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Functional Reconstitution of the γ-Aminobutyric Acid Transporter from Synaptic Vesicles Using Artificial Ion Gradients†

Johannes W. Hell,‡ Lambert Edelmann,§ Joachim Hartinger,§ and Reinhard Jahn*,§
Department of Neurochemistry, Max Planck Institute for Psychiatry, D-8033 Martinsried, Germany
Received April 26, 1991; Revised Manuscript Received September 19, 1991

ABSTRACT: The γ-aminobutyric acid transporter of rat brain synaptic vesicles was reconstituted in proteoliposomes, and its activity was studied in response to artificially created membrane potentials or proton gradients. Changes of the membrane potential were monitored using the dyes oxonol VI and 3,3′-diisopropylthiodicarbocyanine iodide, and changes of the H^+ gradient were followed using acridine orange. An inside positive membrane potential was generated by the creation of an inwardly directed K^+ gradient and the subsequent addition of valinomycin. Under these conditions, valinomycin evoked uptake of [^{3}H]GABA which was saturable. Similarly, [^{3}H]glutamate uptake was stimulated by valinomycin, indicating that both transporters can be driven by the membrane potential. Proton gradients were generated by the incubation of K^+-loaded proteoliposomes in a buffer free of K^+ or Na^+ ions and the subsequent addition of nigericin. Proton gradients were also generated via the endogenous H^+ ATPase by incubation of K^+-loaded proteoliposomes in equimolar K^+ buffer in the presence of valinomycin. These proton gradients evoked nonspecific, nonsaturable uptake of GABA and β-alanine but not of glycine in proteoliposomes as well as protein-free liposomes. Therefore, transporter activity was monitored using glycine as an alternative substrate. Proton gradients generated by both methods elicited saturable glycine uptake in proteoliposomes. Together, our data confirm that the vesicular GABA transporter can be energized by both the membrane potential and the pH gradient and show that transport can be achieved by artificial gradients independently of the endogenous proton ATPase.

Signals are conducted between neurons via the release of small molecules, the neurotransmitters. It is generally accepted that nonpeptide neurotransmitters are stored in high amounts in nerve terminals where they are concentrated in synaptic vesicles. Upon stimulation, synaptic vesicles exocytose and release their content into the synaptic cleft. The synaptic vesicle membrane is then retrieved from the plasma membrane by endocytosis to reform synaptic vesicles. These vesicles are reloaded with neurotransmitter and are reset to undergo another round of exo–endocytosis.

It is well established that synaptic vesicles possess the capacity to sequester and store neurotransmitters. High concentrations of acetylcholine (Whittaker et al., 1964), monoamines [reviewed by Philippu and Matthaei (1988)], glutamate (Burger et al., 1989), GABA,[1] and glycine (Burger et

† Supported by Grant Ja 377/2-3 of the Deutsche Forschungsgemeinschaft.
* Address correspondence to this author at the Howard Hughes Medical Institute, Yale University School of Medicine, Boyer Center for Molecular Medicine, 295 Congress Ave., New Haven, CT 06510.
‡ Present address: Department of Pharmacology, University of Washington, Seattle, WA 98195.
§ Present address: Howard Hughes Medical Institute, Boyer Center for Molecular Medicine, Yale University Medical School, New Haven, CT 06510.

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al., 1991) have been demonstrated in isolated synaptic vesicles. In addition, synaptic vesicles have specific transport systems for monoamines [reviewed in Philippu and Matthaei (1988)], acetylcholine (Parsons & Koenigsberger, 1980), glutamate (Disbrow et al., 1982; Naito & Ueda, 1983), and GABA (Fykse & Fonnum, 1988; Hell et al., 1988). All these transporters are different from the respective Na^+-dependent transporters in the synaptic plasma membrane. The energy required for uptake is provided by a proton electrochemical gradient generated across the vesicle membrane by the activity of a proton ATPase of the vacuolar type. This pump is electrogenic and establishes an electrochemical potential $\Delta\mu_{H^+}$ which is composed of a membrane potential component $\Delta\psi$ and a pH gradient component ΔpH. The ratio of $\Delta\psi$ and ΔpH depends on the availability of permeable counterions, usually chloride ions [for review see Maycox et al. (1990a)].

