Energy Dependence and Functional Reconstitution of the γ-Aminobutyric Acid Carrier from Synaptic Vesicles*

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The energy dependence of γ-aminobutyric acid (GABA) uptake was characterized in rat brain synaptic vesicles and in proteoliposomes reconstituted with a new procedure from vesicular detergent extracts. The proteoliposomes displayed high ATP-dependent GABA uptake activity with properties virtually identical to those of intact vesicles. GABA uptake was similar at chloride concentrations of 0 and 150 mM, i.e. conditions under which either the membrane potential (ΔΨ) or the pH difference (ΔpH) predominates. ΔΨ was gradually dissipated by increasing the concentration of SCN⁻. GABA uptake was reduced by 10 mM SCN⁻, showing less sensitivity to ΔΨ reduction than glutamate uptake but more than dopamine uptake. Dissipation of ΔΨ with NH₄⁺ abolished GABA uptake at pH 7.3, whereas no significant inhibition occurred at pH 6.5. In contrast, dopamine uptake was inhibited more strongly, even at pH 6.5, and glutamate uptake was not reduced in either condition. We conclude that GABA uptake is driven by both components of the proton electrochemical gradient, ΔΨ and ΔpH, and that this is different from the uptake of both dopamine and glutamate, which is more strongly dependent on ΔpH and ΔΨ, respectively. Thus, our data suggest that GABA uptake is electrogenic and occurs in exchange for protons.

Synaptic vesicles are specialized secretory organelles that store neurotransmitters in a concentrated form within the neuron. Upon stimulation, they release their transmitter content by exocytosis. After membrane retrieval, they are re-loaded with their respective transmitter by specific carrier systems and enter another round of exo-endocytotic membrane cycling (for review, see Ceccarelli and Harihur, 1980; Reichardt and Kelly, 1983; De Camilli and Jahn, 1990).

In recent years, evidence has accumulated that synaptic vesicles possess specific carriers for monoamines (Maron et al., 1978; for review, see Njus et al., 1986; Johnson, 1988), acetylcholine (Anderson et al., 1982), glutamate (Naito and Ueda, 1983, 1985; Maycox et al., 1988), GABA (Fykse and Fonnum, 1988; Hell et al., 1988), and glycine (Kish et al., 1989), which are thought to be localized on different vesicle populations (Fischer-Bovenkerk et al., 1988; Kish et al., 1989).

The kinetic properties, substrate specificities, inhibition profiles, and energy dependence of these carriers are clearly different from those of the plasma membrane transporters (Kanner and Schuldiner, 1987). Transmitter uptake by synaptic vesicles is ATP dependent and sensitive to uncouplers. This indicates that all vesicular carriers are driven by a proton electrochemical potential (ΔΨM). The gradient is generated by an H⁺ ATPase of the vacuolar type which is present in the vesicle membrane (Hell et al., 1988; Cidon and Sihra, 1989).

A detailed analysis of the energy dependence has been performed only for the monoamine carrier (Njus et al., 1986; Johnson, 1988) and to some extent for the glutamate carrier (Maycox et al., 1988). Using chromaffin granules as a model system, the monoamine carrier has been shown to be driven by both components of the electrochemical gradient (ΔΨM), the membrane potential (ΔΨ) and the pH gradient (ΔpH), the latter being more effective (for review, see Njus et al., 1986; Kanner and Schuldiner, 1987; Johnson, 1988). In contrast, glutamate uptake by synaptic vesicles is dependent predominantly on ΔΨ with little uptake occurring in the presence of high ΔpH (Maycox et al., 1988).

In the present study, the relative dependence of vesicular GABA uptake on ΔΨ and ΔpH was investigated. Two approaches were used to manipulate ΔΨ and ΔpH. First, the chloride concentration was varied. The proton pump forms a large ΔΨ in the absence of permeant anions, e.g. Cl⁻, which can provide charge balance. An increase in the Cl⁻ concentration results in a gradual shift from ΔΨ to ΔpH while the total electrochemical potential (ΔΨM) remains unchanged (Johnson et al., 1979; Van Dyke, 1988; Maycox et al., 1988). Second, the ΔΨ or ΔpH was selectively dissipated using SCN⁻ and NH₄⁺ ions, respectively (Johnson and Scarpas, 1979). In all experiments, GABA uptake activity was correlated with the relative size of ΔpH and ΔΨ, which was monitored using acridine orange and oxonol VI as indicator dyes, respectively. In addition, the uptake activities of glutamate and dopamine were measured to allow a comparison of all three carriers under identical experimental conditions.

