Active transport of γ -aminobutyric acid and glycine into synaptic vesicles

(neurotransmitter/Mg-activating ATPase/proton gradient/brain/spinal cord)

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ABSTRACT Although γ -aminobutyric acid (GABA) and glycine are recognized as major amino acid inhibitory neurotransmitters in the central nervous system, their storage is poorly understood. In this study we have characterized vesicular GABA and glycine uptakes in the cerebrum and spinal cord, respectively. We present evidence that GABA and glycine are each taken up into isolated synaptic vesicles in an ATP-dependent manner and that the uptake is driven by an electrochemical proton gradient. Uptake for both amino acids exhibited kinetics with low affinity (K_m in the millimolar range) similar to vesicular glutamate uptake. The ATP-dependent GABA uptake was not inhibited by the putative amino acid neurotransmitters glycine, taurine, glutamate, or aspartate or by GABA analogs, agonists, and antagonists. Similarly, ATP-dependent glycine uptake was hardly affected by GABA, taurine, glutamate, or aspartate or by glycine analogs or antagonists. The GABA uptake was not affected by chloride, which is in contrast to the uptake of the excitatory neurotransmitter glutamate, whereas the glycine uptake was slightly stimulated by low concentrations of chloride. Tissue distribution studies indicate that the vesicular uptake systems for GABA, glycine, and glutamate are distributed in different proportions in the cerebrum and spinal cord. These results suggest that the vesicular uptake systems for GABA, glycine, and glutamate are distinct from each other.

γ -Aminobutyric acid (GABA) and glycine are the major inhibitory neurotransmitters in the vertebrate central nervous system (1, 2). Recently, the primary structures of GABA_A and glycine receptors have been deduced; the subunits of these receptors have been shown to have substantial sequence homology, particularly in the region thought to be involved in conducting chloride (3). GABA and glycine are released upon membrane depolarization, both in a calcium-dependent manner (4–6) and in a calcium-independent manner (4, 7). Recent evidence indicates that the calcium-dependent release of GABA originates from a non-cytoplasmic compartment (8). There are also observations indicating that GABA and glycine are concentrated in distinct nerve terminals (9, 10). However, localization of endogenous amino acids in synaptic vesicles has not been clearly demonstrated, either with intact tissues or isolated vesicle preparations. In addition, there has been little study on the vesicular GABA and glycine uptake processes. We have previously provided evidence that glutamate is taken up into synaptic vesicles by a proton-motive force generated by a proton-pump ATPase in the vesicle (11–13). In this communication, we have studied vesicular GABA and glycine uptake, using a synaptic vesicle preparation different from that previously used for vesicular glutamate uptake. We provide evidence that suggests that GABA and glycine are

also accumulated into synaptic vesicles in an ATP-dependent manner and that their uptake is driven by an electrochemical proton gradient. We have characterized these uptake systems with respect to sensitivity to chloride and the specificity of the transporter. Preliminary accounts of this work have been reported in abstract form (14, 15).

EXPERIMENTAL PROCEDURES

Materials. All GABA and glycine analogs were from Sigma or Aldrich. 4-Amino-*n*-[2,3-³H]butyric acid (50 Ci/mmol; 1 Ci = 37 GBq), [2-³H]glycine (19 Ci/mmol), and other tritiated amino acids tested for uptake were obtained from Amersham.