The vesicular transporter for GABA has only recently been discovered and characterized to some extent. It has a low substrate affinity [K_m 5–10 mM, Fykse and Fonnum (1988) and our own unpublished observations] and apparently transports glycine with slightly lower affinity than GABA, suggesting that this carrier is responsible for both GABA and glycine sequestration in the respective nerve terminals (Burger et al., 1991). In addition, we studied the bioenergetic dependence of GABA transport in intact synaptic vesicles and proteoliposomes, using the endogenous proton pump as the driving force (Hell et al., 1990). Experiments in which ΔpH was selectively dissipated with NH_4^+ ions revealed that $\Delta\psi$ can act as the sole driving force. However, under these conditions transport was only observed at pH 6.5 but not at pH 7.4, indicating that the intravesicular proton concentration must be sufficiently high to allow transport to proceed at significant rates. In addition, GABA uptake was observed under conditions where $\Delta\psi$ was selectively reduced, suggesting that also ΔpH can act as the driving force. However, additional evidence for this is required since $\Delta\psi$ could not be completely abolished in these experiments. On the basis of these observations, we suggested that the vesicular GABA carrier operates as an electrogenic GABA–proton exchanger (Hell et al., 1990).

In all previous studies, GABA transport was linked to ATP cleavage via the intermediate action of the endogenous proton pump. This limits the characterization of the transporter to conditions compatible with a sufficiently high proton pump activity. In order to gain independence from the proton pump, we have generated $\Delta\mu_{H^+}$ and ΔpH using artificial ion gradients to energize GABA uptake in proteoliposomes. Our results show that both artificially imposed $\Delta\psi$ and ΔpH are sufficient to obtain carrier-mediated GABA or glycine uptake.

Materials and Methods

Materials. [2,3-^{3}H]GABA (37.5 Ci/mmol), [3-^{3}H]-β-alanine (87.2 Ci/mmol), L-[3,4-^{3}H]glutamate (50 Ci/mmol), and [2-^{3}H]glycine (51.3 Ci/mmol) were obtained from Du Pont–New England Nuclear (Dreieich, Germany). Acridine orange and carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP) were from Serva (Heidelberg, Germany), oxonol VI and 3,3′-diisopropylthiodicarbocyanine iodide [$DiSC_3(5)$] were from Molecular Probes (Eugene, OR), nigericin was from Sigma (Munich, Germany), and valinomycin was from Boehringer (Mannheim, Germany). Sodium cholate (ULTROL grade) was purchased from Calbiochem (Frankfurt, Germany). All other reagents were of high purity and were obtained from standard sources.

Preparation of Liposomes and Proteoliposomes. Synaptic vesicles (fraction P_3) were isolated as described (Hell et al., 1990). For use in the first experiments (Figures 1–4), bovine brain phospholipids were prepared according to Nelson et al. (1988). For the later experiments, the preparation was simplified as follows: 26 g of bovine cerebral cortex was frozen in liquid N_2 and pulverized with a mortar. The powder was resuspended at room temperature in 60 mL of absolute ethanol (preflushed with N_2 for 1 h) using a glass–Teflon homogenizer (10 strokes, 2000 rpm). The suspension was diluted with 1 L of absolute ethanol (preflushed as above) and stirred for 1 h under N_2. Insoluble material was removed by filtration through Whatman 3 MM paper and the solvent reduced to 20 mL by evaporation under vacuum at 30 °C. The phospholipid suspension was stored at −20 °C under N_2 until use.

The reconstitution of the vesicular transporters was modified from our previous protocols (Maycox et al., 1988; Hell et al., 1990). Phospholipid suspension corresponding to 20 mg dry weight was evaporated under a stream of N_2 and further dried under vacuum for 3–4 h. The phospholipids were resuspended in 0.5 mL of 1% (w/v) sodium cholate and 10 mM MOPS–KOH, pH 7.3, as described (Hell et al., 1990). Vesicle proteins were extracted as described (Hell et al., 1990) except that 10 mM MOPS–KOH was used as buffer. For reconstitution, 80 μL of phospholipid suspension was added to 100 μL of vesicle extract. The mixture was kept on ice for 5 min, then quickly frozen, thawed at room temperature, and chromatographed on a Sephadex G-50 superfine column (0.7 cm × 15 cm), using either sucrose buffer (320 mM sucrose, 10 mM MOPS–tetramethylammonium hydroxide, pH 7.3) or KCl buffer (160 mM KCl, 10 mM MOPS–KOH, pH 7.3). Proteoliposomes eluted between 4 and 5.6 mL as a turbid solution. When protein-free liposomes were formed, extraction buffer (Hell et al., 1990) was added to the phospholipid suspension instead of vesicle extracts. For the formation of an outwardly directed K^+ gradient, proteoliposomes or liposomes formed in KCl buffer were sedimented by centrifugation in a Beckman T1 100.2 rotor (15 min, 250000g_{max}) at 4 °C. The pellet was resuspended by three passages through a needle (0.4 mm i.d.) in the same volume of sucrose buffer.

