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GABA and Glycine in Synaptic Vesicles: Storage and Transport Characteristics

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Summary

γ-Aminobutyric acid (GABA) and glycine are major inhibitory neurotransmitters that are released from nerve terminals by exocytosis via synaptic vesicles. Here we report that synaptic vesicles immunoisolated from rat cerebral cortex contain high amounts of GABA in addition to glutamate. Synaptic vesicles from the rat medulla oblongata also contain glycine and exhibit a higher GABA and a lower glutamate concentration than cortical vesicles. No other amino acids were detected. In addition, the uptake activities of synaptic vesicles for GABA and glycine were compared. Both were very similar with respect to substrate affinity and specificity, bioenergetic properties, and regional distribution. We conclude that GABA, glycine, and glutamate are the only major amino acid neurotransmitters stored in synaptic vesicles and that GABA and glycine are transported by similar, if not identical, transporters.

Introduction

The amino acids glutamate, γ-aminobutyric acid (GABA), and glycine are regarded as the major neurotransmitters in the mammalian CNS. They are stored in high concentrations in presynaptic nerve terminals and are released into the synaptic cleft upon depolarization. After interaction with postsynaptic receptors, the amino acids are removed from the extracellular space by specific transporters of high affinity and high capacity located in the plasma membranes of the respective competent neurons and adjacent glial cells (for review see Ottersen and Storm-Mathisen, 1984; Kanner and Schuldiner, 1987; Nicholls, 1989; Nicholls and Attwell, 1990).

Whereas our understanding of amino acid receptors and plasma membrane transporters is progressing rapidly, the elements involved in the presynaptic compartmentalization of these transmitters are less well understood. In recent years, it has been established that synaptic vesicles possess transport activities for glutamate, GABA, and glycine (Disbrow et al., 1982; Naito and Ueda, 1983, 1985; Maycox et al., 1988; Hell et al., 1988; Fykse and Fonnum, 1988; Kish et al., 1989). The properties of these carriers are very different from those of the carriers present in the plasma membrane. Amino acid uptake by synaptic vesicles is driven by a proton electrochemical gradient, $\Delta\mu H^+$, generated by a vacuolar-type proton pump. This is in contrast to the Na^+ gradient–linked transport systems of the plasmalemma. Furthermore, the substrate affinities of the vesicular transporters are low (millimolar range; for review see Maycox et al., 1990). It is not clear how many different transporters exist in the vesicle membrane, and none of the responsible proteins have been identified.

The presence of specific transporters for amino acid neurotransmitters in the membranes of synaptic vesicles strongly supports the concept that these neurotransmitters are stored in synaptic vesicles and are released by exocytosis from this vesicular pool upon depolarization. This implies that synaptic vesicles in situ store amino acid neurotransmitters in high concentrations. Several attempts have been made to demonstrate the presence of amino acid neurotransmitters in synaptic vesicles (De Belleroche and Bradford, 1973; Kontro et al., 1980; Angel et al., 1983; Valcana et al., 1984; Riveros et al., 1986; Orrego et al., 1986; Villanueva and Orrego, 1988). De Belleroche and Bradford (1973) analyzed the amino acid content of a purified vesicle fraction after separating it from free amino acids by gel filtration and found sizeable pools of glutamate and taurine and lower amounts of GABA, aspartate, glutamine, serine, glycine, and alanine. These pools did not exchange with labeled standards added exogenously. However, in contrast to acetylcholine, these amino acids could not be released by osmotic rupture of the membranes. Using a very similar approach, Orrego and co-workers identified glutamate (Riveros et al., 1986), GABA (Orrego et al., 1986), and aspartate (Villanueva and Orrego, 1988). However, since neither the total amino acid composition nor the criteria for vesicle purity were published, these data are difficult to evaluate. Finally, several amino acids were detected by immunogold electron microscopy at high concentrations in nerve terminals (Storm-Mathisen et al., 1983). Although the spatial resolution in these studies does not normally allow any distinction between cytoplasmic and vesicular pools, more abundant labeling for glutamate was observed over clusters of synaptic vesicles than over the adjacent cytoplasm in a giant synapse of the lamprey (Storm-Mathisen and Ottersen, 1990).