Preparation of Synaptic Vesicles. Synaptic vesicles were prepared from 30-day-old rat cerebrum and spinal cord by a modification of the method of Kish and Ueda (16). Cerebrum (6 g) or spinal cord (18 g) was homogenized in 10 volumes of solution A [0.32 M sucrose/0.5 mM Ca(OAc)₂/1 mM Mg(OAc)₂/1 mM NaHCO₃], and the homogenate was centrifuged at $12,100 \times g_{\max}$ for 20 min. The pellet was resuspended with a teflon rod in 20 volumes of ice-cold lysing solution (6 mM Tris maleate, pH 8.1) for 45 min, and then the suspension was centrifuged for 15 min at $43,000 \times g_{\max}$. The resulting supernatant was centrifuged at $200,000 \times g_{\max}$ for 60 min. The crude vesicle pellets were resuspended by homogenization in 25 ml of 25% Percoll/0.25 M sucrose. The suspensions were centrifuged at $75,500 \times g_{\max}$ for 60 min. The vesicle fraction (the top 4 ml of the gradient containing diffuse layers above a distinct yellowish membrane band, with an approximate density of 1.036 g/ml as determined by density marker beads) was removed from the resulting gradient by using a peristaltic pump and was stored frozen under liquid nitrogen until use.

For some experiments a modified method was used to prepare synaptic vesicles from the spinal cord. Heavily myelinated tissues yield synaptic vesicles of lower uptake activity. Removal of the myelin prior to lysis was found to yield purer preparations. The spinal cord (18 g) was homogenized in 10 volumes of solution A. Aliquots (30 ml) were layered over 0.8 M sucrose (30 ml) and centrifuged at $200,000 \times g_{\max}$ for 120 min. The supernatant above the pellet, including accumulated myelin at the 0.32/0.8 M sucrose interface, was carefully removed by suction. The pellet was then suspended in 20 volumes of lysing solution. The remainder of the steps were the same as described above.

Protein was determined by the method of Lowry *et al.* (17) with bovine serum albumin as the standard.

Assay for Vesicular Amino Acid Uptake. The uptake of GABA, glycine, glutamate, phenylalanine, leucine, histidine, and proline was assayed as described for glutamate (11) with a slight modification: the standard sucrose-based uptake

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Abbreviations: FCCP, carbonylcyanide *p*-trifluoromethoxy-phenylhydrazide; GABA, γ -aminobutyric acid.

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medium (final volume, 100 μ l) contained 0.25 M sucrose, 4 mM MgSO_4 , 5 mM Tris maleate (pH 7.4), and synaptic vesicles (100 or 150 μ g of protein). For time-course studies, after synaptic vesicles in the uptake medium (80 μ l) were incubated for 2 min at 30°C, a premixed solution of tritiated amino acid (final concentration, 150 μ M; 0.13 Ci/mmol) and ATP (final concentration, 10 mM; neutralized with Tris base) was added (20 μ l), and the entire mixture was further incubated for the required time at 30°C. For other studies, no preincubation was performed; all assay components were mixed and then incubated for 10 min. Uptake assays were generally carried out in duplicate, and the mean and range of duplicate determinations are presented. Uptake was linear over the protein concentration ranges 50–200 μ g of protein per 0.1 ml and 50–150 μ g of protein per 0.1 ml for GABA and glycine, respectively.

Agents with minimal water solubility were dissolved in dimethyl sulfoxide [carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), oligomycin, muscimol, baclofen, and clonazepam] or ethanol (nigericin). In the experiments where the effect of these reagents was examined, control assay mixtures contained equal amounts of dimethyl sulfoxide (generally 0.5%) or ethanol (generally 0.2%). Dimethyl sulfoxide at this concentration had no effect on the vesicular uptake of glutamate, GABA, or glycine, while ethanol decreased uptake slightly.

Assay for Marker Enzymes. NADPH-cytochrome-*c* reductase and cytochrome-*c* oxidase were assayed by the methods of Omura and Takesue (18) and Sottocasa *et al.* (19), respectively. Na^+/K^+ -transporting ATPase was assayed in the solutions of Medzihradsky *et al.* (20), with liberated ^{32}P determined by the method of Nelson (21).

RESULTS

Time-Course Studies of Vesicular GABA and Glycine Uptake.

Fig. 1 shows the time courses of GABA uptake into cerebral synaptic vesicles (Fig. 1A) and glycine uptake into spinal cord synaptic vesicles (Fig. 1B). Both GABA and glycine uptakes were substantially stimulated by ATP throughout the entire period tested; GABA uptake was stimulated 5-fold, and glycine uptake was stimulated 3.5-fold at 5 min. The maximum levels of uptake were attained at ≈ 10 min.