Synaptic vesicle preparations from the mammalian central nervous system are probably heterogeneous, with only a fraction being GABAergic. It cannot be excluded that changes in ΔΨ and ΔpH are specific for GABAergic vesicles and are different from those of the total vesicle population. Therefore, parallel experiments were performed using proteoliposomes that were prepared from detergent extracts of synaptic vesicles. Since, in this preparation, all protein components are

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The abbreviations used are: ΔΨM, proton electrochemical potential; ΔΨ, transmembrane proton concentration gradient; ΔΨ, transmembrane potential gradient; GABA, γ-aminobutyric acid; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
homogeneously distributed, bulk measurements of $\Delta pH$ and $\Delta V$ should closely reflect the activities of GABA-transporting proteoliposomes. For this purpose, we developed a procedure for the functional co-reconstitution of the GABA carrier and the endogenous proton pump. Our results show that the properties of the vesicular GABA carrier in proteoliposomes are very similar to those in intact synaptic vesicles. They indicate further that GABA uptake can be driven by both $\Delta V$ and $\Delta pH$ and presumably occurs in exchange for protons.

**EXPERIMENTAL PROCEDURES**

**Materials**—$^{2,3}$H]GABA (37.5 Ci/mmol), L-$^{[3,4-3H]}$glutamate (50 Ci/mmol), and $[^3H]$dopamine (40 Ci/mmol) were obtained from Du Pont-New England Nuclear. Acridine orange and oxonol VI were obtained from Serva (Heidelberg, Federal Republic of Germany) and Molecular Probes (Eugene, OR), respectively. Mg-ATP was obtained from Boehringer Mannheim and controlled pore glass beads (3000 Å pore size, glyceryl coated) from Electra-Nucleonics (Fairfield, NJ). All other reagents were of the highest available purity and obtained from commercial sources.

**Preparation of Synaptic Vesicles**—Synaptic vesicles were purified from rat forebrain using a modified procedure of Hell et al. (1988). Briefly, 45 g of rat brain was frozen in liquid nitrogen, crushed, and pulverized to form a fine powder. All subsequent steps were carried out at 0-4 °C. The powder was resuspended in homogenization buffer (0.32 M sucrose, 10 mM HEPES-KOH and magnesium-ATP, 1 mM Mg-ATP, 3 mM Mg-chloride, 100 mM KCl, pH 7.3, 0.5 μg/ml leupeptin) and homogenized in a glass-Teflon homogenizer. A crude vesicle containing supernatant was obtained by centrifugation for 10 min at 47,000 × $g_{av}$ followed by centrifugation at 120,000 × $g_{av}$ for 40 min. This supernatant was layered onto a cushion of 0.05 M sucrose, 10 mM HEPES-KOH and centrifuged at 250,000 × $g_{av}$ for 2 h. The pellet ($P_v$) was resuspended in 8-10 μl of homogenization buffer (without inhibitors). For further purification, insoluble fractions were removed by centrifugation at 27,000 × $g_{av}$ for 10 min, and the supernatant was chromatographed on a controlled pore glass head bead-purified vesicles. All vesicle fractions were stored at -70 °C without loss of activity. When vesicles were prepared at pH 6.5, the HEPES buffer was replaced with 10 mM MES-KOH in all fractionation steps. Vesicle protein was determined according to Bradford (1976).