Recently, we developed a novel immunopurification procedure for synaptic vesicles from the mammalian CNS, allowing the isolation of these organelles under mild conditions in less than 2 hr (Burger et al., 1989). We found that synaptic vesicles isolated from rat cerebral cortex contain high concentrations of glutamate when a proton electrochemical gradient is par-

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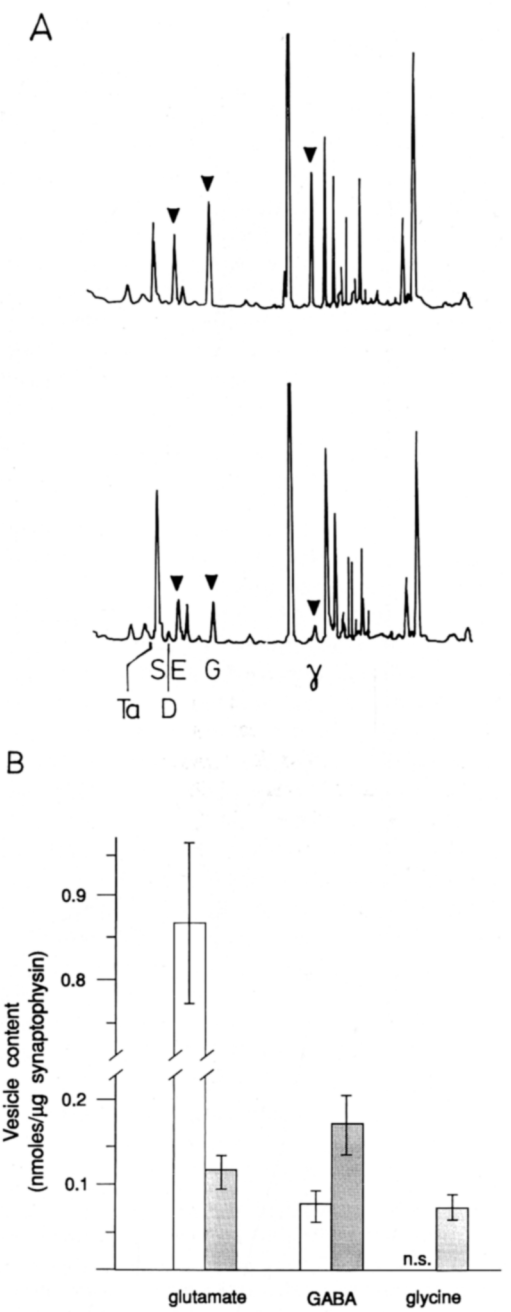

Figure 1. Amino Acid Content of Synaptic Vesicles

(A) Amino acid content of synaptic vesicles isolated from rat medulla oblongata, analyzed by reverse-phase HPLC (DABS-Cl procedure). Synaptic vesicles were immunoisolated using Eupergit C1Z beads coupled with anti-synaptophysin IgG at 0°C (upper trace) and 21°C (lower trace). The positions of amino acid standards are indicated by the single-letter code. Ta, taurine; γ, GABA. Arrowheads point to the positions of glutamate, glycine, and GABA. The additional peaks are due to byproducts of the derivatization reaction. Note that the serine concentration varied as a result of bleeding from the immunobeads.

(B) Comparison of the amino acid content of synaptic vesicles immunoisolated from rat cerebral cortex (open bars) and medulla oblongata (shaded bars). Synaptic vesicles were isolated at 0°C. All values were corrected for nonspecific adsorption as determined by isolating vesicles at 21°C (see [A] and text). The figure shows mean values (± SEM) of 3 independent and representative experiments, normalized to synaptophysin as vesicle marker (Jahn et al., 1985; Wiedenmann and Franke, 1985). n.s. indicates not significant.

tially maintained across the vesicle membrane during isolation. No other amino acids were detected in the vesicle fraction. We have now modified our isolation procedure to prevent the loss of stored solutes irrespective of the maintenance of the energy gradient. Our data show that in addition to glutamate, GABA and glycine, but no other amino acids, are stored in major amounts in synaptic vesicles and that the ratio between these transmitters varies among different brain regions. Furthermore, we provide evidence that GABA and glycine uptake is very similar, suggesting that both amino acids are transported by similar, if not identical, carriers in the vesicle membrane.