Effect of ATPase Inhibitors and Electrochemical Proton-Gradient Dissipaters. The ATP-dependent uptakes of both GABA and glycine were inhibited by *N*-ethylmaleimide and trimethyltin anion (Tables 1 and 2). These agents are known to inhibit the proton-pump ATPase, which is coupled to catecholamine uptake in the chromaffin granule membrane (22, 23) and to glutamate uptake in the synaptic vesicle membrane (12). In contrast, both GABA and glycine were unaffected by the Na^+/K^+ -ATPase inhibitor ouabain and the mitochondrial proton-pumping-ATPase inhibitor oligomycin (Tables 1 and 2). The proton ionophore FCCP inhibited the ATP-dependent GABA and glycine uptake activities (Fig. 1). Likewise, the K^+/H^+ -exchanging ionophore nigericin (in the presence of K^+) and ammonium ions both reduced the ATP-dependent GABA and glycine uptakes (Tables 1 and 2). Ammonium ions produce ammonia, which diffuses into the vesicle and neutralizes internal protons. Moreover, adenosine 5'-[β , γ -methylene]triphosphate, an unhydrolyzable analog of ATP, did not support either the vesicular GABA or glycine uptake (Fig. 1). These results indicate that the ATP-dependent GABA and glycine uptakes are driven by an electrochemical proton gradient generated by a proton-pump ATPase in the synaptic vesicle membrane.

Effect of Chloride on Vesicular GABA and Glycine Uptake. In view of the observation that ATP-dependent vesicular glutamate is markedly stimulated by low millimolar concentrations of chloride (12), we examined the effect of chloride

FIG. 1. Time course of GABA and glycine uptakes into synaptic vesicles. (A) Cortical synaptic vesicles (150 μ g) were incubated at 30°C in the absence of ATP, in the presence of ATP (10 mM), in the presence of ATP (10 mM) and FCCP (10 μ M), and in the presence of adenosine 5'-[β , γ -methylene]triphosphate (AMP-PCP; 2 mM) in the standard assay medium with 150 μ M tritiated GABA. (B) Spinal cord synaptic vesicles (100 μ g) were incubated with 150 μ M tritiated glycine under the same conditions as described in A. The data represent the mean and range of duplicate determinations.

on the uptake of GABA and glycine into synaptic vesicles. Chloride exhibited no significant specific effect on GABA uptake (Fig. 2A). In contrast, glycine uptake (Fig. 2B) was stimulated slightly by low millimolar concentrations of chloride (e.g., 10 mM) but not by the impermeant monovalent anion isethionate. However, this stimulatory effect of chloride is not as large as its effect on glutamate uptake (12). These results indicate that the vesicular GABA and glycine uptake systems are different in response to chloride from the vesicular glutamate uptake system.

Structural Requirements for Vesicular Uptake by GABA and Glycine Translocators. In order to examine the specificity of the vesicular GABA and glycine translocators, various GABA and glycine analogs, agonists, and antagonists were tested for their ability to compete for vesicular uptake of GABA (Table 1) and glycine (Table 2). 2,4-Diaminobutyrate, nipecotic acid, and isonipecotic acid, which have been shown to interact with the Na^+ -dependent GABA uptake system in the plasma membrane (24, 25), had no significant effect on vesicular GABA uptake. Other closely related structural analogs in which a functional group was modified or the carbon chain length was altered, such as hydroxybutyric acid lactone, γ -hydroxybutyrate, *n*-butyrate, D,L- β -aminobutyrate, 3-aminopropylphosphate, 3-amino-1-propanesulfonic acid, 2,3-piperidinedicarboxylic acid, γ -aminobutyryl-L-histidine, β -alanine, and taurine, also showed little or no interaction with the vesicular GABA uptake system. Importantly, the putative amino acid neurotransmitters glutamate, aspartate, and glycine did not interfere with vesicular GABA uptake. Moreover, the GABA_A receptor-agonist muscimol, the GABA_B receptor agonist baclofen, the GABA_A antagonist bicuculline, and other agents known to interact with the GABA_A receptor complex such as clonazepam and picro-