**Measurement of $\Delta pH$ and $\Delta V$—**$\Delta pH$ was measured using the pH-sensitive fluorescent dye acridine orange. Changes in absorbance at 492 nm were measured with an Aminco DW 2000 dual wavelength spectrophotometer, using 530 nm as reference wavelength. $\Delta V$ was measured following the absorption of the potential-sensitive dye oxonol VI, with 625 nm as detection wavelength and 587 nm as reference wavelength. The final concentrations were 10 and 1 μM for acridine orange and oxonol VI, respectively. Unless indicated otherwise, measurements were performed at 32 °C in 700 μl of standard assay buffer (0.32 M sucrose, 4 mM KCl, 10 mM HEPES-KOH, pH 7.3) or buffers with the indicated CI$^-$ concentration (as described below). The additions were made from concentrated stock solutions and added in a volume less than 1/100 of the assay volume, followed by rapid mixing, to minimize dilution effects. All traces were corrected for baseline shifts associated with the additions.

**Uptake of Radiolabeled Neurotransmitters**—Protein was diluted to 1 mg/ml in standard assay buffer that was adjusted to the required CI$^-$ concentration by addition of KCl or by replacing sucrose with the appropriate amount of KCl. 100-μl aliquots were preincubated for 5 min at 32 °C. The reaction was started by the addition of 100 μl of 10 mM MES-KOH in the standard assay buffer. The filters were washed four times with 3 ml of ice-cold assay buffer, and bound radioactivity was determined by liquid scintillation counting.

Filter blanks as shown in Table I were determined by incubating synaptic vesicles on ice for maximally 10 s with their respective radiolabeled neurotransmitter. The samples were filtered and the filters washed as described above. Each experiment was performed in duplicate or triplicate. Specific activities were calculated by subtracting the values obtained in the presence of uncouplers (GABA or glutamate uptake) or reserpine (dopamine uptake; see Table I). There was some variation in the specific activities of the transmitters between individual vesicle preparations (Table I), which is due to the liability of the vesicular proton pump (not shown). To facilitate comparison, we have normalized the activities in every experiment by defining the value obtained under standard conditions (standard assay buffer containing only Mg-ATP) as 100%. All figures show mean values from three to five independent experiments.

**Functional Reconstitution**—The GABA carrier was co-reconstituted with the endogenous H$^+$ pump by a dilution procedure following the addition of solubilized brain phospholipids. Brain phospholipids were prepared according to Nelson et al. (1988) and stored under N$_2$ at -20 °C until use. For reconstitution, phospholipids were resuspended at 125 mg/ml in 10 mM HEPES-KOH, pH 7.3, 1% (v/v) sodium cholate and sonicated for 1-2 min (Branson cell disruptor BI5, microtip, power at position 4) until the solution became clear. Before use, large fragments were removed by centrifugation (1 min at 9,000 × $g_{av}$). 100 μl of synaptic vesicles (10 mg/ml) was solubilized for 10 min on ice after the addition of 25 μl of 500 mM KCl, 50 mM HEPES-KOH, pH 7.3, and 5% sodium cholate. The solution was cleared by centrifugation at 250,000 × $g_{av}$ for 15 min in a Beckman TLA 100.2 rotor. Under these conditions, insoluble material, e.g. intact synaptic vesicles, was completely sedimented. Brain phospholipids (25 μl) were then added to the extract, which was kept on ice for 5 min. It was then incubated at 32 °C for 5 min. For measurement of neurotransmitter uptake, 10-μl aliquots were diluted into 800 μl of standard assay buffer that was adjusted to different CI$^-$ concentrations or pH 6.5 as described above when indicated. After 2 min, the reaction was started by the addition of 100 μl of standard assay buffer supplemented with 7 μCi of $[^3H]$GABA (1.5 mM), Mg-ATP (20 mM), and, if indicated, FCCP (300 μM), (NH$_4$)$_2$SO$_4$ (100 mM), or KSCN (5-100 mM). After 10 min, 2.5 ml of ice-cold standard assay buffer was added. The samples were filtered, washed, and counted as described above. Filter blanks were obtained as described for intact synaptic vesicles. For measurement of $\Delta V$ and $\Delta pH$, 8-μl aliquots of the extract were diluted into 700 μl of the appropriate assay buffer.