Results

GABA and Glycine Are Stored in Synaptic Vesicles in Varying Ratios

In our previous experiments, no evidence for amino acid storage, other than glutamate, was found when synaptic vesicles immunoisolated from rat cerebral cortex were analyzed for their amino acid content (Burger et al., 1989). In these experiments, the temperature during vesicle isolation varied between 6°C and 10°C, resulting in a loss of vesicular amino acid content unless an ATP-regenerating system was added. To eliminate content efflux more efficiently, we modified the preparation procedure by reducing the isolation temperature to 0°C. Control isolations with an equal amount of beads were performed at 6°C–10°C or at 21°C, in the presence or absence of the protonophore FCCP, and at 0°C using immunobeads coupled to bovine IgG.

Vesicle fractions isolated from rat cerebral cortex contained GABA and glutamate in amounts that were significantly enriched over control incubations. Vesicles immunoisolated in parallel from the rat medulla oblongata additionally contained glycine and exhibited different relative amounts of amino acids (Figure 1), with a lower glutamate and a higher GABA content compared with cortical vesicles. The enrichment of GABA, glutamate, and glycine (the latter only in the medulla oblongata) was observed in the absence as well as the presence of an ATP-regenerating system. All control experiments resulted in virtually identical traces, regardless of whether the isolation was performed at elevated temperature or in the presence or absence of FCCP, or whether bovine IgG beads were used (data not shown). Furthermore, the value obtained for glutamate in vesicles isolated from cerebral cortex (0.87 nmol per µg of synaptophysin) at 0°C without an ATP-regenerating system is very similar to that obtained with our previous method (Burger et al., 1989). Amino acid analysis using the OPA procedure yielded amino acid values very similar (less than 10%

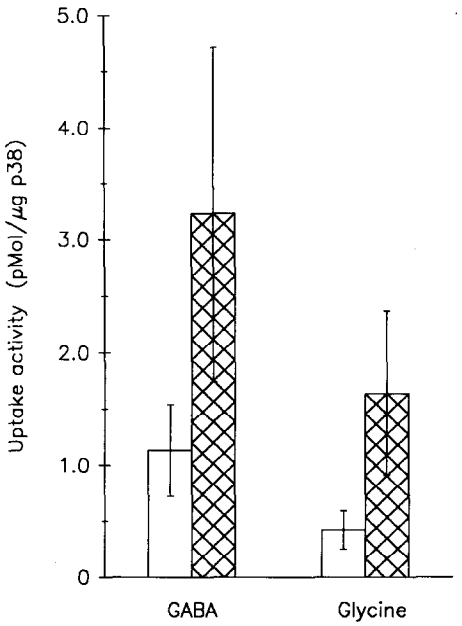

Figure 2. Comparison of the ATP-Dependent, FCCP-Sensitive Uptake Activities for [^{3}H]GABA and [^{3}H]Glycine in Synaptic Vesicles Isolated from Rat Cerebral Cortex and Medulla Oblongata

Cerebral cortex, open bars; medulla oblongata, hatched bars. Synaptic vesicles were immunoisolated at 10°C as described in Experimental Procedures. All values were normalized to the vesicle marker synaptophysin. The figure shows mean values (± SD) from 3 independent experiments.

variation) to those obtained with the DABS-Cl procedure.

Within the sensitivity limits of our methods, no enrichment of other amino acids, in particular taurine or aspartate, was observed. In addition, no glycine enrichment was detected in synaptic vesicles isolated from cerebral cortex. There was some nonspecific adsorption of aspartate and glycine to the carrier beads that was seen consistently in all control experiments (see Figure 1A). The detection threshold was therefore approximately 15 pmol per μg of synaptophysin for aspartate and 25 pmol per μg of synaptophysin for glycine. Despite this limitation, the data indicate that vesicles contain at least 50 times less aspartate than glutamate. This is in sharp contrast to the ratio of the two amino acids in brain homogenate (glutamate: aspartate 3:1; see Kontro et al., 1980; Burger et al., 1989) and indicates that aspartate does not play a major role as an exocytotic neurotransmitter in the mammalian cortex (see Discussion).