Table 1. Effects of GABA analogs, agonists, antagonists, and ATPase inhibitors on the vesicular uptake of GABA

Test agents	Conc.*	GABA uptake, [†] % of control
Analog (5 mM)		
None (control)		100
γ -Aminobutyric acid		21 $\pm$ 3
2,4-Aminobutyric acid		115 $\pm$ 3
Nipecotic acid		89 $\pm$ 3
Isonipecotic acid		93 $\pm$ 3
Hydroxybutyric acid		
lactone		97 $\pm$ 2
γ -Hydroxybutyrate		87 $\pm$ 3
<i>n</i> -Butyric acid		77 $\pm$ 5
D,L- β -Aminobutyric acid		80 $\pm$ 6
3-Aminopropylphosphoric acid		89 $\pm$ 6
3-Amino-1-propanesulfonic acid		77 $\pm$ 4
2,3-Piperidinedicarboxylic acid		84 $\pm$ 5
γ -Aminobutyryl-L-histidine		91 $\pm$ 2
β -Alanine		84 $\pm$ 3
Taurine		102 $\pm$ 0
Glutamine		99 $\pm$ 3
Aspartate		92 $\pm$ 1
Glutamate		98 $\pm$ 5
Glycine		99 $\pm$ 6
Agonist/antagonist		
Muscimol	10 μ M	100 $\pm$ 3
	100 μ M	121 $\pm$ 2
Baclofen	10 μ M	100 $\pm$ 6
	100 μ M	99 $\pm$ 18
Bicuculline	10 μ M	110 $\pm$ 0
	100 μ M	96 $\pm$ 7
Clonazepam	10 μ M	104 $\pm$ 9
	100 μ M	107 $\pm$ 25
Picrotoxin	10 μ M	99 $\pm$ 3
	100 μ M	79 $\pm$ 7
ATPase inhibitor		
Ouabain	200 μ M	108 $\pm$ 8
Oligomycin	5 μ g/ml	143 $\pm$ 9
Trimethyltin chloride	100 μ M	50 $\pm$ 10
<i>N</i> -Methylmaleimide	100 μ M	0 [‡]
pH gradient dissipator		
K ₂ SO ₄	20 mM	65 $\pm$ 3
(NH ₄) ₂ SO ₄	20 mM	0 [‡]
Control		100 [§]
Nigericin	20 μ M	67 $\pm$ 5
Nigericin + K ⁺ ¶	20 μ M	28 $\pm$ 11

Synaptic vesicles (150 μ g of protein) prepared from the rat cerebrum were incubated with 150 μ M [³H]GABA in the presence or absence (control only) of ATP under standard vesicular uptake conditions, except for the addition of test agents. Radioactivity remaining on the filter in the absence of ATP in the control experiment has been subtracted from values observed in the presence of ATP. The values represent ATP-dependent uptake, expressed as % of control.

*Test agents were 5 mM except where otherwise indicated.

[†]A composite of data from several experiments. Values presented for each test agent are % of control from each individual experiment. A representative control value from one of those experiments was 3221 $\pm$ 301 dpm.

[‡]Uptake in the presence of test agent and ATP was lower than the control in the absence of ATP.

[§]Control value obtained in the presence of 0.1% ethanol was 2759 $\pm$ 125 dpm.

[¶]K⁺ was added at a concentration of 5 mM as the aspartate salt; 5 mM potassium aspartate alone had no effect on the uptake.

toxin, all showed lack of interaction with the vesicular GABA uptake system.

