Glutamate uptake into synaptic vesicles of bovine cerebral cortex and electrochemical potential difference of proton across the membrane

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Measurements have been made of the ATP-dependent membrane potential ($\Delta\psi$) and pH gradient (ΔpH) across the membranes of the synaptic vesicles purified from bovine cerebral cortex, using the voltage-sensitive dye bis[3-propyl-5-oxoisoxazol-4-yl]pentamethine oxanol and the ΔpH -sensitive fluorescent dye 9-aminoacridine respectively. A pre-existing small ΔpH (inside acidic) was detected in the synaptic vesicles, but no additional significant contribution by MgATP to ΔpH was observed. In contrast, $\Delta\psi$ (inside positive) increased substantially upon addition of MgATP. This ATP-dependent $\Delta\psi$ was reduced by thiocyanate anion (SCN^-), a $\Delta\psi$ dissipator, or carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP), a protonmotive-force dissipator. Correspondingly, a substantially larger glutamate uptake occurred in the presence of MgATP, which was inhibited by SCN^- and FCCP. A nonhydrolysable analogue of ATP, adenosine 5'-[β -methylene]triphosphate, did not substitute for ATP in either $\Delta\psi$ generation or glutamate uptake. The results support the hypothesis that a H^+ -pumping ATPase generates a protonmotive force in the synaptic vesicles at the expense of ATP hydrolysis, and the protonmotive force thus formed provides a driving force for the vesicular glutamate uptake. The $\Delta\psi$ generation by ATP hydrolysis was not affected by orthovanadate, ouabain or oligomycin, but was inhibited by *N*-ethylmaleimide, quercetin, trimethyltin, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole and 4-acetamido-4'-isothiocyanostilbene-2,2'-disulphonic acid. These results indicate that the H^+ -pumping ATPase in the synaptic vesicle is similar to that in the chromaffin granule, platelet granule and lysosome.

INTRODUCTION

Naito & Ueda (1983, 1985) have recently isolated small, spherical synaptic vesicles from bovine cerebral cortex, using antibodies to a synaptic vesicle-specific protein (Naito & Ueda, 1981), and have demonstrated that glutamate is specifically taken up into these vesicles in an ATP-dependent manner. These results provided biochemical support for the exocytotic release hypothesis of neuroactive glutamate in its synaptic transmission (Fonnum, 1984). The uptake is inhibited by proton ionophores, suggesting that vesicular glutamate uptake is driven by an electrochemical potential difference of H^+ across the membrane, i.e. a 'protonmotive force', generated by a vesicular MgATPase. Although there is good evidence that transport of biogenic amines into storage granules of the chromaffin cell is coupled with H^+ movement (Njus *et al.*, 1981, 1987), little is known about the mechanism of neuroactive amino acid uptake into synaptic vesicles in the central nervous system.

To provide further evidence for the involvement of protonmotive force in ATP-dependent vesicular glutamate uptake, we planned to correlate this uptake with ATP-dependent generation of protonmotive force, which consists of a H^+ gradient (ΔpH) and an electrical

potential difference ($\Delta\psi$) across the vesicular membrane. The limited quantity of the synaptic vesicle preparation available prohibited us from accurately measuring intravesicular volume, ΔpH and $\Delta\psi$ using radioactive chemicals (Njus *et al.*, 1981). Fortunately, some optical probes have been shown to be useful for semi-quantitative measurements of ΔpH and $\Delta\psi$ across biological membranes, using only a small amount of sample preparation. For example, the acridine dye, 9-aminoacridine, has been used to measure ΔpH in chloroplast (Schuldiner *et al.*, 1972), liposomes (Salama *et al.*, 1980) and secretory vesicles (Russell, 1984); and bis[3-propyl-5-oxoisoxazol-4-yl]pentamethine oxanol (Oxanol VI) has proved to be valuable for measuring $\Delta\psi$ of the neurosecretory vesicle membrane (Russell, 1984). In this study, we have used these probes to measure ΔpH and $\Delta\psi$ across the synaptic vesicle membrane and to correlate the protonmotive force and the vesicular glutamate uptake.

