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High Affinity Transport of Taurine and β-Alanine and Low Affinity Transport of γ-Aminobutyric Acid by a Single Transport System in Cultured Glioma Cells*

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David L. Martin and William Shain

From the Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20014, and Department of Chemistry, University of Maryland, College Park, Maryland 20742

Transport of radioactive taurine, β-alanine, and γ-aminobutyrate was studied in a rat spinal glioma cell line, LRM55. Kinetic analyses showed that LRM55 cells possess only low affinity γ-aminobutyrate transport (K_m = 709 ± 61 μM) and only high affinity taurine and β-alanine transport (K_m = 30 ± 3 and 79 ± 12 μM, respectively). No high affinity γ-aminobutyrate or low affinity taurine or β-alanine transport systems were observed. Maximum rates of uptake were 4.2 ± 2.1, 4.6 ± 1.4, and 1.7 ± 0.3 nmol/min/mg of protein for γ-aminobutyrate, β-alanine, and taurine, respectively. Net transport of γ-aminobutyrate and taurine was demonstrated by amino acid analysis of cells. Control cells contained approximately 38 mM taurine but identification of γ-aminobutyrate and β-alanine were uncertain and the amounts too small to be quantified. Influxes of 1 mM taurine, 1 mM β-alanine, and 0.025 μM to 2 mM γ-aminobutyrate were nearly linear for 2 h, whereas influxes of 1 μM taurine and 1 μM β-alanine had nearly ceased after 1 h. These differences among the time courses were attributable to the differences in the kinetic parameters of the three compounds as indicated by the accurate fit of a simple computer model (consisting of Michaelis-Menten influx and first order efflux components) to the time courses for all three compounds. Graded replacement of NaCl with choline chloride strongly reduced the influxes of taurine and γ-aminobutyrate in a nearly identical way. Taurine, β-alanine and γ-aminobutyrate were mutually competitive inhibitors of the transport of each other. Sixteen amino acids and structural analogs of taurine, β-alanine, and γ-aminobutyrate were found to have very similar inhibitory effects on the influxes of all three substrates. The mutual competition and similarities in sodium dependency and inhibition by analogs indicate that low affinity γ-aminobutyrate and high affinity taurine and β-alanine transports occur by the same system in LRM55 cells. This suggests that low affinity γ-aminobutyrate uptake in other preparations such as brain slices or synaptosomes is carried out, at least in part, by high affinity transport systems for taurine and β-alanine.

Two classes of membrane transport systems which are distinguished by their Michaelis constants for substrates and are commonly called high and low affinity systems have been described for a number of compounds in nervous tissue. Taurine transport has been studied in a variety of brain preparations as part of an effort to understand the physiological role of this compound in brain. Both high affinity ($K_m \simeq$ 20 to 60 μM) and low affinity ($K_m \simeq$ 170 to 6100 μM) transport systems have been reported to occur in synaptosomes, brain slices, retina, and glia cells (1-7). High affinity GABA[1] transport (K_m < 5 × 10^{-5} M) has been studied extensively and has been demonstrated in a wide variety of nervous tissue preparations including glia cells from several sources (8). The primary function of high affinity GABA transport is thought to be termination of GABA-mediated synaptic transmission by removal of GABA from the extracellular fluid at the synapse (8). Low affinity GABA transport (K_m ~ 10^{-4} to 10^{-3} M) has been much less thoroughly studied although it has been found in brain slices (9-11), synaptosomes (10), and glia cell preparations (12, 13). The physiological function, if any, of low affinity GABA transport is unknown. The possibility that the low affinity system might participate in termination of synaptic transmission has been discussed but there is no supporting evidence (8). It has also been suggested that nonspecific transport of GABA by some other transport system might account for low affinity GABA uptake but no such system has been identified so far.

We report here studies of low affinity GABA transport by a clonal glioma cell line which has no detectable high affinity GABA transport. The results show that low affinity GABA transport in these cells is carried out by the same transport system that carries out high affinity transport of taurine and β-alanine. Since low affinity GABA and high affinity taurine transport are found throughout the central nervous system (5, 11), these results suggest that much if not all low affinity GABA transport might be attributed to high affinity taurine transport.

MATERIALS AND METHODS

Cell Line—LRM55 cells were obtained as a clone from an ethyl nitrosourea-induced mixed glioma isolated from the spinal cord of a Fisher rat and grown in cell culture (14). Cells were grown routinely in modified Ham's F_{12} medium (15) supplemented with 5% fetal calf serum in 100-mm plastic Petri dishes (Falcon 3003). All experiments were carried out with cells from Passages 2 to 15.

Uptake of Labeled Amino Acid—LRM55 cells adhere very tightly to plastic substrates so the approach of Richelson (16) could be used for transport studies. Briefly, individual wells (16 mm diameter) of 24-well culture dishes (Costar 3524) were seeded with about 10^4 cells and the cells allowed to grow to confluency (4 to 6 days). Unless stated otherwise, wells contained 64 to 161 μg of protein at the time transport was measured. At the start of the experiment, growth medium was removed and the cells were rinsed two times with Hanks'

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[1] The abbreviations used are: GABA, γ-aminobutyric acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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solution (see below) by means of a syringe pipettor which was modified to direct the rinse solution evenly around the walls of the well and which incorporated an aspirator tube to remove the rinse solution. Uptake was then initiated by removing the last rinse solution and adding the appropriate labeled compound in 0.2 ml of Hanks' solution. The wells were sealed with No. 2 neoprene stoppers to prevent evaporation of the fluid and incubated at 37°C. Uptake was stopped by drawing off the medium and rapidly rinsing the wells three times with 1 ml of Hanks' solution using the syringe pipettor. This required 6 to 8 s. One-half-milliliter of 0.4 M NaOH was then added to each well. The samples were allowed to stand overnight and then transferred to counting vials along with an additional 0.5 ml of 0.4 M NaOH and 0.5 ml of water used to rinse the wells. Radioactivity was measured by liquid scintillation using Triton X-100 counting solution (17).