Uptake of Radiolabeled Neurotransmitters. The proteoliposome or liposome suspension was preincubated for 5 min at 32 °C. Incubation was started by adding uptake buffer containing the ^{3}H-labeled amino acids (see below). At the end of the reaction, the amount of the sequestered substrate was determined by a filtration assay as described (Hell et al., 1990).

For the generation of an inside positive diffusion potential (Figures 1–4), 200 μL of proteoliposomes formed in sucrose buffer (adjusted to pH 6.5 for GABA uptake) were added to 200 μL of uptake buffer containing K_2SO_4 (110 mM), $MgCl_2$ (8 mM), 4 μCi of [^{3}H]GABA (1.2 mM) or 2 μCi of [^{3}H]-glutamate (0.2 mM), and MOPS–KOH (10 mM), adjusted to pH 6.5 (GABA uptake) or pH 7.3 (glutamate uptake). For the formation of an artificial ΔpH (inside acidic, Figures 5–8), an outwardly directed potassium gradient was created as described above. Proteoliposomes (200 μL) or liposomes were added to 100 μL of uptake buffer containing sucrose (320 mM), $MgCl_2$ (6 mM), 2 μCi each of either [^{3}H]GABA, [^{3}H]-β-alanine, or [^{3}H]glycine (each 1.8 mM), and MOPS–tetramethylammonium hydroxide pH 7.3 (10 mM). For the

[1] Abbreviations: $\Delta\mu_{H^+}$, proton electrochemical potential; ΔpH, transmembrane proton concentration gradient; $\Delta\psi$, transmembrane potential gradient; $DiSC_3(5)$, 3,3′-diisopropylthiodicarbocyanine iodide; DCCD, *N*,*N*′-dicyclohexylcarbodiimide; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; GABA, γ-aminobutyric acid; MOPS, 3-(*N*-morpholino)propanesulfonic acid.

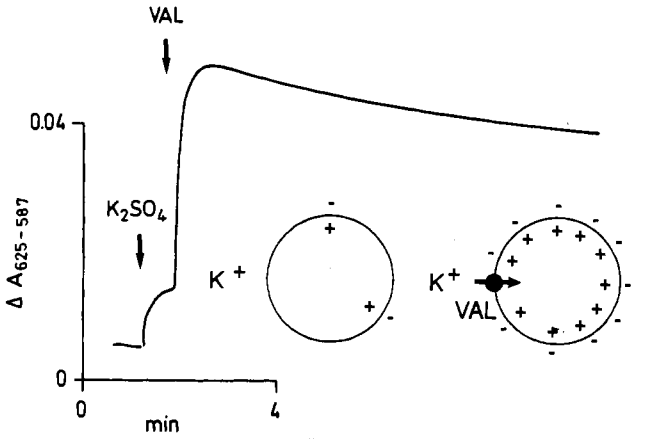

FIGURE 1: Generation of $\Delta\psi$ in proteoliposomes formed in potassium-free sucrose buffer by application of an inwardly directed K^+ concentration gradient. Potential changes were measured with oxonol VI (increase of the signal indicates increase of $\Delta\psi$, inside positive). K_2SO_4 and the K^+ ionophore valinomycin (VAL) were added at final concentrations of 60 mM and 125 nM, respectively. Note that K_2SO_4 elicits a small increase of $\Delta\psi$ which is greatly enhanced by valinomycin.

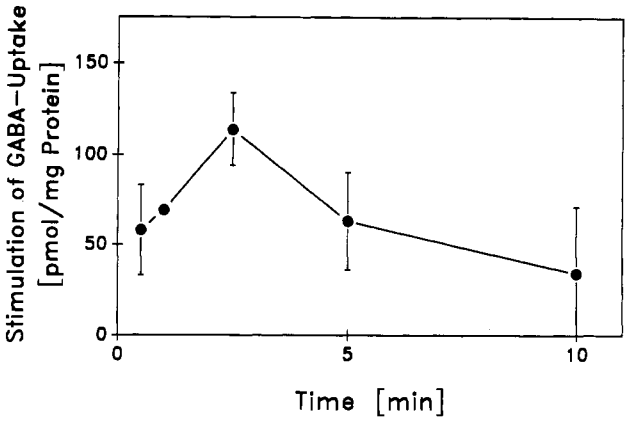

FIGURE 2: Stimulation of GABA uptake by valinomycin-induced $\Delta\psi$ in proteoliposomes formed in sucrose buffer in the presence of a K^+ concentration gradient (see Figure 1). Valinomycin was added at a final concentration of 125 nm. The figure shows the difference between GABA uptake observed in the presence and absence of valinomycin. The data are mean values (±SEM) of 5 independent experiments.