**RESULTS**

**Functional Reconstitution of the GABA Carrier in Proteoliposomes**—The GABA carrier was reconstituted using the endogenous H$^+$ pump to generate the proton gradient. The procedure is similar to the reconstitution of the vesicular glutamate carrier (Maxvax et al., 1988). Vesicle proteolipids were solubilized with sodium cholate, cleared from insoluble material by ultracentrifugation, and reconstituted by dilution after addition of solubilized brain phospholipids. Fig. 1 shows
the time course of ATP-dependent GABA uptake in proteoliposomes. The kinetics of uptake was similar to that of intact synaptic vesicles (not shown; see also Fykse and Fonnum, 1988). Uptake was almost complete after 10 min, and therefore this was the chosen incubation period in all further experiments involving proteoliposomes. The uptake activity recovered after reconstitution was higher than in the intact synaptic vesicles used as starting material (Table I). As in intact synaptic vesicles, uptake in proteoliposomes was inhibited by the uncoupler FCCP. Together, these data show that the carrier was reconstituted in an active form and that all components required for uptake are constituents of the synaptic vesicle membrane.

Chloride Dependence of GABA Uptake—The Cl− concentration was varied in order to study the relative dependence of the vesicular GABA uptake on ΔpH and ΔΨ. Maximal uptake occurred in a concentration range of 4–50 mM Cl− (Fig. 2). When no Cl− was present, GABA uptake was reduced by about 40% of maximal activity. A similar reduction was observed at a Cl− concentration of 150 mM. To study the effect of Cl− in more detail, changes of ΔΨ and ΔpH were monitored at three Cl− concentrations (0, 4, and 150 mM) and correlated with GABA uptake. Parallel experiments were performed with proteoliposomes (4 and 150 mM Cl−; since the reconstitution procedure involves Cl−-reconstituted vesicles could not be tested under Cl−-free conditions). In addition, GABA uptake was compared with that of glutamate (which has been shown to be solely dependent on ΔΨ; Maycox et al., 1988) and dopamine (which is preferentially driven by ΔΨ; Njus et al., 1986; Johnson, 1988).

In the absence of Cl−, ΔΨ was maximal in intact vesicles (Fig. 3a). In addition, a small preexisting ΔpH was present (Fig. 4a). At 4 mM Cl−, a slight ATP-dependent acidification was observed (Fig. 4a), which was associated with a significant reduction of ΔΨ (Fig. 3a). At 150 mM Cl−, ΔpH was maximal, whereas ΔΨ was barely detectable (Figs. 3a and 4a). Similar traces were obtained when proteoliposomes were used (Figs. 3b and 4b). GABA uptake was observed at 0 and 150 mM Cl−, suggesting that both ΔpH and ΔΨ can act as the driving force. A comparison of the uptake of glutamate and dopamine (Fig. 5) revealed differences in the Cl− dependence of the three carrier systems. Dopamine uptake was observed at 0 and 4 mM Cl− but displayed 2-fold higher activity at 150 mM, which is in agreement with its preference for ΔpH as the driving force. In contrast, glutamate uptake was maximal at 4 mM Cl− and was strongly reduced at 150 mM, which is in agreement with its dependence on ΔΨ. The reduced activity in the absence of chloride probably reflects a direct involvement of Cl− in the glutamate uptake process. These findings are in agreement with earlier reports (Johnson et al., 1979; Naito and Ueda, 1985; Maycox et al., 1986).

Influence of NH4+:—The experiments described above suggest that GABA uptake is active under conditions in which either ΔpH or ΔΨ predominates. In order to support this view further, we analyzed the effects of reagents that are commonly used to dissipate ΔpH or ΔΨ selectively. These experiments were performed at a Cl− concentration of 4 mM, which is close to the intravesicular concentration of this ion within mammalian neurons (Hansen, 1985).

NH4+ was used to dissipate ΔpH since it equilibrates the intravesicular pH with that of the incubation medium. As shown in Fig. 4, 20 mM NH4+ is sufficient to destroy ΔpH completely at 4 mM Cl− in intact and reconstituted vesicles. Further additions of NH4+ had no effect. At 150 mM Cl−, a slight residual pH difference was observed which was dissipated by a further addition of 20 mM NH4+. At 4 mM Cl−, 20 mM NH4+ induced an increase in the membrane potential which was similar in both intact and reconstituted vesicles (Fig. 6, a and b, left). This corresponds with the complete dissipation of ΔpH indicating that ΔΨ+pH, the sum of ΔpH and ΔΨ, remains constant. Virtually identical traces were obtained at pH 6.5 instead of pH 7.3 (data not shown).