Uptake of Unlabeled Amino Acids—Growth medium was removed from confluent LRM55 cells in 100-mm plastic Petri dishes and the cells were rinsed two times with 6 ml of Hanks' solution. The cells were then incubated in 6 ml of Hanks' solution containing the appropriate amino acid (or none in the control) at 37°C for 1 h and then rinsed three times with 6 ml of Hanks' solution. To remove the cells, 0.4 ml of Hanks' solution was added to one dish and the cells were scraped from the bottom with a Teflon-covered scraper. The scrapings from the first dish were transferred to a second and the scraping procedure was repeated. The combined material from the two dishes was then analyzed. Protein was precipitated by adding 5-sulfosalicyclic acid to a final concentration of 5%. Amino acid compositions of the protein-free extracts were determined by Dr. Gary Beecher, Protein Nutrition Laboratory, Nutrition Institute, United States Department of Agriculture, Beltsville, Md., using an automated analyzer developed in his laboratory (18). Precipitated protein was washed with 5% trichloroacetic acid and dissolved in 0.1 M NaOH prior to determination of cell protein.

Protein Analyses—Protein was determined by a modification of the method of Lowry *et al.* (19).

Cell Volume—Internal volume of LRM55 cells per mg of protein was determined by measuring the amount of [^{14}C]urea entering the cells using the procedures described for transport except that six-well plates were used (Linbro) and each well received 1 ml of Hanks' solution containing about 2 μCi of [^{14}C]urea. Cells were incubated for 1 h. The internal volume was calculated from the amount of radioactivity in the cells and the concentration of radioactivity in the external medium. Control experiments showed that urea distributed passively across the membrane. The half-time for urea equilibration was about 5 min. Since the rinsing procedure required less than 20 s the amount of urea lost during the rinse was negligible. The volume figure used in this report, 2.3 μl/mg of protein, was obtained by linear regression analysis of 14 measurements (Fig. 1).

Materials—The modified Hanks' solution used in these experiments contained 10 mM Hepes, 11 mM glucose, 130 mM Na^+, 4.5 mM K^+, 136 mM Cl^-, 1.1 mM Ca^{2+}, 0.5 mM Mg^{2+}, 0.2 mM SO_4^{2-}, and 1.4 mM phosphate, and was adjusted to pH 7.3. Aminooxyacetic acid (10 μM) was added in GABA uptake experiments to prevent metabolism of GABA (8, 20). [2,3-^{3}H]GABA (25 to 40 Ci/mmol), β-[3-^{3}H]alanine (30 to 50 Ci/mmol), [2-^{3}H]taurine (2 to 40 Ci/mmol), and [^{14}C]urea (40 to 60 mCi/mmol) were obtained from New England Nuclear, Boston, MA. [^{35}S]Taurine (32 mCi/mmol) was obtained from Amersham Corp., Arlington Heights, IL.

Calculations—The simple Michaelis-Menten rate law was fitted to uptake data using a computer program based on the method of Eisenthal and Cornish-Bowden (21). Values of K_i were calculated using Dixon plots for competitive inhibition (22). A computer program was used to fit lines to the data by linear regression and to calculate points of intersection. Time courses of tracer uptake were calculated by numerically integrating the equations for a simple model of the transport process using a self-readjusting Runge-Kutta-Adams-Moulton routine. In the model, tracer and substrate movement were considered to consist of an influx component described by the Michaelis-Menten rate equation and a first order efflux component. The basic equations for the model were:

$$\frac{dA_0}{dt} = k_e[A_i] - \frac{V_{\max}[A_0]}{K_m + [A_0]} \quad (1)$$

$$\frac{dL_0}{dt} = k_e[A_i]\cdot S_i - \frac{V_{\max}[A_0]\cdot S_0}{K_m + [A_0]} \quad (2)$$

The subscripts i and o denote the inside and outside of the cell, respectively, A is the substrate, L is the label, and S is the specific activity of the substrate (L/A). Separate equations for movement of label and substrate are necessary because in the experiments modeled, the isotope was initially present only outside the cell whereas substrate (particularly taurine) was initially present both outside and inside. Values of $V_{\max}$ and K_m which fit the data were within the ranges of values obtained by fitting the Michaelis-Menten rate equation to data from initial rate experiments as described above (Tables I and II). No independent estimates of k_e were available and values were merely selected to fit the data. Initial concentrations of substrate inside the cell were based on the data in Table III.

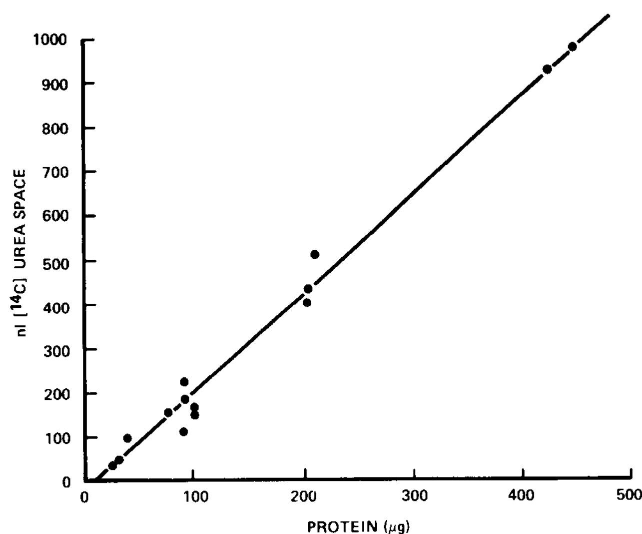

FIG. 1. **Correlation of [^{14}C]urea space and total protein of LRM55 cells.** Urea space was determined as described under "Materials and Methods." The line was calculated by linear regression analysis. Each point represents a single value.

RESULTS

Influxes of taurine, β-alanine, and GABA were highly temperature-dependent. Uptake of label by cells in dishes placed on ice averaged only 1 to 2% of uptake at 37°C. These results indicated that the amounts of substrates remaining in extracellular spaces or adsorbed to the dish were very small and, therefore, uptake values were not corrected for these factors.