EXPERIMENTAL

Preparation of synaptic vesicles

Bovine brains were obtained from a local slaughterhouse and brought to the laboratory on ice. The cerebral

Abbreviations used: protonmotive force, electrochemical potential difference of proton across the membrane; ΔpH , $\Delta\psi$, the transmembrane pH and electrical potential difference respectively; ΔA , absorbance difference of Oxanol VI at wavelengths 625 and 587 nm; H^+ -ATPase, proton-translocating ATPase; Oxanol VI, bis[3-propyl-5-oxoisoxazol-4-yl]pentamethine oxanol; NEM, *N*-ethylmaleimide; $\text{ADP}(\text{CH}_2)_2\text{P}$, adenosine 5'-[β -methylene]triphosphate; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodi-imide; Nbd-Cl, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; SITS, 4-acetamido-4'-isothiocyanostilbene-2,2'-disulphonic acid.

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cortex was trimmed clean of membranes and white matter. Synaptosomes were prepared using a single sucrose density gradient (0.8 M-sucrose layer) based on the procedure of Hajos (1975). Briefly, the cerebral cortex was minced and homogenized in 0.32 M-sucrose/1 mM- NaHCO_3 /1 mM- MgCl_2 /0.5 mM- CaCl_2 . The homogenate was centrifuged at 1500 *g* (max.) for 10 min, and the supernatant was then collected, while a fluffy white layer above the pellet was carefully discarded. The supernatant was centrifuged at 21 500 *g* (max.) for 10 min. The pellet was layered onto 0.8 M-sucrose and centrifuged at 14 500 *g* (max.) for 20 min. A layer of 0.8 M-sucrose containing the synaptosomal fraction was collected, diluted with 1.5 vol. of cold deionized water and centrifuged at 22 000 *g* (max.) for 30 min. The pellet containing synaptosomes was suspended in a hypotonic 6 mM-Tris/HCl solution, pH 8.1, and synaptic vesicles released were collected from the lysate as described previously (Naito & Ueda, 1983) with a minor modification. The lysate was centrifuged at 39 100 *g* (max.) for 15 min, and the resulting supernatant was then centrifuged at 71 000 *g* (av.) for 100 min. The pelleted crude synaptic vesicle fraction was dispersed in a solution containing 0.32 M-sucrose and 1 mM- NaHCO_3 using a Teflon homogenizer (900 rev./min, 5 strokes) and centrifuged at 74 500 *g* (av.) for 100 min on a discontinuous sucrose density gradient (0.4 M-, 0.6 M- and 0.8 M-sucrose). The band at the 0.32 M-/0.4 M-sucrose interface and the 0.4 M-sucrose layer were combined, and the mixture was diluted with cold deionized water to bring the final sucrose concentration to 0.25 M, and spun at 150 000 *g* (av.) for 50 min. The pellet was resuspended in 0.32 M-sucrose containing 1 mM- NaHCO_3 . Small portions of this synaptic vesicle preparation were stored at -70°C . The yield was 4 mg of protein per 120 g of cerebral cortex, on average. For some experiments (e.g. see Fig. 1), synaptic vesicles were further purified by immunoprecipitation using anti-Synapsin I antibodies, as described previously (Naito & Ueda, 1983). Our minor modification of the preparation method by Naito & Ueda (1983) changed neither the yield of immunoprecipitation nor the specific activity of ATP-dependent glutamate uptake in the same assay conditions (however, the assay conditions were modified as described below).

Measurement of membrane potential using Oxanol VI

Membrane potential ($\Delta\psi$) was monitored by measuring changes in difference absorbance (ΔA) of Oxanol VI (5 μM) at wavelengths of 625 and 587 nm with an Aminco DW-2 dual-wavelength spectrophotometer. Dual-wavelength techniques provide a highly stable and sensitive readout of small specific absorbance changes in turbid biological material (Russell, 1984). Synaptic vesicles (136 μg of protein) were suspended in a standard solution (final vol., 1.7 ml) containing 5 mM-Tris/maleate, pH 7.4, 0.25 M-sucrose, 0.5 mM-ATP and 1 mM- MgSO_4 . This concentration of ATP was used instead of 2 mM because the latter gave a large fluorescence quenching in ΔpH measurements (see Results section). For some experiments, KCl was added to see the effect of Cl^- (Naito & Ueda, 1985). The temperature was maintained at 30°C by circulating water through the thermostated jacket of the cell holder. In some experiments, ΔA was calibrated against $\Delta\psi$ generated in synaptic vesicles by varying the Nernst potential for