GABA and Glycine Uptake Occurs by a Similar Transport Activity in the Vesicle Membrane

In the following experiments, we analyzed the vesicular transport activities for GABA and glycine in synaptic vesicles from the cerebral cortex and the medulla oblongata. As shown in Figure 2, synaptic vesicles isolated from the cerebral cortex displayed uptake activity not only for GABA, but also for glycine. Glycine

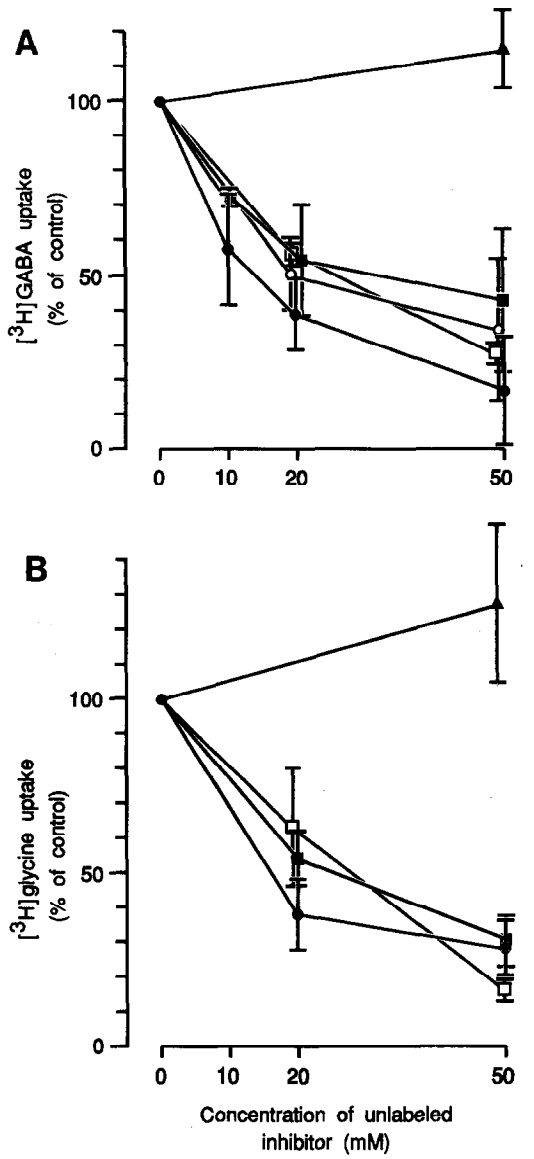

Figure 3. Inhibition of the ATP-Dependent, FCCP-Sensitive Uptake of [^{3}H]GABA and [^{3}H]Glycine by Increasing Concentrations of Unlabeled GABA, Glycine, β-Alanine, and δ-Aminovaleric Acid

GABA, closed circles; glycine, closed squares; β-alanine, open squares; δ-aminovaleric acid, open circles. For control, the effect of taurine (closed triangles), a structural analog of GABA, is shown. Synaptic vesicles were either isolated from cerebral cortex according to Hell et al. (1988) (A) or by immunoisolation from spinal cord (B). Results identical to those shown here were obtained for glycine uptake when synaptic vesicles were immunoisolated from cerebral cortex and for GABA uptake when synaptic vesicles were immunoisolated from spinal cord, ruling out any artifactual effect of the isolation procedure. The values were normalized to the activities observed under standard uptake conditions (0.3 mM GABA or glycine, respectively). The figure shows mean values (± SD) of 3 independent experiments.

uptake activity was approximately 60% lower than GABA uptake activity. It was dependent on the presence of ATP and was sensitive to FCCP (Table 1), indicating a proton gradient-driven uptake process. Analysis of synaptic vesicles from the medulla oblongata

Table 1. [^{3}H]Glycine Uptake by Synaptic Vesicles Isolated from Cerebral Cortex

Condition	[^{3}H]Glycine Uptake
ATP	290 ± 69
FCCP	148 ± 27
Control	129 ± 10

Isolation was according to Hell et al. (1988). All values are given in pmol per mg of protein (n = 3, ± SD).