Initial rates of uptake of GABA, β-alanine, and taurine were accurately described by the simple Michaelis-Menten equation over wide ranges of concentrations (Figs. 2–4). The values of K_m for β-alanine and taurine were typical of high affinity transport systems for these compounds (Table I). We found no significant deviation of the data from fitted lines even at substrate concentrations greater than 10 times K_m. Thus, there appears to be no significant contribution from low affinity transport of these compounds by these cells under the conditions used in this study. However, the K_m for labeled GABA uptake was typical of values reported for low affinity uptake (Table I). We found no evidence of high affinity GABA uptake in these cells. Reported K_m values for high affinity GABA uptake in glia cells range from about 0.2 to about 50 μM (12, 13, 23–28). The close fit of the data to the Michaelis-Menten equation down to 1.25 μM (Fig. 4) appears to eliminate the possibility of high affinity GABA uptake with a K_m near 50 μM. In separate experiments we found that the rate of GABA uptake was a linear function of the GABA concentration over the range 2.5×10^{-8} to 1.0×10^{-6} M (Fig. 4C). At concentrations this far below the apparent K_m, the slope of the line should be equal to $V_{\max}/K_m$ if the uptake is described by a single saturation function. The slope (± S.E.) from linear regression analysis, 6.8 ± 0.1 μl/min/mg of protein, was not significantly different from the values 6.1 ± 2.0 calculated from independent estimates of K_m and $V_{\max}$ (Tables I and II).

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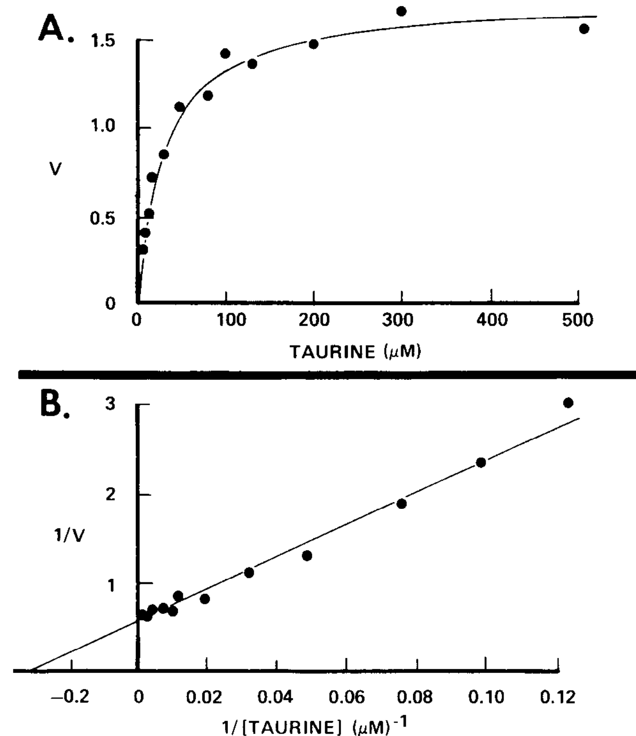

FIG. 2. **Initial rates of uptake of taurine.** *A*, fit of initial rates of taurine influx in LRM55 cells to the Michaelis-Menten rate equation. *B*, double reciprocal plot of the data in *Panel A*. Lines in both panels were calculated using apparent values of K_m (31 μM) and V_{max} (1.7 nmol/min/mg of protein) obtained as described under "Materials and Methods." Uptake by cells equivalent to 74 μg of protein/well was measured after 10 min of incubation. Rates are in nanomoles per min per mg of protein. Each point is the average of two values.

Although K_m for GABA differed by a factor of about 10 from the K_m for β-alanine and 20 from the K_m for taurine, the values of V_{max} were similar (Table II).

The time course of influx of labeled GABA, taurine, and β-alanine was studied over a wide range of concentrations (Fig. 5). GABA uptake was nearly linear for almost 2 h using concentrations from 0.025 to 2000 μM. In contrast, uptakes of 1 μM taurine and β-alanine had almost come to a stop in 1 h but the uptake of millimolar levels of these two amino acids continued almost linearly for the duration of the experiments. It is notable that about 80% of 1 μM β-alanine and taurine were taken up within 1 h but less than 30% of 2 μM labeled GABA was taken up in 2 h. Thus, the time courses of uptake of taurine and β-alanine were different from those for GABA uptake, particularly at low concentrations. To examine the reasons for this, we developed a simple computer model of the uptake process (see "Materials and Methods"). This model accurately described the time courses of uptake of all three substrates (Fig. 5). The results indicate that the differences among the time courses for the three amino acids are attributable to the differences in the kinetic parameters for the three compounds and not to differences in mechanism. The values of K_m for taurine and β-alanine are only 4 and 11% of the K_m for GABA whereas the values of V_{max} are similar for all three compounds. As a result, the transport rates of micromolar concentrations of taurine and β-alanine are approximately 10 to 20 times higher than the corresponding rate of GABA transport. Thus, the cells rapidly accumulate a high percentage of the small amount of taurine or β-alanine present in the well but do not do so with GABA. At millimolar concentrations where the rates of uptake of all three compounds are more nearly the same, the wells contain much larger amounts of the amino acids and the transport system cannot rapidly reduce the external concentrations of any of the amino acids. In addition to the kinetic parameters, another major factor which determined the rapidity of uptake was the number of cells per well. This factor accounts for the more rapid accumulation of β-alanine than taurine in the experiments shown in Fig. 5. In another experiment the uptake of both 1 and 100 μM taurine reached a steady state within 1 h when the wells contained twice as many cells as in the experiment in Fig. 5 (data not shown).

Ten-minute incubation periods were selected for uptake studies with taurine and β-alanine to accommodate the manipulations necessitated by the use of the 24-well dishes. The time course experiments also indicated 10 min was an adequate incubation period for kinetic studies of taurine and β-