The uptake of GABA was almost completely inhibited by 20 mM NH4+ at pH 7.3 in both intact and reconstituted vesicles (Fig. 7, left), which parallels the dissipation of ΔpH (cf. Fig. 4). In contrast, no inhibition by NH4+ was observed at pH 6.5 (Fig. 7, right). This indicates that a rise in the intravesicular proton concentration (at pH 6.5, it is 9-fold higher than at pH 7.3) is sufficient to allow GABA uptake to proceed in the complete absence of ΔpH. Under these conditions, it is solely driven by ΔΨ.

A comparison of GABA uptake with that of glutamate and dopamine confirmed the different dependence of the three carriers on ΔpH and ΔΨ. Addition of NH4+ increased glutamate uptake at pH 7.3 (Fig. 7, left). This parallels the slight increase in the membrane potential (Fig. 6, left) and confirms the strict ΔΨ dependence of the glutamate carrier. In contrast, dopamine uptake was strongly reduced by NH4+ at both pH 7.3 and pH 6.5, which is in accordance with the preference of the monoamine carrier for ΔpH.

Influence of SCN−—In the following experiments, the lipophilic anion SCN− was used to dissipate ΔΨ. SCN− penetrates membranes easily, thereby neutralizing the positive charge within the vesicle. SCN− can act as a chaotrope at higher concentrations. It is known to affect hydrophobic interactions and has adverse effects on membrane protein function. Therefore, the SCN− concentration was titrated to analyze its effect on ΔΨ in detail. As shown in Fig. 6, a and

| Table I |
| Uptake of 3H]GABA, L-[3H]glutamate, and [3H]dopamine in synaptic vesicles (fraction P) and of 3H]GABA in proteoliposomes under standard assay conditions |
| The incubation times were: [3H]GABA (synaptic vesicles), 6 min; [3H]GABA (proteoliposomes), 10 min; L-[3H]glutamate, 5 min; [3H]dopamine, 10 min. All data are given in pmol/mg of protein and are means of five independent experiments. |
| | GABA | Glutamate (intact vesicles) | Dopamine (intact vesicles) |
| | pmol/mg protein |
| | Intact vesicles | Proteoliposomes | Intact vesicles | Proteoliposomes |
| Control (ATP) | 322 ± 80 | 770 ± 267 | 849 ± 226 | 18.4 ± 5.1 |
| FCCP (20 μM) | 144 ± 55 | 298 ± 79 | 103 ± 39 | 2.3 ± 0.8 |
| Gramicidin D (10 μM) | 170 ± 23 | ND | 81 ± 31 | ND |
| Reserpine (1 μM) | ND | ND | ND | 2.9 ± 0.9 |
| Reserpine + FCCP (20 μM) | ND | ND | ND | 2.4 ± 0.7 |
| Filter blank | 102 ± 70 | 240 ± 42 | 87 ± 47 | 1.1 ± 0.1 |
| * ND, not determined. |
GABA Uptake of Synaptic Vesicles

Fig. 2. Cl⁻ dependence of [³H]GABA uptake by intact synaptic vesicles under standard assay conditions. GABA uptake in this and the following experiments was normalized to that observed at 4 mM Cl⁻.

Fig. 3. ATP-dependent changes of ΔΨ in synaptic vesicles (a, 500 μg of protein) and in proteoliposomes (b, approximately 100 μg of protein) at different Cl⁻ concentrations. Potential changes were measured with oxonol VI at pH 7.3. FCCP or gramicidin D (150 mM KCl) was used to uncouple the proton gradient and was added at a final concentration of 10 or 20 μM, respectively.