Table 2. Effects of Cl^- and $(NH_4)_2SO_4$ on ATP-Dependent, FCCP-Sensitive [^{3}H]Glycine Uptake by Synaptic Vesicles Immunoisolated from Spinal Cord or Cerebral Cortex

Condition	[^{3}H]Glycine Uptake
No Cl^-	30 ± 20
4 mM Cl^- (standard conditions)	100
50 mM Cl^-	100 ± 3
150 mM Cl^-	90 ± 28
10 mM $(NH_4)_2SO_4$	30 ± 5

Isolation was according to Hell et al. (1988). Uptake was normalized to that observed under standard conditions (4 mM Cl^-, 0.3 mM glycine). The numbers are mean values (± SD) from 3 independent experiments.

revealed uptake activities for both GABA and glycine that were higher than those in the cerebral cortex, but the activity ratio was similar (Figure 2). These findings contrast with our content data for GABA and glycine in these two regions and suggest that GABA and glycine are transported by similar transporters. For further analysis, the properties of the two transport activities were compared in competition experiments. As shown in Figure 3A, the uptake of [^{3}H]GABA was inhibited by increasing concentrations of unlabeled GABA or glycine in a comparable manner, GABA being slightly more potent. Also, two structural analogs with a C3 and C5 backbone, β-alanine and δ-aminovaleric acid, respectively, inhibited GABA uptake with similar potency. Correspondingly, the uptake of [^{3}H]glycine was inhibited by unlabeled glycine and GABA in a similar manner, GABA again being slightly more potent (Figure 3B). No inhibition was observed when up to 50 mM taurine, a structural analog of GABA, was added (Figure 3). This profile was observed independently of the source of the vesicles, i.e., the cerebral cortex (Figure 3A), the spinal cord (Figure 3B), or the medulla oblongata (data not shown). It was also independent of the vesicle isolation procedure, since similar results were obtained with vesicles isolated according to either Hell et al. (1990) (Figure 3A; and data not shown), Kish and Ueda (1989) (data not shown), or the immunoisolation procedure described here (Figure 3B).

For further characterization, the bioenergetic properties of the uptake activities for [^{3}H]glycine and [^{3}H]GABA were compared. We have shown previously that GABA uptake is dependent on both components of the electrochemical proton gradient $\Delta\mu H^+$, the membrane potential $\Delta\psi$ and the proton concentration gradient ΔpH (Hell et al., 1990), in contrast to the purely potential-dependent glutamate uptake (Maycox et al., 1988). To test whether the bioenergetic characteristics of glycine and GABA uptake are indeed similar, we examined the effects of Cl^- and NH_4^+ on glycine uptake. With the aid of these ions it is possible to discriminate between the potential component $\Delta\psi$ and the pH component ΔpH of the electrochemical gradient. Increased Cl^- concentrations result in a gradual shift from $\Delta\psi$ to ΔpH while $\Delta\mu H^+$ remains constant (Maycox et al., 1988; Hell et al., 1990). As shown in Table 2, glycine uptake has a broad activity maximum with respect to Cl^- concentration and is reduced in the absence of Cl^-. This profile is similar to that of GABA uptake (Hell et al., 1990) and differs from that of glutamate uptake (Naito and Ueda, 1985; Maycox et al., 1988), which is greatly reduced at 150 mM KCl (at which $\Delta\mu H^+$ consists almost exclusively of ΔpH). Therefore, this indicates that both $\Delta\psi$ and ΔpH can act as the driving force for glycine uptake. For further characterization, the effect of 10 mM $(NH_4)_2SO_4$ was examined. $(NH_4)_2SO_4$ quantitatively dissipates ΔpH while leading to a compensatory increase of $\Delta\psi$ (Hell et al., 1990). Glycine uptake was strongly reduced by $(NH_4)_2SO_4$ at pH 7.4 (Table 2). This is similar to the effect of $(NH_4)_2SO_4$ on GABA uptake (Hell et al., 1990) and clearly different from the effect on glutamate uptake, which is not inhibited by $(NH_4)_2SO_4$ (Hell et al., 1990). Thus, the results obtained for vesicular glycine uptake are typical for a transporter dependent on both the membrane potential and the proton gradient.

Taken together, our data show that the vesicular transport activities for GABA and glycine have similar properties, both with respect to substrate specificity and with respect to their dependence on $\Delta\psi$ and ΔpH.