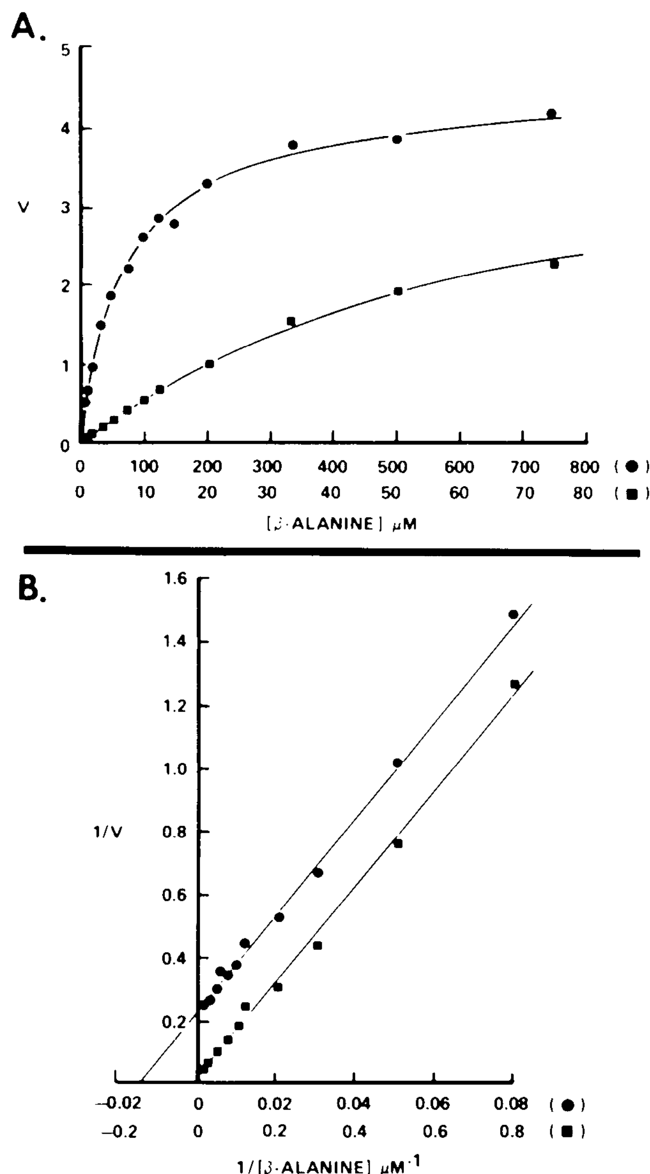

FIG. 3. **Initial rates of uptake of β-alanine.** *A*, fit of initial rates of β-alanine influx in LRM55 cells to the Michaelis-Menten rate equation. *B*, double reciprocal plot of the data in *Panel A*. Lines in both panels were calculated using apparent values of K_m (68 μM) and V_{max} (4.4 nmol/min/mg of protein) obtained as described under "Materials and Methods." Ordinate values for lower concentration range in *Panel B* must be multiplied by 10. Transport by cells equivalent to 64 μg of protein/well was measured after 10 min of incubation. Rates are in nanomoles per min per mg of protein. Each point is the average of two values.

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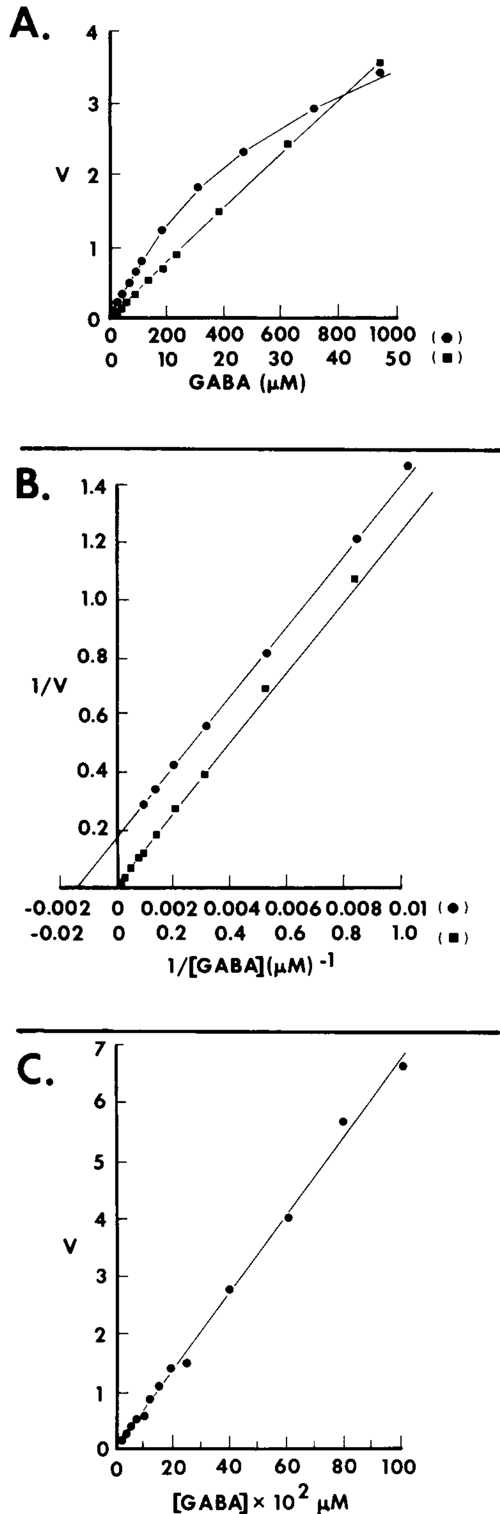

FIG. 4. **Initial rates of uptake of GABA.** *A*, fit of initial rates of GABA influx in LRM55 cells to the Michaelis-Menten rate equation. *B*, double reciprocal plot of the data in *Panel A*. *C*, initial rates of GABA uptake at 1 μM and lower concentrations of GABA. Rates in *Panels A* and *B* are in nanomoles per min per mg of protein. Ordinate values for lower concentration range must be divided by 10 in *Panel A* and multiplied by 100 in *Panel B*. Rates in *Panel C* are in picomoles per min per mg of protein. Lines in *Panels A* and *B* were calculated using apparent values of K_m (760 μM) and V_{max} (6.1 nmol/min/mg of protein) obtained as described under "Materials and Methods." The line in *Panel C* was calculated by linear regression analysis. Transport by cells equivalent to 94 (*Panels A* and *B*) or 81 μg of protein/well (*Panel C*) was measured after 30 min of incubation. Each point is the average of two values.

TABLE I

Comparison of inhibition and Michaelis constants

Michaelis and inhibition constants were calculated as described under "Materials and Methods." Values of K_m are average ± S.D. from four experiments. Values of K_i are average ± S.D. of 5 to 12 values from Dixon plots.

Substrate	K_m* or K_i† (μM)		
	Inhibitor		
	GABA	β-Alanine	Taurine
GABA	*709 ± 61	†81 ± 11	†27 ± 4
β-Alanine	†1050 ± 170	*79 ± 12	†29 ± 17
Taurine	†1280 ± 260	†85 ± 20	*30 ± 3

TABLE II

Maximum rates of uptake in LRM55 cells

Values are average ± S.D. of three or four determinations.