potassium according to the published method (Russell, 1984); ΔA was measured at various concentrations (1–100 mM) of $[\text{K}^+]_{\text{out}}$, in the presence of 1 μM -valinomycin in the synaptic vesicle suspension. A plot of ΔA vs K^+ diffusion potential calculated from the Nernst equation was linear. For a quantitative comparison, the steady-state level or maximum level of ΔA was measured.

Measurement of pH gradient using 9-aminoacridine

Excitation and fluorescence emission (at 397 nm and 450 nm respectively) of 9-aminoacridine (1 μM), and fluorescence quenching by synaptic vesicles (400 μg of protein/ml) in the same media as described above, were measured with an Aminco-Bowman fluorescence spectrophotometer (Salama *et al.*, 1980; Russell, 1984). Nigericin and Triton X-100 were added to final concentrations of 7 μM and 0.04% respectively. Control experiments were carried out to ensure that the reagents used in the experiments did not alter the 9-aminoacridine fluorescence.

Vesicular uptake of L-glutamate

The uptake of glutamate into synaptic vesicles was assayed as described previously (Naito & Ueda, 1983) with a slight modification. Synaptic vesicles (20 μg of protein) were suspended in the media described above (final vol., 100 μl). After 5 min of incubation at 30°C , L- $[\text{^3H}]$ glutamate (potassium salt, final concentration, 50 μM ; specific radioactivity, 0.4 Ci/mmol) was added, and the reaction mixtures were filtered at 1.5 min or at intervals as indicated. Glutamate uptake at higher concentrations was shown in our previous report (Naito & Ueda, 1985). Values were usually expressed as means $\pm$ S.E.M.

Assay of ATPase and protein determination

ATPase activity and protein concentration were measured as previously described (Naito & Ueda, 1985).

Materials

9-Aminoacridine, ATP (Tris salt), 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (Nbd-Cl), *N,N'*-dicyclohexylcarbodi-imide (DCCD) and adenosine 5'- $[\beta,\gamma\text{-methylene}]$ triphosphate ($\text{ADP}(\text{CH}_2)_2\text{P}$) were purchased from Sigma. Oxanol VI was obtained from Molecular Probes, Junction City, OR, U.S.A. Carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP), nigericin and trimethyltin were gifts from Dr. R. Holz, Department of Pharmacology, University of Michigan, MI, U.S.A. 4-Acetamido-4'-isothiocyanostilbene-2,2'-disulphonic acid (SITS) was purchased from Pierce Chemicals, Rockford, IL, U.S.A. Sodium orthovanadate was from Aldrich, Milwaukee, WI, U.S.A. L-[2,3- ^3H]Glutamic acid (20 Ci/mmol) was obtained from Amersham.

RESULTS

Measurement of membrane potential with Oxanol VI

The changes in difference absorbance (ΔA) of Oxanol VI in a synaptic vesicle suspension were continuously monitored in the dual-wavelength mode at $\lambda = 625\text{--}587\text{ nm}$ (Fig. 1a). The addition of MgATP resulted in a large time-dependent increase in ΔA , reaching the steady state in 3–5 min. This suggests the generation of an inside positive membrane potential ($\Delta\psi$), since the K^+ diffusion

Fig. 1. Time courses of (a) the generation of membrane potential and (b) glutamate uptake in purified synaptic vesicles

ATP ($\circ$, ∇) or ADP(CH₂)P ($\square$) (final concentration 0.5 mM) was added at time 0 min to synaptic vesicles (136 μ g of protein/1.7 ml for the measurement of $\Delta\psi$ and 20 μ g of protein/100 μ l for uptake measurements) suspended in 5 mM-Tris/maleate, pH 7.4/0.25 M-sucrose, plus 1 mM-MgSO₄ ($\circ$, $\square$) or 1 mM-calcium acetate (∇). (a) Absorbance difference of Oxanolo VI (final concentration 5 μ M) at 625 nm and 587 nm was measured as an indicator of $\Delta\psi$ in synaptic vesicles. FCCP was added at 10 μ M (final concentration) to the ATP-treated sample at 10 min, and ATP was added to the ADP(CH₂)P-treated sample at 11 min. (b) Vesicular uptake of L-[³H]glutamate (final concentration 50 μ M) was measured by a filtration method as described in the Experimental section.