Fig. 4. Acidification of synaptic vesicles (a, 500 μg of protein) and of proteoliposomes (b, approximately 100 μg of protein) at different Cl⁻ concentrations after addition of 2 mM Mg-ATP. Acidification was monitored by following the absorbance of acridine orange at pH 7.3. When indicated, a final concentration of 20 mM NH₃ was added from a stock solution of 3 M (NH₄)₂SO₄.

b, (right), the effects of increasing concentrations of SCN⁻ on ΔΨ in intact synaptic vesicles (Fig. 6a) were similar to those obtained in reconstituted vesicles (Fig. 6b). Two mM SCN⁻ reduced the oxonol signal by about 40%. Higher SCN⁻ concentrations led to a further reduction, reaching 80% at 10 mM.

Fig. 5. Cl⁻ dependence of [³H]GABA uptake by synaptic vesicles (closed bars) and proteoliposomes (cross-hatched bars). For comparison, the uptake of L-[³H]glutamate and [³H]dopamine by synaptic vesicles was determined. The activities were normalized to those observed at 4 mM Cl⁻.

Fig. 8 shows the effects of SCN⁻ on GABA uptake in intact and reconstituted vesicles. At 2 mM SCN⁻, GABA uptake was only slightly inhibited (less than 20% inhibition). This contrasts with glutamate uptake, which is already reduced by 50% at 2 mM SCN⁻, parallel to the reduction of ΔΨ (see Fig. 6a and b, right). At 10 mM SCN⁻, GABA uptake is inhibited almost completely in intact vesicles and by more than 50% in reconstituted vesicles. In contrast, dopamine uptake is only slightly reduced by 10 mM SCN⁻ (see also Apps et al., 1980). This shows that the inhibition of GABA uptake at this SCN⁻ concentration is not due to a dissipation of the overall energy gradient ΔμH⁺. It further supports the view that the dopamine carrier is predominantly dependent on ΔpH. Together, the data indicate that GABA uptake is less sensitive to SCN⁻ than the ΔΨ-dependent glutamate uptake but more sensitive than dopamine uptake.

DISCUSSION

In the present study, we have analyzed the energy dependence of GABA uptake by synaptic vesicles in intact and reconstituted vesicle preparations. Our reconstitution procedure involves the formation of proteoliposomes from soluble detergent extracts after addition of exogenous phospholipids. This ensured a random distribution of all protein components.
It resulted in high activities of the endogenous proton pump as well as of the GABA carrier and allowed a precise comparison of the intact and reconstituted systems. Under all experimental conditions, the two systems exhibited similar properties with respect to $A\Psi$, $A\Delta\phi$, and GABA uptake. These observations allow the conclusion that the subpopulation of GABAergic vesicles responds to perturbations of the energy gradient in a way similar to the whole vesicle population. Preliminary results indicate that this applies also to the glutamate carrier. Several approaches were used to modify the relative proportions of $A\Delta\phi$ and $A\Psi$. In addition to varying the chloride concentration of the assay buffer, we used SCN$^-\$ and NH$_4$ to dissipate $A\Psi$ and $A\Delta\phi$, respectively. The concentration of SCN$^-\$ or NH$_4$ was kept as low as possible to avoid adverse effects on the integrity of membrane proteins. Neither ion affected the ATPase activity of the H$^+$ pump at the maximal concentrations used (10 and 20 mM, respectively) (data not shown). However, higher concentrations of NH$_4$ and SCN$^-\$ led to nonspecific effects including reduction of the energy gradient and inhibition of the carrier activities. These findings emphasize the necessity for a careful control of all parameters in experiments involving these ions.

Our results indicate that both components of $\Delta\mu_{H^+}$, $A\Psi$ and $A\Delta\phi$, are able to energize the vesicular GABA carrier. The following observations demonstrate that under appropriate experimental conditions, GABA uptake can be driven solely by $A\Psi$, without involvement of $A\Delta\phi$. First, addition of NH$_4$ did not inhibit GABA uptake at pH 6.5, whereas $A\Delta\phi$ was completely abolished. Similarly, glutamate uptake, which is known to be $A\Psi$ dependent, was not affected. Second, GABA uptake was only reduced by 40% in the absence of chloride, whereas $A\Delta\phi$ is barely measurable under these conditions. It could not be documented with the same stringency that $A\Delta\phi$ can act as the driving force for GABA uptake. However, it is supported by the following observations. First, GABA uptake was only reduced to 40–50% of its maximal activity at 150 mM Cl$^-$, whereas $A\Delta\phi$ was reduced to a far greater extent, which was reflected by a parallel inhibition of glutamate uptake (>80%). Second, GABA uptake was less sensitive to inhibition by low concentrations of SCN$^-\$ than glutamate uptake (Fig. 8). Since there is still a residual amount of $A\Psi$ in both experimental conditions, it remains to be established whether $A\Psi$ can drive GABA uptake in the complete absence of $A\Delta\phi$. This is currently being investigated in our laboratory.