Substrate	V_{max}
	nmol/min/mg protein
GABA	4.2 ± 2.1
β-Alanine	4.6 ± 1.4
Taurine	1.7 ± 0.3

alanine uptake. Even though a significant percentage of taurine and β-alanine was accumulated by 10 min (Fig. 5), computer studies indicated that rates calculated from uptake measured at 10 min were only 5 to 10% lower than rates determined with shorter incubation periods (5 min).

Net transport of GABA and taurine was demonstrated by direct measurement of the amino acid composition of cell extracts (Table III). Although internal cell volume measurements were not made in these particular experiments, the amino acid content expressed per mg of protein was converted to internal concentrations using the value, 2.3 μl/mg of protein determined previously for the internal volume of the cells (see "Materials and Methods"). The amount of GABA, if present in LRM55 cells, was too small to be quantified in these experiments. There was a small peak that appeared in all samples where standard GABA appears but we have made no attempts to determine whether this material was, in fact, GABA. Cells incubated for 1 h with 700 μM GABA accumulated an amount equivalent to an internal concentration of about 29 mM.

LRM55 cells contained more taurine than any other amino acid except glutamate which was present in about an equal amount. Incubation with 30 μM taurine for 1 h raised the taurine content about 50% from an amount equivalent to an internal concentration of 38 mM to about 57 mM. Incubation with GABA had no significant effect on the taurine content of the cells. This result suggests that at most only a small fraction of GABA uptake may take place by GABA-taurine heteroexchange. The amounts of other amino acids in LRM55 cells were not significantly affected by incubation with either GABA or taurine.

As in the case of GABA, there was a small peak in the position where standard β-alanine appeared. However, the amount was too small to be quantified accurately and no effort was made to identify this material positively.

Net transport of taurine was also demonstrated by experiments with gramicidin. LRM55 cells exposed to high concentrations of this antibiotic (0.25 mM) for 5 min remained attached to the dish and retained their general microscopic appearance but were rendered permeable to trypan blue dye.[2] The same treatment released more than 99% of labeled taurine from the cells indicating that an intact plasma membrane was required for taurine retention by the cells.

[2] B. E. Seligman, W. Shain, and D. L. Martin, unpublished work.

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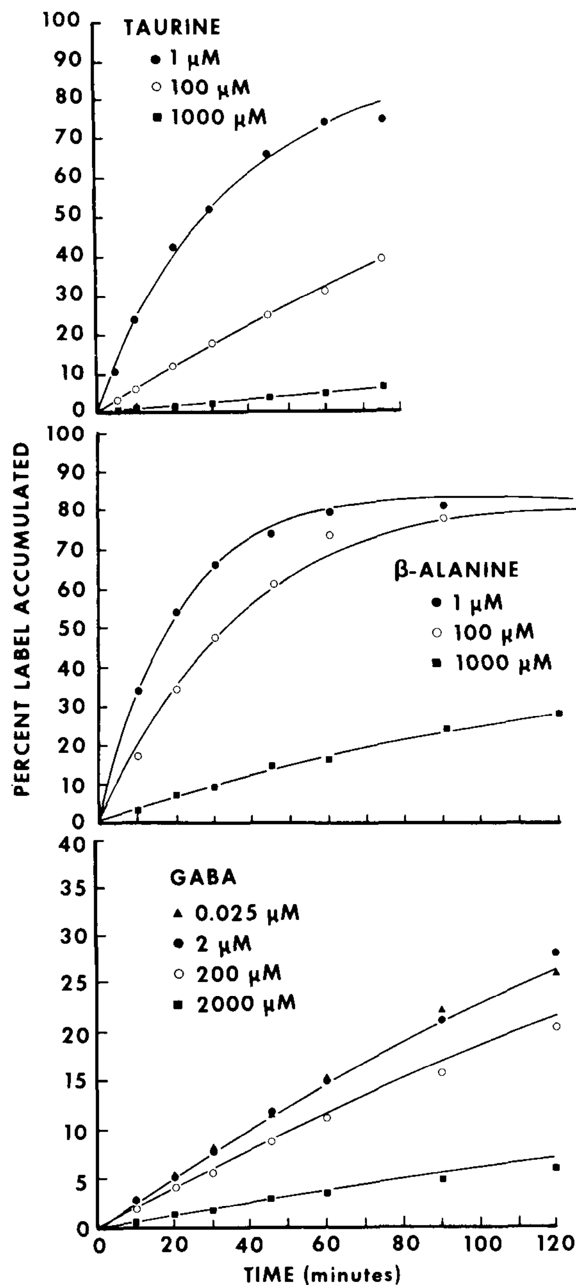

FIG. 5. **Experimental and calculated transport of taurine, β-alanine, and GABA as functions of time of incubation.** Cells equivalent to 76, 161, and 94 μg of protein were incubated with taurine, β-alanine, and GABA, respectively, at the substrate concentrations indicated on the figure. Lines were calculated by numerical integration of transport equations as described under "Materials and Methods." Uptake is reported as the percentage of the label present in wells. When reported in this way, calculated uptakes of 0.025 and 2 μM GABA were almost superimposable so only a single line is shown for both concentrations. Values of K_m (μM), V_{max} (nmol/min/mg of protein), and k_e (nl/min/mg of protein) used in the calculations for these particular experiments were 30, 2.1, and 5.3 for taurine; 94, 5.0, and 18 for β-alanine; and 650, 3.9, and 2.6 for GABA. Initial internal concentrations were assumed to be 38 mM for taurine (Table III and text) and 0 for β-alanine and GABA. Each experimental point is the average of two values.

Taurine and GABA transport in LRM55 cells showed very similar Na^+ dependencies (Fig. 6). In both cases, plots of initial rates as a function of the Na^+ concentration were sigmoidal. The rates of uptake at 10 mM Na^+ were less than 5% of the rate at 144 mM Na^+. There was no clear saturation of the Na^+ effect at the highest concentrations tested. In these experiments NaCl was replaced with choline Cl to maintain osmotic pressure but similar results were obtained when sucrose was substituted for NaCl. The sigmoidal character of the sodium dependency curves for uptake by LRM55 cells is similar to that observed for GABA uptake by synaptosomes (29) and suggests that the mechanism of taurine and GABA transport involves the binding of more than one Na^+ ion.