potential produced by valinomycin plus 100 mM-K⁺ also gave an absorbance change in the same direction (see the Experimental section). The subsequent addition of FCCP (10 μ M) reduced ΔA . When the experiment was carried out in the absence of Mg²⁺, no significant increase in ΔA was noticed (results not shown). Mn²⁺ was more effective than Mg²⁺ (about 1.5 times), but Ca²⁺ was far less effective (Fig. 1a). ADP(CH₂)P, a non-hydrolysable analogue of ATP, could not substitute for ATP (Fig. 1a), indicating that ATP hydrolysis is required for the generation of $\Delta\psi$. It might be noted that ΔA increased with subsequent addition of ATP, even in the presence of ADP(CH₂)P.

Thiocyanate anion, SCN⁻, is membrane-permeable and is known to abolish the inside positive $\Delta\psi$ of vesicles (Salama *et al.*, 1980). The MgATP-induced ΔA was decreased by thiocyanate in a dose-dependent manner (Fig. 2), which supports the notion that the ΔA observed here reflects changes in $\Delta\psi$. Chloride ion also, though less potently, inhibited the ATP-induced ΔA (49% inhibition at 20 mM). FCCP, a potent proton ionophore known to abolish the protonmotive force, also decreased ΔA in a dose-dependent fashion (Fig. 3). These results are consistent with the idea of $\Delta\psi$ generation by an electrogenic H⁺-pumping ATPase. Adding ATP to a synaptic vesicle suspension containing Oxanolo VI resulted in an increase in ΔA in a concentration-dependent manner (results not shown). Approximately 1 mM-ATP produced a half-maximal response; this is similar to the K_m value of ATP for glutamate uptake reported by Naito & Ueda (1985). ΔA was converted to $\Delta\psi$ (mV) values using a calibration line derived from the K⁺ diffusion potential (see the Experimental section). The maximum

internal positive potential created by ATP under the standard conditions (see the Experimental section) was estimated to be approx. +125 mV.

Measurement of vesicular pH gradient with 9-aminoacridine

The excitation and fluorescence emission spectra of 9-aminoacridine in sucrose media have been reported (Salama *et al.*, 1980; Russell, 1984). In a series of experiments, we observed that 9-aminoacridine fluorescence was quenched upon addition of synaptic vesicles in 0.25 M-sucrose/5 mM-Tris/maleate (pH 7.4) (16.2 $\pm$ 6.4% by synaptic vesicles containing 400 μ g protein/ml). Quenching was diminished by the addition of (1) Triton X-100 or (2) nigericin, a K⁺/H⁺-antiporter, in the presence of K⁺. Furthermore, extravesicular alkalization by Tris-base enhanced the quenching. Control experiments showed that KCl, nigericin, or Tris-base alone had no effect on the 9-aminoacridine fluorescence, whereas Triton X-100 caused minor quenching. These observations indicate that the fluorescence quenching measured was caused by a Δ pH.

In control solutions without synaptic vesicles, ATP itself caused substantial quenching (about 20% at 0.5 mM). Mg²⁺ did not affect quenching in control solutions, but decreased the quenching due to synaptic vesicles in the absence of ATP. Although these changes complicated the analysis of the effect of MgATP, correction for these contributions to the quenching did not show any significantly greater increase in Δ pH by the addition of MgATP under standard assay conditions.