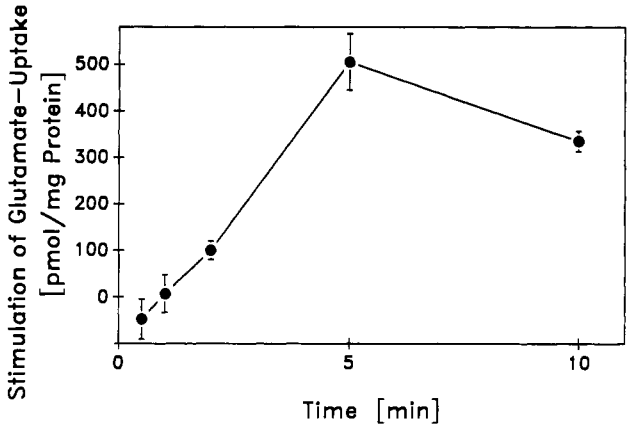

FIGURE 3: Stimulation of glutamate uptake by $\Delta\psi$ in proteoliposomes as described in Figure 2. The figure shows the difference between glutamate uptake observed in the presence and absence of valinomycin. The data are mean values (±SEM) of 3 independent experiments.

generation of ΔpH using the endogenous proton pump (Figure 9), 200 μL of proteoliposomes formed in KCl buffer was added to 100 μL of uptake buffer containing KCl (160 mM), 2 μCi of [^{3}H]glycine (1.8 mM), sodium ATP (6 mM), $MgCl_2$ (12 mM), and MOPS–KOH (10 mM). Control incubations contained in addition FCCP (50 μM final concentration).

Other Methods. Measurements of ΔpH and $\Delta\psi$ were performed as described, monitoring changes of absorbance of acridine orange, oxonol VI, or $DiSC_3(5)$ in the dual-wavelength mode (Hell et al., 1990). All dyes were used at a final concentration of 10 μM. The final sample volume was 0.5 mL containing 0.1 mL of column eluate. For $DiSC_3(5)$, the detection wavelength was set to 685 nm, with 655 nm as reference wavelength. Traces were corrected for base-line shifts associated with the additions. Protein concentration was determined according to Bradford (1976).

Results

GABA Uptake by Proteoliposomes Driven by Transient, Inside-Positive, Diffusion Potentials. In the first series of experiments we created transient diffusion potentials by imposing potassium gradients over the membrane using the K^+ ionophore valinomycin. For this purpose, proteoliposomes were formed by gel filtration from detergent extracts of synaptic vesicles in potassium-free sucrose buffer. The proteoliposomes were then transferred into isoosmotic K_2SO_4-containing buffer, and valinomycin was added subsequently. $\Delta\psi$ was monitored using oxonol VI as a dye indicator.

Addition of K_2SO_4 or KCl (not shown) elicited a slight $\Delta\psi$ as indicated by an increase in absorbance (Figure 1). This indicates a slight K^+ permeability of the proteoliposome membrane since no such increase was observed when choline chloride was added (not shown). As expected, addition of valinomycin caused a fast and strong increase in $\Delta\psi$ (Figure 1) which slowly decreased during prolonged incubation.

In the following experiments, GABA uptake was monitored in proteoliposomes under conditions similar to those presented in Figure 1. Figure 2 shows the time course of GABA uptake following the generation of an inwardly directed potassium gradient. Addition of valinomycin evoked a stimulation of GABA uptake. This is shown in Figure 2 which shows the difference between valinomycin-induced and control uptake of GABA. The stimulatory effect of valinomycin is maximal 2.5 min after addition. For comparison, the uptake of glutamate was assayed under the same conditions. Glutamate transport by synaptic vesicles is electrogenic and is driven by $\Delta\psi$ but not by ΔpH (Maycox et al., 1988, 1990b; Hell et al., 1990). As expected, uptake of glutamate was also stimulated by the addition of valinomycin, with a maximum after 5 min (Figure 3).