Inhibition studies showed that taurine and β-alanine were potent inhibitors of GABA transport and similarly, that GABA strongly inhibited taurine and β-alanine transport. Kinetic examination of these inhibitory effects showed that taurine, β-alanine, and GABA were mutually competitive inhibitors of transport (Fig. 7). Values of apparent K_m for the transported substrate were increased about 2.5-fold at the highest concentrations of inhibitors used. The inhibition constants for taurine and β-alanine were nearly identical to the values of K_m for these compounds as substrates (Table I). However, values of K_i for GABA were higher than the K_m for GABA transport.

We examined the effects of a number of compounds of the transport of taurine, β-alanine, and GABA to characterize further the specificity of the transport system. To facilitate

TABLE III

Amino acid content of LRM55 cells

Cells incubated as described under "Materials and Methods" with no added amino acids (control) or with 30 μM taurine or 700 μM GABA, were harvested and the amino acid content determined with an automated amino acid analyzer as described under "Materials and Methods." Question mark indicates the compound was not positively identified or quantified. Values are average ± S.D. of three determinations.

Amino acid	Control	Taurine	GABA
		nmol/mg protein	
Taurine	88 ± 11	131 ± 6	80 ± 12
GABA	?	?	66 ± 12
Glutamate	89 ± 9	74 ± 5	79 ± 8
Glutamine	26 ± 2	19 ± 4	23 ± 5
Glycine	30 ± 8	26 ± 3	25 ± 2
α-Alanine	19 ± 3	15 ± 1	14 ± 3
β-Alanine	?	?	?

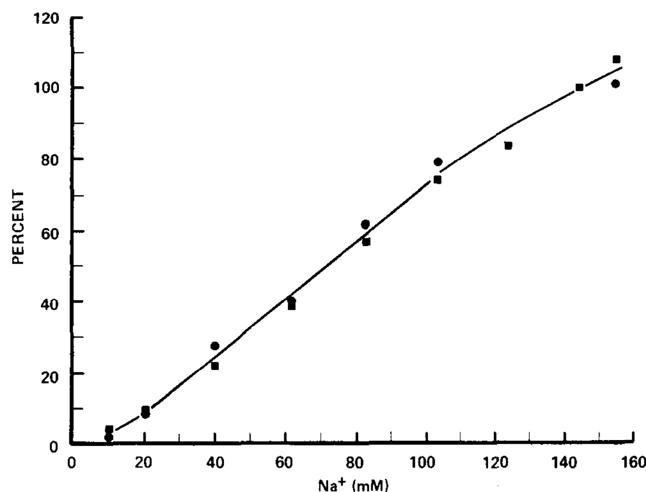

FIG. 6. **Na^+ dependencies of taurine (●) and GABA (■) transport by LRM55 cells.** Uptakes are expressed as per cent of initial rate of uptake at 144 mM Na^+. Sodium chloride was replaced with choline chloride to maintain osmotic pressure. To make the substrate concentrations kinetically equivalent, concentrations equal to K_m for each substrate were used, *i.e.* 30 μM taurine and 700 μM GABA. Uptake was measured after 15 min of incubation. Each point is the average of two values.

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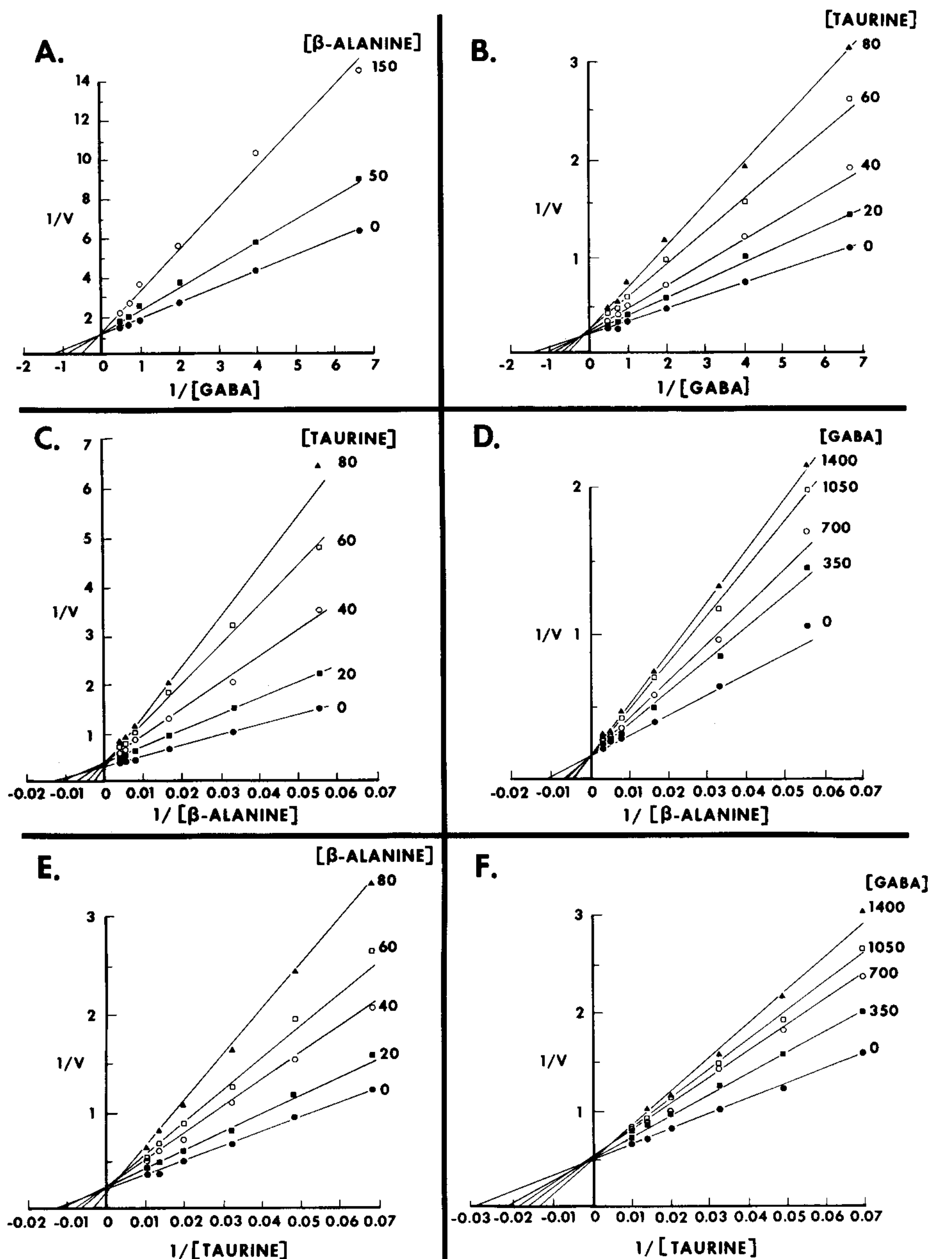