The addition of 4 mM-KCl to the assay system did not

Fig. 2. Effect of various concentrations of thiocyanate on the synaptic vesicular membrane potential and glutamate uptake

Various amounts of potassium thiocyanate were added to the vesicle suspension, and the mixtures were preincubated for 5 min and then incubated for another 1.5 min with MgATP, as described in the Experimental section. Both $\Delta\psi$ (●) and uptake activities (○) are expressed as a percentage of control values obtained in the absence of thiocyanate.

enhance the quenching by synaptic vesicles in the presence of MgATP. Even the addition of 54 mM-KCl increased the net quenching by only 2–4 %, indicating very minor ATP-dependent changes in ΔpH even in the presence of permeable anions.

Relationship between membrane potential and synaptic vesicular glutamate uptake

It has previously been observed that isolated synaptic vesicles take up L-glutamate when MgATP is added to the medium (Naito & Ueda, 1983, 1985). The data presented in Fig. 1 show that vesicular L-glutamate uptake is preceded by the generation of $\Delta\psi$. When Mg^{2+} was replaced by Ca^{2+} , there was little glutamate uptake, similar to the effect on $\Delta\psi$. ADP(CH₂)P, a non-hydrolysable analogue of ATP, is ineffective in both increasing glutamate uptake and generation of $\Delta\psi$. In a separate experiment, Mn^{2+} showed stronger activity than Mg^{2+} in glutamate uptake (496 ± 63 and 219 ± 31 pmol/1.5 min per mg of protein in the presence of Mn^{2+} and Mg^{2+} respectively, at 50 μM -L-glutamate), which corresponds well to an ATP-dependent generation of $\Delta\psi$ in the presence of these ions (see the previous section). Moreover, MgATP-induced glutamate uptake was inhibited in parallel with the reduction of $\Delta\psi$ which was caused by either thiocyanate or FCCP (Figs. 2 and 3). MgATP-stimulated uptake of other neurotransmitters such as glycine, GABA (γ -aminobutyrate) and aspartate in this synaptic vesicle preparation has been very small in comparison with that of glutamate (Naito & Ueda, 1983). Similarly, only minor uptakes of acetylcholine, serotonin and dopamine were observed in the absence and presence of ATP (results not shown). These results

Fig. 3. Effect of various concentrations of FCCP on the synaptic vesicular membrane potential and glutamate uptake

For measurements of $\Delta\psi$ (●), the synaptic vesicles were incubated with MgATP for 4.5 min, and then small amounts of concentrated FCCP were successively added at 0.5 min intervals. For uptake measurements (○), the synaptic vesicles were preincubated for 5 min with various concentrations of FCCP. Then ATP was added and the reaction mixtures were incubated for an additional 1.5 min. Results are expressed as a percentage of the maximum inhibition produced by FCCP.

suggest that $\Delta\psi$ (positive inside) is an important element of the driving force specific for the vesicular glutamate uptake.

Proton-pumping ATPase activity

The highly purified synaptic vesicles from the bovine cerebral cortex possessed MgATPase activity. The activities were 371 ± 1 and 405 ± 3 nmol of P_i /1.5 min per mg of protein at 30°C in the absence and presence of 4 mM-KCl respectively, at 2 mM-ATP. These values are comparable to the specific activity of ATPase obtained in highly purified chromaffin granules (200–500 nmol/min per mg of protein at 30 °C, Apps *et al.*, 1982). The ATPase activity was shown to be Mg^{2+} -dependent, and was inhibited by 0.1 mM-NEM (70 % inhibition) and by 25 μM -trimethyltin (50 % inhibition), agents known to block H^+ -ATPase activity in chromaffin granules. The ATPase activity was unaffected by oligomycin and ouabain. These results suggest the presence of H^+ -ATPase in the synaptic vesicle. Further evidence for this comes from the presence of a ΔpH and the generation of MgATP-dependent $\Delta\psi$, which is FCCP-sensitive (see Fig. 1). Consistent with this notion, the MgATPase activity in the vesicle fraction was increased 2-fold by 1 μM -FCCP. FCCP dissipates the proton motive force, thereby activating the enzyme.