It should be noted that a time-dependent increase of glutamate and GABA uptake was also observed in the absence of valinomycin (data not shown). To examine whether the increase in the absence of valinomycin is also carrier-mediated, the uptake of [^{3}H]GABA and [^{3}H]glutamate was competed with excess unlabeled substrate. In fact, addition of 30 mM GABA reduced the [^{3}H]GABA uptake not only in the presence but also in the absence of valinomycin. The remaining, uncompetable uptake was virtually identical in both cases (Figure 4). A very similar inhibition was found for [^{3}H]-glutamate uptake when 10 mM unlabeled glutamate was added. Together, these data indicate that the valinomycin-induced uptake of both neurotransmitters is carrier-mediated. In addition, the reduction of uptake in the absence of valinomycin suggests that even under these conditions carrier-mediated uptake occurs which is probably due to a small $\Delta\psi$ (compare Figure 1).

GABA and Glycine Uptake by Liposomes and Proteoliposomes Driven by Imposed pH Gradients. To generate artificial pH gradients, proteoliposomes were loaded with KCl during

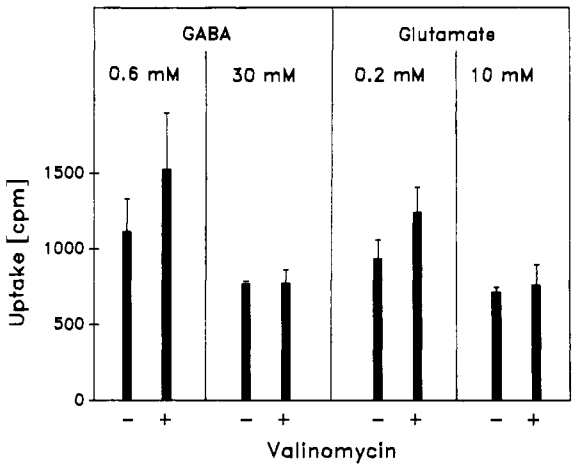

FIGURE 4: Competition of [^{3}H]GABA and [^{3}H]glutamate uptake in proteoliposomes by a 50-fold excess of unlabeled substrate. The conditions were as described in Figure 2. Osmotic balance was maintained by reducing the sucrose concentration accordingly. Incubation was for 2.5 and 5 min for GABA and glutamate uptake, respectively. The values show the mean (±range) of 2 independent experiments. Note that no difference between uptake in the presence or absence of valinomycin is observed when unlabeled substrate is added.

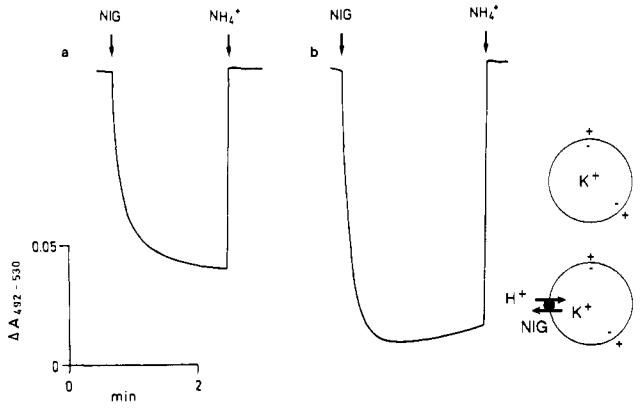

FIGURE 5: Generation of ΔpH (inside acidic) by nigericin (NIG, 250 nM final concentration) in liposomes (a) and proteoliposomes (b) which were formed in KCl buffer and incubated in sucrose buffer. ΔpH changes were measured with acridine orange. $(NH_4)_2SO_4$ (NH_4^+) was added at a final concentration of 10 mM.

reconstitution, collected by centrifugation, and resuspended in potassium- and sodium-free sucrose buffer. For comparison, protein-free liposomes were prepared in parallel. Under these conditions, the ionophore nigericin operates as a K^+/H^+ exchanger and causes a net uptake of protons which is driven by the outwardly directed potassium gradient. Both liposomes and proteoliposomes displayed a strong nigericin-induced acidification which was reversed by NH_4^+ (Figure 5), an ion that dissipates pH gradients at elevated concentrations (Hell et al., 1990). In contrast, nigericin addition did not alter the membrane potential (Figure 6b,c). However, a small, inside-negative diffusion potential was present. This was uncovered by a reduction of the absorbance of the indicator dye $DiSC_3(5)$ upon KCl addition to the extracellular medium (Figure 6b) which was also observed when KCl was added before nigericin (not shown). This potential difference is probably due to a small K^+ permeability, corresponding to that shown above (Figure 1). As expected, addition of valinomycin generated a large, inside-negative diffusion potential which was reversed by addition of KCl (Figure 6a). Thus, nigericin allows the generation of a large and stable ΔpH in this system, without affecting $\Delta\psi$. In addition, the small $\Delta\psi$ observed is