FIG. 7. **Double reciprocal plots showing mutual competitive inhibition of taurine, β-alanine, and GABA uptakes by each other.** Concentrations of inhibitors (μM) are shown at right end of the corresponding line on each panel. Units of abscissae are reciprocal micromolar for taurine and β-alanine as substrates and reciprocal millimolar for GABA. Initial velocities are given as reciprocal nanomoles per min per mg of protein in all panels except *A* where the values are reciprocal nanomoles per min per well. Lines were calculated from apparent values of K_m and V_{max} obtained as described under "Materials and Methods." Each point is the average of two values.

comparisons of the inhibitors, the transport substrates were studied at kinetically equivalent concentrations. Specifically, each substrate was present at the concentration equal to its K_m. Under these conditions, each inhibitor should have very nearly the same effect on the transport of all three substrates if all three are transported by the same system. This was observed with all of the inhibitors tested (Fig. 8). All inhibitors were added at the same two concentrations (1 and 5 mM) so their relative inhibitory potencies could be assessed. The β-amino acids, taurine and β-alanine, were the most potent inhibitors by far, indicating that the transport system was highly specific for these compounds. Aside from the two β-amino acids, GABA was the most potent inhibitor among those compounds tested which occur naturally in the brain. The α-amino acids tested included those which are most closely related structurally to the substrates: glycine, α-alanine, cysteiate, aspartate, and glutamate. None was an effective inhibitor. To examine the structural requirements of the transport system further, structural analogs of taurine, β-alanine, and GABA were tested. The results showed that replacement of the carboxylate or sulfonate group on the transport inhibitor with a phosphonate group greatly reduced the inhibitory potency. The β-amino acid analog, 2-aminoethylphosphonate was similar in potency to GABA rather than to taurine or β-alanine and the GABA analog, 3-aminopropylphosphonate was not inhibitory. On the other hand, a sulfate analog of GABA, ethanolamine-*O*-sulfate, had about the same inhibitory effectiveness as GABA, as might have been expected in view of the high affinity of the system for the sulfonate, taurine. Nipecotic acid and 2,4-diaminobutyric acid, which are both strong inhibitors of high affinity GABA uptake in synaptosomes or brain slices (30, 31) were somewhat less effective inhibitors than GABA in this system. Nipecotic acid is of particular interest in this respect since its carboxyl and amino groups are separated by 2 methylenic carbon atoms and it might be considered an analog of β-alanine.

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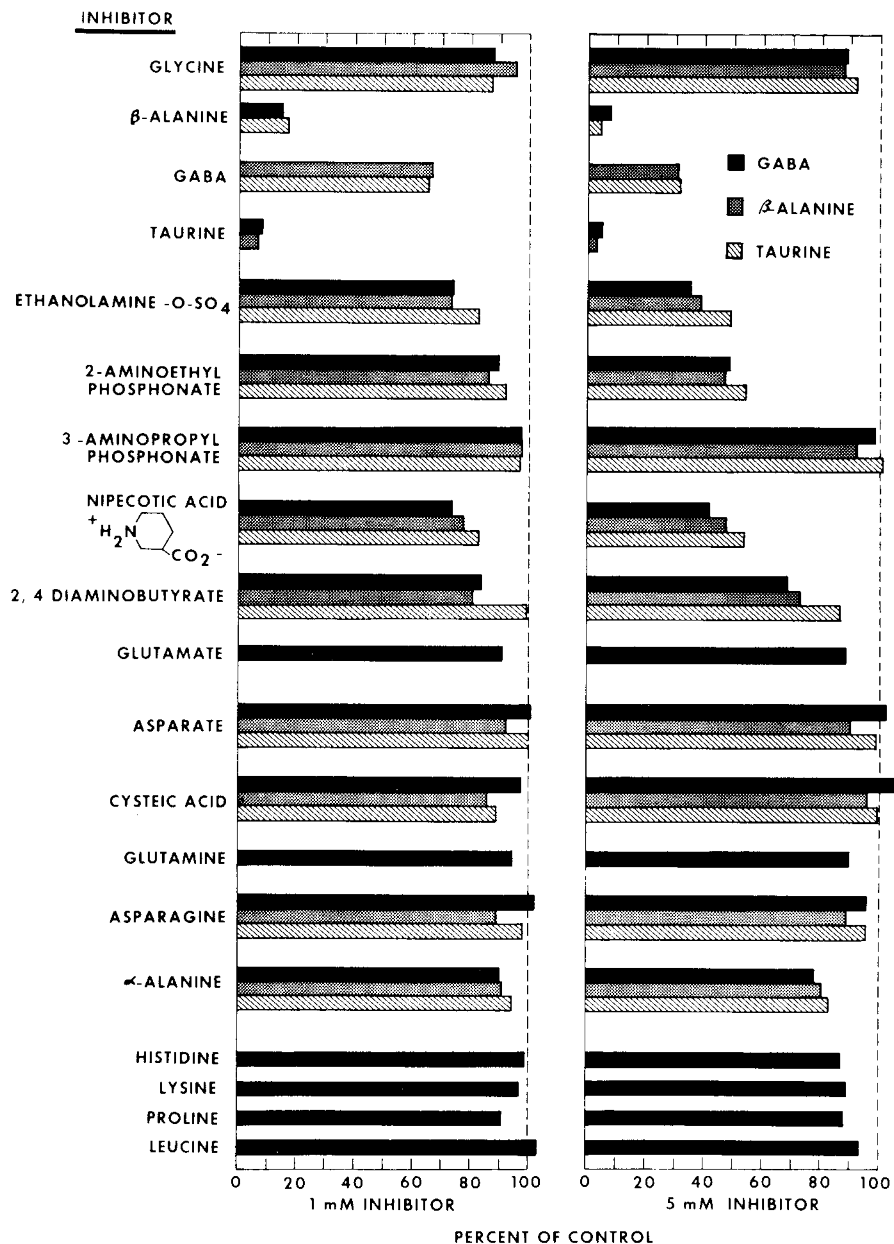

