of the autoradiogram. After a 3-day exposure, bands of the same size were also visible in liver and pancreas (data not shown). Rehybridization of the blot with the cDNA encoding cyclophilin (15) confirmed that roughly equal amounts of RNA were present in each sample except that of retina, which was significantly degraded (data not shown). Thus, taurine transporter mRNA levels were highest in brain and lung, intermediate in kidney, heart, and spleen, and lowest in liver and pancreas. The abundance and pattern of distribution of the taurine transporter mRNA by Northern blot are consistent with data obtained using PCR (Fig. 5A); further, the same size transcript is present in all tissues evaluated. These findings suggest that a single taurine transporter functions in both the brain and peripheral tissues; however, we cannot exclude the existence of additional taurine transporters.

Conclusion. Taurine is abundant in the central nervous system and is involved in a variety of neural activities. Unlike classical neurotransmitters, the effects of taurine are mediated both intra- and extracellularly. A major regulator of taurine

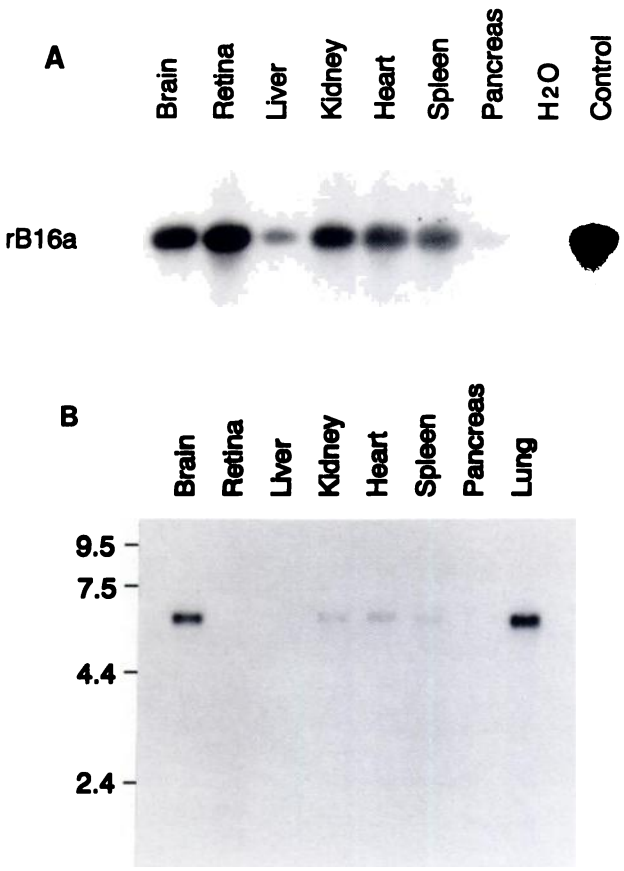

Fig. 5. Localization of the taurine transporter. A, Tissue distribution of mRNA encoding the taurine transporter as determined by PCR. Single-stranded cDNA converted from poly(A)$^+$ RNA was used for PCR amplification (30 cycles) of taurine transporter cDNA from a variety of rat tissues. A plasmid containing the cloned taurine transporter was amplified under identical conditions as a control. Amplified products were detected by hybridization with an oligonucleotide probe specific to the taurine transporter; an autoradiogram of the Southern blot is shown. Equivalent samples of poly(A)$^+$ RNA (not treated with reverse transcriptase) subjected to identical PCR conditions showed no hybridization with the transporter probe (data not shown), indicating that the signals obtained with cDNA were not a result of genomic DNA contamination. The experiment was repeated with similar results. B, Northern blot analysis of mRNA encoding the taurine transporter. Poly(A)$^+$ RNA (5 μg) from a variety of rat tissues was separated by formaldehyde-agarose gel electrophoresis, blotted to a nylon membrane, and hybridized at high stringency with ^{32}P-labeled taurine transporter cDNA (rB16a). The autoradiogram was developed after an overnight exposure. Size standards are indicated at the *left* in kb. The hybridizing transcript is ~6.2 kb.

levels, both within cells and in the synaptic cleft, is the transport of taurine across the plasma membrane. Our cloning of a high affinity taurine transporter represents a critical step in defining the role of taurine in both neural and non-neural tissues and in developing therapeutic agents that alter taurine and GABA neurotransmission. In addition, the identification of a new member of the set of inhibitory amino acid transporters will aid in the elucidation of the molecular structure-function relationships within the transporter family.