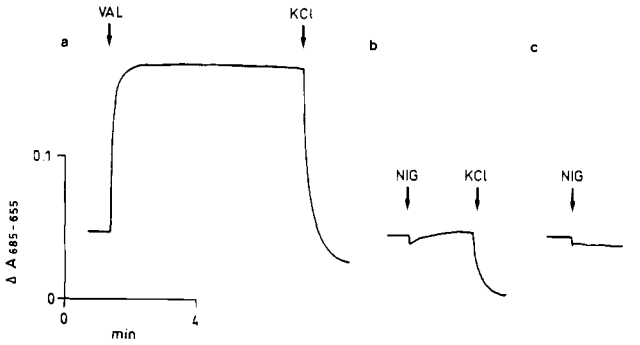

FIGURE 6: Changes of $\Delta\psi$ in liposomes (a, b) and proteoliposomes (c) which were formed in KCl buffer and incubated in sucrose buffer, monitored with $DiSC_3(5)$ as indicator (increase of the signal indicates increase of $\Delta\psi$, inside negative). Valinomycin (VAL) and nigericin (NIG) were added as described in the legends of Figures 1 and 5, respectively. Note that KCl addition (60 mM final concentration) causes reversal of the signal which is due to the collapse of the K^+ gradient.

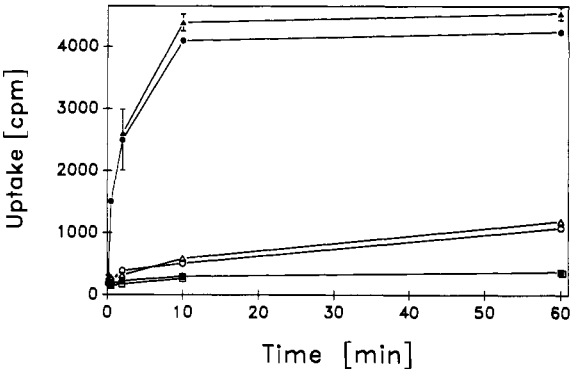

FIGURE 7: Stimulation of the uptake of [^{3}H]GABA (●, ○), [^{3}H]-β-alanine (▲, △), and [^{3}H]glycine (■, □) by nigericin-induced ΔpH in liposomes prepared and incubated as described in Figure 6. Closed symbols: Uptake in the presence of nigericin. Open symbols: Uptake in the absence of nigericin. The data shown are means (±SEM) of triplicate determinations obtained in a representative experiment. Note that glycine is the only substrate that is not sequestered by the liposomes when ΔpH is generated.

of reversed polarity to that generated in the experiments using an inwardly directed K^+ gradient.

In the next series of experiments, we analyzed the dependence of [^{3}H]GABA uptake on nigericin-induced pH gradients. Unexpectedly, control phospholipid liposomes, i.e., liposomes formed without adding synaptic vesicle proteins, displayed a rapid uptake of [^{3}H]GABA upon addition of nigericin (Figure 7) which could not be competed with 30 mM unlabeled GABA (not shown). This nonspecific uptake was independent of the source and composition of the phospholipids used for liposome formation, e.g., bovine brain phospholipids, asolectin, or defined mixtures of synthetic phospholipids. Addition of KCl to the extravesicular medium abolished GABA accumulation (not shown). These data suggest that GABA in its zwitterionic form equilibrates with the vesicle interior where it is protonated and trapped due to the lower pH inside the liposome. Since protonated GABA is presumably impermeant, the generation of a ΔpH (inside acidic) causes a net enrichment of GABA similar to that of other weak bases. It implies, however, that the zwitterionic form of GABA is membrane-permeant, at least in artificial membrane systems. To analyze this phenomenon further, we tested the uptake of [^{3}H]-β-alanine and [^{3}H]glycine under identical conditions. Both compounds are structural analogues of GABA and are transported with similar efficiency by the vesicular GABA transporter. As shown in Figure 7, β-alanine, but not glycine,