Discussion

In the present study we show that synaptic vesicles contain significant amounts of GABA and glycine when isolated under appropriate conditions. Our data show that the loss of GABA, glycine, and glutamate from synaptic vesicles can be prevented or at least slowed down when vesicle isolation is carried out at 0°C. A similar content preservation was observed when vesicles were loaded with amino acids by ATP-dependent transport in vitro and then maintained at 0°C in the presence of uncouplers of the energy gradient $\Delta\mu H^+$ (Hartinger and Jahn, unpublished data). This indicates that the losses observed at higher isolation temperatures are due to rapid efflux via reversed transport through the carriers in the vesicle membrane (see also below; Figure 4). In the experiments presented here it was essential to control the temperature during all steps, including centrifugation, requiring an elaborate experimental procedure.

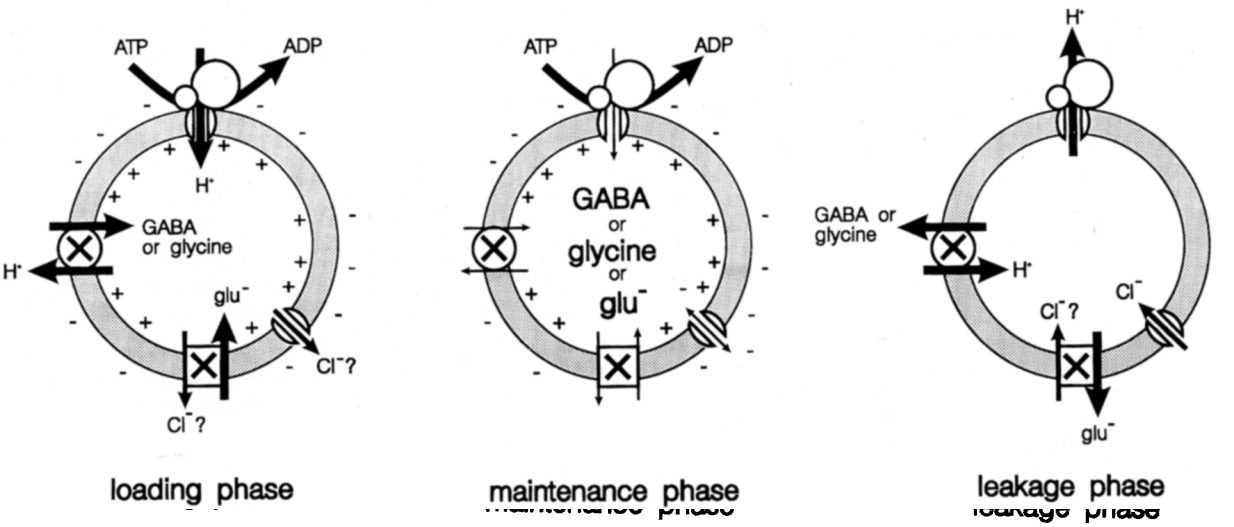

Figure 4. Model Summarizing Our Current View of Amino Acid Uptake and Storage by Synaptic Vesicles

For comparison, the transporters for glutamate and for GABA and glycine are drawn into the same vesicle. The coupling of glutamate uptake to Cl^- efflux either by direct (substoichiometric) counter-transport or by the action of the vesicular Cl^- channel is hypothetical (see Maycox et al., 1990, for discussion). glu^- indicates glutamate ions. Loading phase and maintenance phase depict normal conditions occurring in intact nerve terminals; leakage phase describes the solute movements under conditions of an inactive proton pump, e.g., resulting from energy depletion. See text for details.

The vesicle content for all amino acids was greatly reduced at slightly elevated isolation temperature (above 2°C) and reached background values at 6°C (unpublished data). This provides an explanation for the previous problems encountered in demonstrating convincingly the presence GABA and glycine in vesicle fractions. It further suggests that the association of amino acids with vesicle fractions reported in previous studies (see Introduction) does not represent vesicular content that would have been lost during the long isolation procedures. We cannnot exclude that even under our controlled conditions, some efflux of transmitter occurred. The actual vesicular content may therefore be higher than that found in our experiments, and an accurate estimate of the intravesicular concentration of these amino acids is not possible.