Acknowledgments

The authors would like to thank Steven Fried, Marc Dizon, Anastasia Kokkinakis, and Golshanak Mehrazar for expert technical assistance and Drs. Richard Miller and J. Barry Lombardini for helpful discussions. We also thank Mr. George Moralishvili and Mr. Ernest Lilley for producing the illustrations.

References

1. Huxtable, R. J. Taurine in the central nervous system and the mammalian actions of taurine. *Prog. Neurobiol.* **32**:471–533 (1989).
2. van Gelder, N. M. Neuronal discharge hypersynchrony and the intracranial water balance in relation to glutamic acid and taurine redistribution: migraine and epilepsy. *Prog. Clin. Biol. Res.* **351**:1–20 (1990).
3. Shain, W., and D. L. Martin. Review: uptake and release of taurine: an overview. *Prog. Clin. Biol. Res.* **351**:243–252 (1990).
4. Kontro, P., E. R. Korpi, and S. S. Oja. Taurine interacts with $GABA_A$ and $GABA_B$ receptors in the brain. *Prog. Clin. Biol. Res.* **351**:83–94 (1990).
5. Quinn, M. R. Taurine allosterically modulates binding sites of the $GABA_A$ receptor. *Prog. Clin. Biol. Res.* **351**:121–127 (1990).
6. Wu, J.-Y., C. Liao, C. J. Lin, Y. H. Lee, J.-Y. Ho, and W. H. Tsai. Taurine receptor in mammalian brain. *Prog. Clin. Biol. Res.* **351**:147–156 (1990).
7. Lombardini, J. B. Effects of taurine and mitochondrial metabolic inhibitors on ATP-dependent Ca^{2+} uptake in synaptosomal and mitochondrial subcellular fractions of rat retina. *J. Neurochem.* **51**:200–205 (1988).
8. Huxtable, R. J. Review: taurine interactions with ionic conductances in excitable membranes. *Prog. Clin. Biol. Res.* **351**:157–161 (1990).
9. Sturman, J. A. Review: taurine deficiency. *Prog. Clin. Biol. Res.* **351**:385–395 (1990).
10. Guastella, J., N. Nelson, H. Nelson, L. Czyzyk, S. Keynan, M. C. Miedel, N. Davidson, H. A. Lester, and B. I. Kanner. Cloning and expression of a rat brain GABA transporter. *Science (Washington D. C.)* **249**:1303–1306 (1990).
11. Smith, K. E., L. A. Borden, P. R. Hartig, T. A. Branchek, and R. L. Weinshank. Cloning of a glycine transporter reveals co-localization with NMDA receptors. *Neuron* **8**:915–926 (1992).
12. Sanger, S. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467 (1977).
13. Miller, J., and R. N. Germain. Efficient cell surface expression of class II MHC molecules in the absence of associated invariant chain. *J. Exp. Med.* **164**:1478–1489 (1986).
14. Lopata, M. A., D. W. Cleveland, and B. Sollner-Webb. High-level expression of a chloramphenicol acetyltransferase gene by DEAE-dextran-mediated DNA transfection coupled with a dimethyl sulfoxide or glycerol shock treatment. *Nucleic Acids Res.* **12**:5707–5717 (1984).
15. Danielson, P. E., S. Forss-Petter, M. A. Brow, L. Calavetta, J. Douglass, R. J. Milner, and J. G. Sutcliffe. p1b15: a cDNA clone of the rat mRNA encoding cyclophilin. *DNA* **7**:261–267 (1988).
16. Pacholczyk, T., R. D. Blakely, and S. G. Amara. Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. *Nature (Lond.)* **350**:350–354 (1991).
17. Yamauchi, A., S. Uchida, H. M. Kwon, A. S. Preston, R. B. Robey, A. Garcia-Perez, M. B. Burg, and J. S. Handler. Cloning of a Na^+- and Cl^--dependent betaine transporter that is regulated by hypertonicity. *J. Biol. Chem.* **267**:649–652 (1992).