Table 2. Effects of glycine analogs, antagonists, and ATPase inhibitors on the vesicular uptake of glycine

Test agents	Conc.*	Glycine uptake, [†] % of control
Analog		
None (control)		100
Glycine		33 $\pm$ 2
Alanine		105 $\pm$ 11
<i>n</i> -Methylglycine		108 $\pm$ 9
Serine		106 $\pm$ 1
Taurine		100 $\pm$ 1
<i>N</i> -Methylalanine		101 $\pm$ 1
β -Alanine		80 $\pm$ 3
Nipecotic acid		96 $\pm$ 2
Glutamine		102 $\pm$ 3
Aspartate		101 $\pm$ 1
Glutamate		90 $\pm$ 4
γ -Aminobutyric acid		85 $\pm$ 6
Antagonist		
Strychnine	10 μ M	100 $\pm$ 6
	2.5 mM	99 $\pm$ 18
Picrotoxin	10 μ M	100 $\pm$ 3
	2.5 mM	121 $\pm$ 2
ATPase inhibitor		
Ouabain	200 μ M	105 $\pm$ 5
Oligomycin	5 μ g/ml	111 $\pm$ 1
Trimethyltin chloride	100 μ M	57 $\pm$ 4
<i>N</i> -Methylmaleimide	100 μ M	0
pH gradient dissipator		
K ₂ SO ₄	20 mM	65 $\pm$ 7
(NH ₄) ₂ SO ₄	20 mM	30 $\pm$ 12
Control		100 [§]
Nigericin	20 μ M	67 $\pm$ 5
Nigericin + K ⁺ ¶	20 μ M	4 $\pm$ 12

Synaptic vesicles (150 μ g of protein) prepared from the rat spinal cord (by the modified procedure described) were incubated with 150 μ M [³H]glycine in the presence or absence (control only) of ATP under standard vesicular uptake conditions, except for the addition of test agents. Radioactivity remaining on the filter in the absence of ATP in the control experiment has been subtracted from values observed in the presence of ATP. The values represent ATP-dependent uptake, expressed as % of control.

*Test agents were 5 mM except where otherwise indicated.

[†]A composite of data from several experiments. Values presented for each test agent are % of control from each individual experiment. A representative control value from one of those experiments was 2618 $\pm$ 136 dpm.

[‡]Uptake in the presence of test agent and ATP was lower than the control in the absence of ATP.

[§]Control value obtained in the presence of 0.1% ethanol was 867 $\pm$ 40 dpm.

[¶]K⁺ was added at a concentration of 5 mM as the aspartate salt; 5 mM potassium aspartate alone had no effect on the uptake.

Likewise, vesicular glycine uptake was hardly affected by most of the structural analogs of glycine tested; these included alanine, *N*-methylglycine (sarcosine), serine, taurine, and *N*-methylalanine. β -Alanine, which is known to interact with the glycine receptor (26), caused 20% inhibition. However, the glycine receptor antagonists strychnine and picrotoxin had no effect on vesicular glycine uptake. Moreover, GABA, glutamate, and aspartate did not interfere with the vesicular glycine uptake system. These results suggest that the vesicular GABA and glycine uptake systems are distinct from each other as well as from the vesicular glutamate uptake system.

Tissue and Subcellular Distributions of ATP-Dependent GABA, Glycine, and Glutamate Uptake Activities. The vesicular GABA and glycine uptake activities were both higher in the spinal cord than in the cerebrum; in particular, the glycine uptake activity was 10-fold higher in the spinal cord (22.8

FIG. 2. Effect of varying the concentration of chloride or isethionate on the ATP-dependent uptake of GABA and glycine. GABA uptake (A) and glycine uptake (B) were determined by incubating 150 μ g of synaptic vesicles for 10 min in the presence or absence of 10 mM ATP in the standard uptake medium. The ATP-dependent GABA and glycine uptakes in the absence of test agents were 12 and 18 pmol, respectively, and each was expressed as 100%.

versus 2.1 pmol/mg of protein) (Table 3). This is in contrast to the glutamate uptake system; the specific uptake activity of glutamate was about 2 times higher in the cerebrum than in the spinal cord (506 versus 249 pmol/mg of protein). Table 3 also shows that the relative uptake activities GABA/glutamate, glycine/glutamate, and glycine/GABA are substantially different between these regions of the central nervous system.