Despite these caveats, it is evident that the ratio of individual amino acids in synaptic vesicles varies between brain regions. Compared with the cerebral cortex, the vesicular glutamate content is greatly reduced and the GABA content is increased in the medulla oblongata, which agrees well with the distribution of the vesicular transporters (Fykse and Fonnum, 1989; and our own unpublished data). Also, glycine was found in medullar vesicles but was not detectable in cortical vesicles. We assume that glutamate, GABA, and glycine reside in different vesicle populations. This implies that the variation of amino acid ratios between brain regions reflects a variation in the composition of the respective nerve terminals. The relative distribution of vesicular amino acids found by us agrees well with that of glutamatergic, GABAergic, and glycinergic afferents in these regions.

One of the most striking findings in our experiments is that apart from glutamate, GABA, and glycine, no other amino acids were detectable in synaptic vesicles. This is particularly noteworthy for aspartate and taurine, which are present in high concentrations in brain homogenate (see e.g., Kontro et al., 1980; and unpublished data) but were never enriched over background values in synaptic vesicles. Clearly, the inherent sensitivity limits of our method do not allow the exclusion of small populations of synaptic vesicles containing aspartate or other amino acids. Furthermore, we cannot exclude that, even under our isolation conditions, vesicular aspartate or other amino acids are leaking from the vesicles. Nevertheless, we conclude that aspartate does not play a major role as an exocytotic neurotransmitter in the two brain regions studied. These findings agree well with the lack of evidence for an aspartate-specific vesicular carrier (Naito and Ueda, 1985; Hell, Maycox, and Jahn, unpublished data) and for aspartate release from synaptosomal preparations (McMahon and Nicholls, 1990).

Our analysis of [^{3}H]GABA and [^{3}H]glycine uptake in isolated vesicles suggests that in vitro both amino acids are transported by similar, if not identical, transporters which display preference for GABA. This is supported by the constant ratios of GABA versus glycine uptake by vesicles from different brain regions and in different types of vesicle preparations. In particular, glycine uptake by cortical vesicles is difficult to reconcile with separate transporters, since glycine is not a major transmitter in this region and is consequently lacking from these vesicles. In addition, the profiles of [^{3}H]GABA and [^{3}H]glycine uptake in competition experiments and bioenergetic studies are very

similar. The existence of only one vesicular transporter for both amino acids is not inconsistent with the expression of GABAergic and glycinergic phenotypes. The expression of glutamate decarboxylase and the Na^+-dependent high affinity GABA transporter in the plasma membrane, both providing a high intracellular GABA concentration, would be sufficient to determine the phenotype of a GABAergic neuron. We assume that in GABAergic neurons the GABA:glycine ratio is high in the cytoplasm, resulting in preferential transport of GABA into the vesicle. Conversely, glycine is the only available substrate for the vesicular transporter in glycinergic neurons, in which GABA is not synthesized as the result of the lack of glutamate decarboxylase expression.

It should be noted that our data contradict a recent report by Kish et al. (1989) concerning a specific glycine transporter present in synaptic vesicles from the spinal cord. These authors demonstrated selective competition of [^{3}H]GABA and [^{3}H]glycine by the respective unlabeled amino acids and different uptake ratios in the cerebral cortex and the spinal cord. Presently, we do not have an explanation for this discrepancy, as even a precise repetition of the published experiments in our hands did not provide evidence for any divergence between glycine and GABA uptake (Figure 2; Figure 3; and unpublished data). Furthermore, Christensen et al. (1990) found an inhibition of [^{3}H]glycine uptake by GABA in synaptic vesicles from spinal cord which is similar to that reported here.