It is evident, however, that GABA uptake is strongly influenced by the intravesicular proton concentration. This is demonstrated by the response of GABA uptake to NH$_4$ at different pH values. NH$_4$ equilibrates the internal pH with that of the extravesicular medium, inhibiting GABA uptake at pH 7.5 but not at pH 6.5. $A\Delta\phi$ is completely dissipated.

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**Fig. 7.** Influence of NH$_4$ (20 mM) on ATP-dependent uptake of $[^{3}H]$GABA by synaptic vesicles (closed bars) and proteoliposomes (cross-hatched bars). For comparison, the uptake of L-$[^{3}H]$glutamate and $[^{3}H]$dopamine by synaptic vesicles was determined. All assays were performed under standard assay conditions.

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$[^{3}H]$GABA Uptake of Synaptic Vesicles
under both conditions. This indicates that the intravesicular proton concentration must be sufficiently high for GABA uptake to proceed at significant rates. These findings can be explained in two ways. First, it is possible that protonation of a side group of the GABA carrier protein(s) is required for its activation. Second, protons may be directly involved in GABA uptake by a coupled exchange mechanism. In this case, the increased activity at low pH (in the absence of ΔpH) reflects the affinity of the carrier to protons as co-substrate. We favor the latter interpretation, as an exchange of GABA for protons offers a plausible mechanistic explanation since both Δψ and ΔpH can probably act as the driving force (see below).

The model in Fig. 9 summarizes our results and depicts a possible mechanism of the GABA carrier. For comparison, models of the glutamate and the dopamine carriers are shown as well. As discussed above, our data can be explained most readily by a model involving a coupled exchange of GABA for protons. In this model, transport is associated with the translocation of a net positive charge out of the vesicle. This explains why an inside positive membrane potential can act as the driving force. Furthermore, it also explains why an outwardly directed proton gradient leads to GABA accumulation. In contrast, glutamate uptake does not seem to involve protons and is clearly independent of ΔpH. This is indicated by the observation that dissipation of ΔpH by NH₃ does not inhibit but rather activates glutamate uptake. Additionally, glutamate uptake is very similar at pH 7.3 and 6.5 in the presence of NH₃, indicating that it is not influenced by the intravesicular proton concentration. It is interesting to note that glutamate uptake is strongly reduced in the absence of chloride (Fig. 5; see also Naito and Ueda, 1985) although Δψ is maximal under this condition (Fig. 3). We assume that a minimal concentration of Cl⁻ is required for optimal activity of the carrier. It is possible that Cl⁻ is directly involved in glutamate uptake, e.g. by a coupled substoichiometric exchange with glutamate. However, more evidence is needed to support this view.

In the case of the monoamine carrier, the view that one positively charged monoamine molecule is exchanged for two protons has received strong support (Fig. 9; Johnson et al., 1979 and 1981; Knoth et al., 1981). Despite this, an alternative model involving the exchange of one uncharged monoamine molecule for one proton has been proposed (e.g. Scherman and Henry, 1981; for review see Njus et al., 1986 and Johnson, 1988). In our experiments, dopamine uptake appears to be more dependent on ΔpH than on Δψ. It is 2-fold higher at 150 mM Cl⁻ compared with 4 or 0 mM Cl⁻, hardly affected by 10 mM SCN⁻, and reduced by NH₃ even at pH 6.5. These findings are in agreement with earlier studies and support the idea of the participation of two protons in the transport cycle. These models are still hypothetical and need further support by additional experiments. We hope that our analysis of the GABA carrier and its functional reconstitution in proteoliposomes will provide an experimental basis for its further characterization at the molecular level.

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