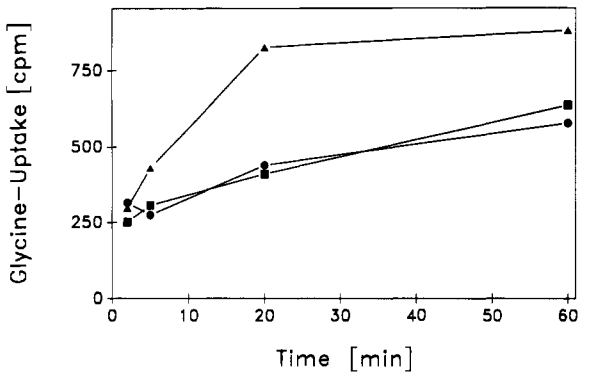

FIGURE 8: Stimulation of glycine uptake by nigericin-induced ΔpH in proteoliposomes, prepared and incubated as described in Figure 6. The figure shows the time course of [^{3}H]glycine uptake. (▲) Uptake under standard conditions, plus nigericin, (■) uptake in the presence of 50 mM unlabeled GABA, plus nigericin, and (●) uptake under standard conditions, no nigericin. The data are obtained from a representative experiment. Note that addition of excess unlabeled GABA completely abolishes the nigericin-induced stimulation of [^{3}H]glycine uptake.

displayed a nigericin-induced enrichment similar to that of GABA. These results indicate that liposomes as well as proteoliposomes (not shown) are, at least under certain conditions, permeable for GABA and β-alanine whereas glycine is impermeant. Since glycine is transported by the vesicular GABA transporter with an efficiency comparable to GABA, it was used as substrate in the following experiments. As shown in Figure 8, glycine uptake into proteoliposomes reconstituted with synaptic vesicle proteins was significantly stimulated by nigericin. This stimulatory effect was long lasting and paralleled the nigericin-induced ΔpH. Furthermore, it was abolished in the presence of unlabeled GABA. These data indicate that artificially imposed pH gradients are capable of driving the GABA transporter, even in the presence of a slight Δψ of reversed (inside-negative) polarity.

To further confirm that ΔpH can energize glycine uptake, we designed experiments in which ΔpH was generated by the endogenous proton pump and Δψ was clamped to 0 mV by compensating K^+ fluxes. We have shown earlier that the vesicular proton pump is electrogenic and that charge balance is provided by a chloride channel present in the vesicle membrane (Maycox et al., 1988). Therefore, the proton pump was coreconstituted in proteoliposomes with the GABA transporter using KCl buffer. Under these conditions, ATP-dependent proton pumping results in the generation of a large ΔpH (net influx of HCl), but Δψ (inside positive) is still measurable [Figure 9; see also Hell et al. (1990)]. This residual Δψ was dissipated by addition of valinomycin (Figure 9b) which neutralizes any unbalanced charge movement over the membrane by a compensatory K^+ ion flux. In addition, ΔpH is increased (Figure 9a) after valinomycin addition. This indicates that $\Delta\mu_{H^+}$ generated by the proton pump remains constant, allowing a compensation of Δψ dissipation by an increase of ΔpH. In the presence of valinomycin, glycine uptake was only slightly (35%) reduced (data not shown), supporting the view that ΔpH can act as the sole driving force for the vesicular GABA transporter.

Discussion

The aim of this study was to investigate the bioenergetic properties of the GABA transporter of synaptic vesicles independently of the endogenous proton pump by applying artificially imposed Δψ and ΔpH. For this purpose, proteoliposomes were formed in different buffers to allow the generation of suitable ion gradients.