In summary, the present data allow us to form an integrated view of the involvement of synaptic vesicles in the storage of amino acids in nerve terminals (Figure 4). The three major amino acid transmitters—glutamate, GABA, and glycine—are sequestered by specific transporters in the vesicle membrane: one glutamate transporter and probably only one transporter type for GABA and glycine. This transport is driven by the energy of an electrochemical proton gradient over the vesicle membrane. Glutamate transport is solely dependent on $\Delta\psi$, whereas GABA and glycine transport can be driven by both $\Delta\psi$ and ΔpH (for review see Maycox et al., 1990). The precise ionic mechanisms of transport and storage remain to be elucidated. However, it is clear that amino acids are in osmotic and ionic equilibrium with the cytoplasm. A filled vesicle therefore will retain its amino acid content only as long as the vesicular energy gradient remains intact. This differs from the storage of acetylcholine or catecholamines (for review see Whittaker, 1982; Philippu and Matthaei, 1988), which appear to survive prolonged isolation procedures under energy-depleted conditions. In this maintenance state, the exchange of amino acids with the cytoplasm appears to be slow (see, e.g., Sanchez-Prieto et al., 1987; Sihra and Nicholls, 1987). A reduction of intracellular ATP will lead to a rapid efflux of vesicular amino acids into the cytoplasm by carrier reversal. This may bear relevance for situations of neuronal damage (ischemia, etc., or experimental situations with nonoptimal energy preservation) in which the exocytotic pool of amino acids would be expected to disappear rapidly via leakage into the cytoplasm. In general terms, the mechanism of amino acid storage by synaptic vesicles is principally similar to that of Na^+-dependent solute storage in cells. In both cases, the electrochemical gradient of a leading ion (H^+ or Na^+, respectively) provides the basis not only for the generation but also for the maintenance of solute gradients over the membrane. The collapse of the leading ion gradient, in both cases, causes a dissipative readjustment of the solute gradients depending on the presence of impermeable ions (Donnan equilibrium) and may result in a complete equilibration of the solute pools on both sides of the membrane.

Experimental Procedures

Isolation of Synaptic Vesicles

Immunoisolation of synaptic vesicles was performed as described previously using monoclonal antibodies for the vesicle protein synaptophysin that were coupled to Eupergit C1Z microbeads as an immunoadsorbent (Burger et al., 1989). The following modifications were introduced: After coupling purified monoclonal antibodies or bovine IgG to Eupergit C1Z beads, the remaining active groups were quenched with serine rather than glycine to eliminate glycine bleeding from the beads during the experiments. In addition, all steps of the vesicle isolation (homogenization, centrifugation, incubation with the immunobeads and washing steps) were carried out at a constant temperature of 0°C (maximum deviation ± 2°C), using precooled equipment, ice-water baths and continuous control of the sample temperature throughout the procedure. In some experiments, the sucrose buffer (Burger et al., 1989) was replaced by 150 mM sodium acetate (pH 7.4) in the last washing step and extraction of amino acids was performed with 50% (v/v) acetonitrile in 50 mM NaCl instead of sulfosalicylic acid to reduce reagent peaks in the HPLC analysis. These changes did not affect the results. Immunobeads coupled to bovine IgG were used as a control. As an additional control, vesicles were isolated at 6°C–10°C or at 21°C.

For the analysis of vesicular GABA and glycine uptake, synaptic vesicles were also isolated according to Huttner et al. (1983), Hell et al. (1988), and Kish and Ueda (1989).

Uptake of [^{3}H]GABA and [^{3}H]Glycine

When vesicle preparations isolated by one of the conventional methods were used, vesicular uptake of [^{3}H]GABA and [^{3}H]glycine was measured by a filtration assay essentially as described (Hell et al., 1990). When immunoisolated vesicle preparations were used (isolated at 6°C), the procedure was modified. For each data point, bead-bound vesicles corresponding to 2.5 mg of beads were incubated in the presence of [^{3}H]GABA and [^{3}H]glycine in 150 μl of incubation buffer as described (Hell et al., 1990). At the end of the incubation with the respective radioligand, 1 ml of ice-cold sucrose buffer (Burger et al., 1989) was added, and the bead-bound vesicles were sedimented by centrifugation in a refrigerated minifuge (30 s, 12,000 × g). The sediment was subsequently washed three times at 0°C with 1 ml of sucrose buffer. The radioactivity in the final pellet was determined by liquid scintillation counting.

Other Analytical Procedures

Amino acids were analyzed by reverse-phase HPLC as described (Burger et al., 1989), using precolumn derivatization with either o-phthaldialdehyde (OPA procedure; Godel et al., 1984) or dimethylaminoazobenzenesulfonyl chloride (DABS-Cl procedure; Honegger et al., 1989). Synaptophysin was quantitated by dot immunobinding (Jahn et al., 1984). Protein was quantitated according to Bradford et al. (1976).

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