18. Blakely, R. D., H. E. Berson, R. T. Fremeau, Jr., M. G. Caron, M. M. Peek, H. K. Prince, and C. C. Bradley. Cloning and expression of a functional serotonin transporter from rat brain. *Nature (Lond.)* **354**:66–70 (1991).
19. Hoffman, B. J., E. Mezey, and M. J. Brownstein. Cloning of a serotonin transporter affected by antidepressants. *Science (Washington D. C.)* **254**:579–580 (1991).
20. Kilty, J. E., D. Lorang, and S. G. Amara. Cloning and expression of a cocaine-sensitive rat dopamine transporter. *Science (Washington D. C.)* **254**:578–579 (1991).
21. Shimada, S., S. Kitayama, C.-L. Lin, A. Patel, E. Nanthakumaar, P. Gregor, M. Kuhar, and G. Uhl. Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. *Science (Washington D. C.)* **254**:576–578 (1991).
22. Hediger, M. A., E. Turk, and E. M. Wright. Homology of the human intestinal Na^+/glucose and *Escherichia coli* Na^+/proline cotransporters. *Proc. Natl. Acad. Sci. USA* **86**:5748–5752 (1989).
23. Lombardini, J. B. High-affinity transport of taurine in the mammalian central nervous system, in *Taurine and Neurological Disorders* (A. Barbeau and R. J. Huxtable, eds.). Raven Press, New York, 119–135 (1978).
24. Yorek, M. A., and A. A. Spector. Taurine transport and metabolism in human retinoblastoma cells, in *Taurine: Biological Actions and Clinical Perspectives* (L. Ahtee, P. Kontro, M. Paasonan, and F. Oja, eds.). Alan R. Liss, Inc., New York, 361–370 (1985).
25. Lambert, I. H., and E. K. Hoffman. Taurine transport and cell volume regulation in a mammalian cell. *Prog. Clin. Biol. Res.* **351**:267–276 (1990).
26. Larsson, O. M., R. Griffiths, I. C. Allen, and A. Schousboe. Mutual inhibition kinetic analysis of γ-aminobutyric acid, taurine, and β-alanine high-affinity transport into neurons and astrocytes: evidence for similarity between the taurine and β-alanine carriers in both cell types. *J. Neurochem.* **47**:426–432 (1986).
27. Kilberg, M. S. Amino acid transport in isolated rat hepatocytes. *J. Membr. Biol.* **69**:1–12 (1982).
28. Hruska, R. E., R. J. Huxtable, and H. I. Yamumura. High-affinity, temperature-sensitive, and sodium-dependent transport of taurine in rat brain, in *Taurine and Neurological Disorders* (A. Barbeau and R. J. Huxtable, eds.). Raven Press, New York, 109–117 (1978).
29. Choquet, D., and H. Korn. Does β-alanine activate more than chloride channel associated receptor? *Neurosci. Lett.* **84**:329–340 (1988).

30. Mathers, D. A., A. Grewal, and Y. Wang. β-Alanine induced ion channels in the membrane of cultured spinal cord neurons. *Neurosci. Lett.* **108**:127–131 (1990).
31. Sandberg, M., and I. Jacobson. β-Alanine, a possible neurotransmitter in the visual system? *J. Neurochem.* **37**:1353–1356 (1981).
32. Kihara, M., Y. Misu, and T. Kubo. Release by electrical stimulation of endogenous glutamate, γ-aminobutyric acid, and other amino acids from slices of the rat medulla oblongata. *J. Neurochem.* **52**:261–267 (1989).

Send reprint requests to: Richard L. Weinshank, Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, NJ 07652.