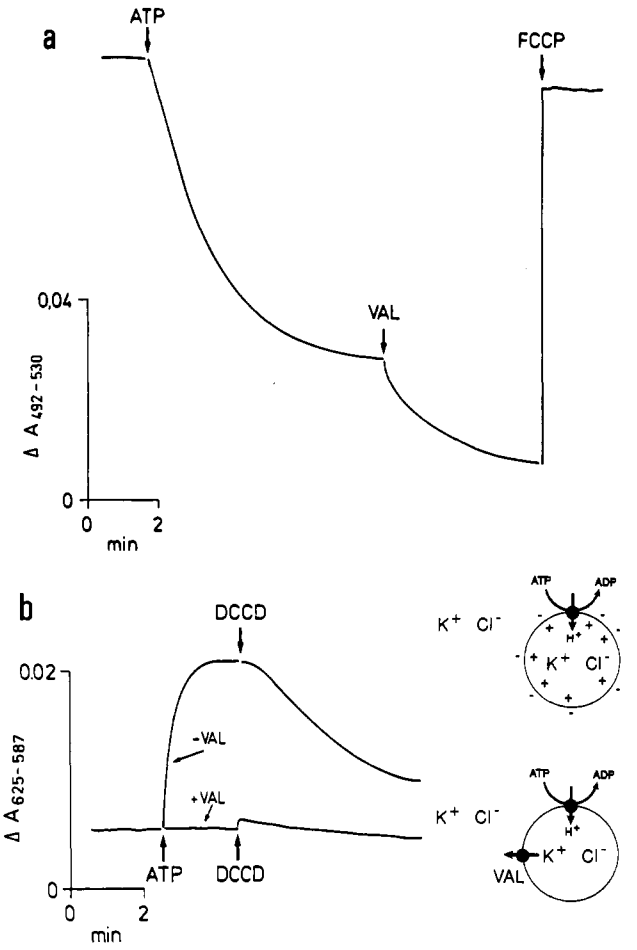

FIGURE 9: Effects of valinomycin (VAL) on ΔpH (a) and Δψ (b), created by the endogenous proton pump in proteoliposomes formed and incubated in KCl buffer. ΔpH and Δψ (inside positive) were measured with acridine orange and oxonol VI, respectively. ATP (2 mM final concentration), DCCD (0.2 mM final concentration), and FCCP (20 μM) were added where indicated. In (b), DCCD was used to inhibit the endogenous proton pump, resulting in a dissipation of Δψ. Note that valinomycin prevents the generation of ATP-dependent membrane potential (b) which is paralleled by a compensatory increase of ΔpH (a).

A transient Δψ was created by the formation of an inwardly directed potassium diffusion potential. This Δψ evoked [^{3}H]GABA uptake which was competed with excess unlabeled GABA, demonstrating that this uptake is transporter-mediated. Similarly, saturable [^{3}H]glutamate uptake was observed. Glutamate is transported by a carrier different from the GABA transporter. Glutamate uptake is entirely dependent on Δψ (Maycox et al., 1988, 1990b; Hell et al., 1990) and is transiently activated by a K^+ diffusion potential in intact synaptic vesicles (Shioi & Ueda, 1990). Together, our data show that the vesicular GABA transporter, like the vesicular glutamate transporter, can be driven by Δψ. This confirms our conclusions obtained previously with the endogenous proton pump as energy source (Hell et al., 1990).

To study whether the GABA transporter can be driven by ΔpH, an H^+ gradient was created by nigericin-induced K^+/H^+ exchange using an outwardly directed K^+ gradient as driving force. This analysis was complicated by the finding that induction of ΔpH caused a nonspecific enrichment of GABA and β-alanine in proteoliposomes as well as in pure phospholipid vesicles. This indicates that GABA and β-alanine, in their zwitterionic form, penetrates the liposome membrane. It is possible that both molecules can form intramolecular ion pairs between the COO^- and NH_3^+ groups which would result in

a six- and five-ring structure, respectively. In this form, the molecules would neutralize their charges and expose their hydrophobic backbone to the lipid phase of the bilayer, explaining their ability to cross the membrane. In contrast, glycine cannot form a stable ring structure and is therefore membrane-impermeant, allowing us to use [^{3}H]glycine to differentiate carrier-mediated uptake from nonspecific uptake. The unspecific ΔpH-dependent accumulation of GABA and β-alanine is probably due to a protonation of their weakly basic COO^- groups in the acidic liposome interior, resulting in the accumulation of their charged and membrane-impermeant forms. This mechanism is common to other membrane-permeant weak bases, e.g., amines (Rottenberg, 1989). We do not know whether native biological membranes are also permeant for GABA and β-alanine. The answer to this question is of some importance since even a limited membrane permeability of GABA would provide a leak pathway for GABA efflux from neurons and glia cells.

With glycine as a substrate, our data show that an artificially created ΔpH can act as a driving force for the vesicular GABA transporter. In this system, a small, inside-negative $\Delta\psi$ was present, probably due to a low K^+ permeability of the membrane. This potential should have an adverse effect (if any) on the transport rate. To entirely rule out interference by $\Delta\psi$, we analyzed glycine uptake in a system where the potential was clamped to 0 mV and ΔpH was generated by the endogenous proton pump. Again, carrier-mediated glycine uptake proceeded at a significant rate. We conclude that ΔpH can function as the sole driving force for the transporter.

Together, the experiments described here demonstrate that the GABA transporter of synaptic vesicles can be energized independently of ATP cleavage by artificially created $\Delta\psi$ and ΔpH. The data strongly support our view (Hell et al., 1990) that the transporter operates as an electrogenic GABA–proton exchanger.

ACKNOWLEDGMENTS

We are greatly indebted to Drs. Nils Brose and Peter R. Maycox (Martinsried, Germany) for helpful discussions and for critically reading the manuscript.

Registry No. GABA, 56-12-2; H^+, 12408-02-5; ATPase, 9000-83-3; glutamic acid, 56-86-0; glycine, 56-40-6.

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