FIG. 8. **Inhibition of taurine, β-alanine, and GABA transport by structural analogs and other amino acids.** To make substrate concentrations kinetically equivalent, a concentration equal to the K_m for each substrate was used, *i.e.* 30 μM taurine, 80 μM β-alanine, and 700 μM GABA. Inhibitors were tested at two concentrations (1 mM, *left panel*; 5 mM, *right panel*) and were added at the same time as the substrates.

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DISCUSSION

The necessity of removing neurotransmitters from the extracellular space to limit their effects in the central nervous system has been widely recognized. The discovery and localization of high affinity GABA transport in synaptosomes focused attention on that system and it is now widely accepted that the high affinity GABA system serves to terminate synaptic transmission (8). The strong emphasis on that high affinity system left the occurrence and possible physiological function of low affinity GABA transport in brain an enigma.

GABA transport by brain slices and other preparations has been studied under conditions in which low affinity GABA transport should be an important contributor to total GABA uptake. However, the exact contribution of low affinity transport to total uptake is difficult to evaluate in these studies (8). More exact study of this transport mechanism would be possible in a homogeneous population of cells in which the ambiguities of diffusion barriers and loss of cell integrity were eliminated. A number of single cell colonies were isolated from an ethyl nitrosourea-induced mixed glioma (14) which contained multiple transport systems for GABA. One of these, LRM55, was found to possess only low but no high affinity transport for GABA and only high but no low affinity transport for the β-amino acids, taurine and β-alanine. This cell line provides an opportunity to study unambiguously the properties of these transport systems.

The results presented in this paper demonstrate that a single transport system carries out both low affinity transport of GABA and high affinity transport of the β-amino acids, taurine and β-alanine. The apparent Michaelis constant for the low affinity GABA system is 700 μM and is well within the range of reported values for low affinity GABA transport (310 to 1620 μM; see Ref. 8). The apparent Michaelis constant for the high affinity transport of taurine is 30 μM. This value is similar to most reported values for high affinity taurine transport (10 to 60 μM) (1, 2, 4–7). Hruska *et al.* (3), however, have recently reported a high affinity taurine transport system in

rat brain synaptosomes with an apparent K_m of 3.2 μM. The apparent Michaelis constant for transport of β-alanine by LRM55 cells is 79 μM. The evidence that strongly supports the hypothesis that all three of these amino acids are transported by means of a single transport system is found in Table I and Figs. 7 and 8. Fig. 7 shows that each of these amino acids competitively inhibits the transports of either of the other two amino acids. The similarities of the apparent values for K_m and K_i reported in Table I also suggest that the transport of all three of these amino acids is mediated by a single carrier. Additional evidence supporting the hypothesis is that all three of the amino acids show the same susceptibility to inhibition by a variety of analogs and other amino acids (see Fig. 8). Furthermore, the transport of taurine and GABA showed nearly identical sodium dependencies (see Fig. 6).

Low affinity GABA transport has not been associated previously with high affinity β-amino acid transport. Extensive studies of amino acid transport in brain slices under low affinity conditions by Lajtha and co-workers (*i.e.* 0.4-mm-thick slices and millimolar GABA concentrations) led them to conclude that GABA was transported by a separate system from those for the α-amino acids (32). However, as they pointed out, they made no attempt to study β-amino acid transport in detail or to relate GABA transport to β-amino acid transport. The very small amount of data available from their studies is consistent with the idea that low affinity GABA transport and β-amino acid transport occur by means of a common system in brain slices (33, 34). Our results are also consistent with other studies of high affinity taurine transport. In these cases, GABA was reportedly a relatively weak inhibitor of taurine uptake (1, 3, 4, 6, 7). However, the effects of GABA were not characterized kinetically.

The relationships between β-amino acid transport and GABA transport *in vivo* are not limited to the one described in this paper, however. β-alanine has previously been reported to be a potent inhibitor of high affinity GABA transport by glia cells in rat sensory ganglion and superior cervical ganglion (20, 24, 35). However, high affinity GABA uptake by cultured astrocytes and glia cells in rat retina were not strongly inhibited by β-alanine (28, 36). Thus, β-alanine and GABA might share either high or low affinity GABA transport systems in different tissue preparations.

The apparent identity of low affinity GABA transport with high affinity β-amino acid transport helps to clarify the possible physiological roles of low affinity GABA transport. The possibility that low affinity GABA transport might be important in rapidly lowering extracellular GABA levels after synaptic release has been discussed (8). In this scheme, low affinity transport would be most active immediately following release when the extracellular level of GABA was highest and high affinity transport would be responsible for reducing extracellular GABA to very low levels. However, the finding that high and low affinity GABA transport systems have different distributions in brain suggests that this scheme is not correct (8, 11). Our results suggest that the presence of low affinity GABA transport in various brain preparations is an inescapable accompaniment to high affinity β-amino acid transport. This appears to be the case in brain slices for example. Only low affinity GABA uptake is observed in thick (0.4-mm slices) but not in smaller prisms (0.1 × 0.1 × 2 mm) (10). Also taurine and β-alanine uptake are greater in thick than in small slices (37). These changes in uptake with slice size have been attributed to increased survival of glial elements in large slices and hence, increased uptake by glia (10, 37, 38). Thus low affinity GABA uptake in thick slices is probably carried out by the β-amino acid transport system in glia. It is doubtful that transport systems for α-amino acids contribute appreciably to low affinity GABA transport since a variety of α-amino acids which are representative of the various transport groups had little effect on GABA transport in this (Fig. 9) or other studies (32). Thus, the widespread occurrence of taurine and taurine transport systems may provide an explanation for occurrence of low affinity GABA transport and it seems likely that there is no specific low affinity GABA transport system with a special function in the neurochemical peregrinations of GABA.

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