To determine the nervous system specificity of the ATP-dependent uptake, we have examined other tissues such as kidney, heart, liver, lung, and spleen for ATP-dependent uptake of these amino acids. Subcellular fractions were prepared in a manner identical to the synaptic vesicle preparation from the cerebrum. No ATP-dependent uptake was detected (data not shown).

Table 3. Vesicular amino acid neurotransmitter uptakes in the cerebrum and spinal cord

Neurotransmitter	Uptake activity, pmol/150 μ g of protein	
	Cerebrum*	Spinal cord†
Glutamate	506.4 $\pm$ 75.8	249.0 $\pm$ 6.4
GABA	16.3 $\pm$ 1.4	29.6 $\pm$ 2.8
Glycine	2.1 $\pm$ 3.7	22.8 $\pm$ 1.0
GABA/glutamate	0.03	0.12
Glycine/glutamate	0.00	0.09

Synaptic vesicles were prepared from the rat cerebrum and spinal cord, and aliquots (150 μ g of protein) were assayed for ATP-dependent uptake of [3 H]GABA, [3 H]glycine, or [3 H]glutamate at a concentration of 150 μ M, under standard uptake conditions.

*Values are means $\pm$ SD of the experiments using three preparations.

†Values are means and range of the experiments using two preparations.

Although we have used purified synaptic vesicle fractions in this study, to ensure that the ATP-dependent uptake we have observed represents a synaptic vesicular process, we have analyzed the various subcellular fractions for ATP-dependent uptake of GABA, glycine, and glutamate. These studies showed essentially no ATP-dependent uptake of GABA, glycine, or glutamate into the microsomal fraction, myelin fraction, or the combined mitochondrial and plasma membrane fraction (data not shown).

Marker enzyme analysis indicated that there was very little activity (<10%) of Na^+/K^+ -ATPase and cytochrome-c oxidase in the synaptic vesicle fraction either from the cerebrum or spinal cord, compared to the corresponding activities in the lysed synaptosomal membrane fraction ($43,000 \times g_{\text{max}}$ pellet), which contains mitochondria and synaptic plasma membranes. These results suggest that the synaptic vesicular fraction, prepared from either the cerebrum or spinal cord, has little plasma membrane or mitochondrion contamination. NADPH-cytochrome-c₂ reductase activity in the synaptic vesicle fraction was $\approx 50\%$ of that in the crude microsomal fraction. Although this suggests a significant contamination of microsomes in the synaptic vesicle preparation, it might be noted that relatively high cytochrome-c reductase activity has been found in vesicle preparations extensively purified by immunoprecipitation with antibodies to the vesicular protein synapsin I (11). This raises the possibility that NADPH-cytochrome-c₂ reductase or a similar electron-transfer enzyme might be present in the synaptic vesicle membrane. Compatible with this notion, chromaffin granule membranes have been shown to have an electron-transfer system capable of reducing cytochrome c (27). It is also important to point out that no significant ATP-dependent uptake was found in the microsomal fraction. Therefore, even if microsomal contamination remains in the vesicle fraction, it would not be expected to contribute to uptake.

DISCUSSION

This study provides evidence that GABA and glycine are each taken up into a purified synaptic vesicle preparation in an ATP-dependent manner. The ATP-dependent GABA uptake is in accord with the observation made earlier by Phillippu and Matthaei (28) using a crude synaptic vesicle preparation.

We have shown in this study that the ATP-dependent GABA and glycine uptakes are inhibited by either FCCP, nigericin (in the presence of K^+), or ammonium. This suggests that the vesicular GABA uptake and glycine uptake are both driven by an electrochemical proton gradient, generated by a proton-pump ATPase in the synaptic vesicle membrane. Thus, the nature of the driving force for the vesicular uptake of the inhibitory amino acid neurotransmitters is likely to be very similar to that involved (i) in the vesicular uptake of glutamate (11, 12), (ii) in catecholamine uptake into chromaffin granules (22, 23) and brain synaptic vesicles (29), or (iii) in acetylcholine uptake into *Torpedo* synaptic vesicles (30). In contrast, other amino acids such as phenylalanine, leucine, histidine, and proline showed no ATP-dependent uptake into synaptic vesicles prepared from the cerebrum (data not shown). There has been no good evidence for a neurotransmitter role for these amino acids. These lines of evidence all suggest that vesicular uptakes of most if not all of the low molecular weight neurotransmitter substances share a common mechanism.

Neither the GABA nor glycine uptake into synaptic vesicles is greatly stimulated by low millimolar chloride concentrations. This is in great contrast to the vesicular glutamate uptake, which is markedly potentiated by low concentrations of chloride in various synaptic vesicle preparations (12, 16, 31). The vesicular GABA and glycine uptake systems also

differ in sensitivity to chloride from the Na⁺-dependent GABA and glycine uptake systems in the plasma membrane, both of which require chloride (32, 33).

The vesicular GABA and glycine uptake systems differ from each other with respect to substrate specificity. Their putative translocators have each exhibited narrow specificity for their ligands. Thus, vesicular GABA and glycine uptakes were not affected by GABA and glycine analogs, agonists or antagonists, nor by compounds known to interact with the Na⁺ gradient-dependent plasma membrane uptake systems for these amino acids. This suggests that these translocators are also distinct from their postsynaptic receptors as well as from the Na⁺ gradient-dependent transporters in the plasma membrane responsible for cellular uptake. Compatible with this notion are the kinetic data (not shown), which indicate that these uptake systems have much lower affinity for GABA and glycine than their receptors (34, 35) and Na⁺-dependent plasma membrane uptake systems (32, 36, 37). For vesicular uptake, the *K_m* values for GABA and glycine were determined to be 6.5 and 8.9 mM, respectively. The *V_{max}* values of their uptake systems were 2.6 and 3.2 nmol/min per mg, respectively. These vesicular amino acid uptake systems have strict structural requirements for their ligands. Thus, the γ -amino group and the α -carboxyl group as well as the four-carbon skeleton of GABA are all essential for recognition by the vesicular GABA translocator. Likewise, the primary α -amino group, the α -carboxyl group, and the two-carbon skeleton of glycine are all required for interaction with the putative glycine translocator.

It has been shown that the vesicular glutamate translocator is highly specific for glutamate and imposes strict structural requirements on its ligand (12). Based upon these unique properties of the glutamate transport system, it has been proposed (13) that the presence of the specific ATP-dependent vesicular glutamate translocator in the synaptic vesicles in a given nerve ending determines whether glutamate serves as a neurotransmitter. The same notion may apply to glycine, another common amino acid. In contrast, for GABA, both the synthetic enzyme glutamate decarboxylase and the vesicular GABA translocator would be necessary to render a neuron GABAergic.

The tissue distribution studies indicate that the vesicular GABA, glycine, and glutamate uptake systems are distributed in the cerebrum and spinal cord in different proportions. Of particular interest is that the vesicular glycine uptake activity in the spinal cord is 10 times higher than in the cerebrum. This uneven distribution of the glycine uptake system is in accord with the high concentration of glycine (2), glycine receptors (35), and the Na⁺-dependent high-affinity glycine uptake system (37, 38) in the spinal cord. Moreover, the differential distribution of these vesicular uptake systems is consistent with the observations that GABA, glycine, and glutamate are accumulated in different populations of nerve endings (9, 10, 39). These lines of evidence are compatible with the notion that the vesicular uptake system for GABA, glycine, and glutamate are present in three functionally different types of nerve terminals and support the neurotransmitter role of these amino acids.

Note Added in Proof. After submission of the original manuscript, three papers on vesicular GABA uptake have appeared (40–42).

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