Effects of other agents on the vesicular H^+ -pump and glutamate uptake

Various ATPase inhibitors were tested for their ability to inhibit the H^+ -pumping and L-glutamate uptake

Table 1. Effects of various inhibitors on synaptic vesicular membrane potential and L-glutamate uptake

The MgATP-dependent membrane potential and glutamate uptake were determined using immunopurified synaptic vesicles in the standard sucrose-based medium as described in the Experimental section, except that various test agents were included in the preincubation medium (5 min preincubation). Membrane potential was determined after the absorbance difference of Oxanol VI reached a maximum. Glutamate uptake was measured for 1.5 min incubation. Membrane potential and glutamate uptake in the absence of 0.25% ethanol and inhibitors (control 1) was expressed as 100%. Glutamate uptake is expressed as means $\pm$ S.E.M. n.d., not determined

Inhibitors	Concentration	Relative activities (% of control 1)	
		Membrane potential	Uptake
Control 1		100 %	100 $\pm$ 7
Control 2	*	70	105 $\pm$ 9
Oligomycin	2.5 μ g/ml	114	112 $\pm$ 11
DCCD	0.2 mM*	47	75 $\pm$ 5
Ouabain	1 mM	96	96 $\pm$ 12
Vanadate	0.25 mM	73	113 $\pm$ 12
	0.5 mM	n.d.	131 $\pm$ 7
NEM	0.1 mM	n.d.	0 $\pm$ 10
	1 mM	0	0 $\pm$ 3
Trimethyltin	5 μ M	n.d.	91 $\pm$ 12
	50 μ M*	36	18 $\pm$ 1
SITS	0.5 mM	0	0 $\pm$ 1
Nbd-Cl	50 μ M	27	0 $\pm$ 3
Quercetin	0.5 mM	5†	56 $\pm$ 4

* 0.25 % ethanol was present.

† Quercetin produced an inhomogeneous suspension of synaptic residues.

activities in the synaptic vesicle (Table 1). Oligomycin, an inhibitor of mitochondrial H^+ -ATPase, and ouabain and orthovanadate, inhibitors of Na^+/K^+ -ATPase, had no significant effect on either the generation of $\Delta\psi$ or glutamate uptake. DCCD, a potent inhibitor of mitochondrial H^+ -ATPase, inhibited these activities only partially, even at 200 μ M. In contrast, NEM, trimethyltin, Nbd-Cl and SITS, inhibitors of H^+ -ATPase in the chromaffin granule (Cidon & Nelson, 1986), platelet (Fishkes & Rudnick, 1982) and lysosome (Moriyama *et al.*, 1984), exhibited potent inhibition of both $\Delta\psi$ generation and glutamate uptake in synaptic vesicles. The results are consistent with the idea that glutamate uptake is coupled with the efflux of H^+ down the electrochemical potential gradient generated by a H^+ -ATPase in the vesicle membrane. Also suggested is the possibility that the vesicular H^+ -ATPase is similar to that in the chromaffin granule, lysosome and platelet granule membranes, but distinct from that in the mitochondrial and plasma membranes.

DISCUSSION

In order to demonstrate chemi-osmotic coupling between ATP hydrolysis and glutamate transport into the synaptic vesicle, it is essential to satisfy three criteria:

(1) agents which dissipate the protonmotive force inhibit ATP-dependent transport; (2) ATP hydrolysis generates protonmotive force; and (3) protonmotive force imposed by other means than ATP also induces glutamate transport. Naito & Ueda (1985) provided evidence to satisfy criterion (1). Criterion (2) is addressed in this paper. The work dealing with criterion (3) will be presented in a separate paper. This study provides evidence that a $\Delta\psi$, inside positive, is generated as a result of ATP hydrolysis, and that its variation leads to changes in the amount of glutamate taken up into the synaptic vesicle, indicating that $\Delta\psi$ generated by a H^+ -ATPase is an important element of the driving force for vesicular glutamate uptake. The importance of $\Delta\psi$ in catecholamine uptake into chromaffin granules has been demonstrated by Holz (1978), Njus & Radda (1979) and Johnson & Scarpa (1979). To our knowledge, the results presented here represent the first direct evidence for the involvement of $\Delta\psi$ in vesicular glutamate uptake.

Many lines of evidence support the view that the ATP-induced $\Delta\psi$ studied here is not a resultant of contamination by other organelles or plasma membranes. (1) The specific activities of a marker enzyme for mitochondria and plasma membranes are minimal in the purified synaptic vesicle preparation (Naito & Ueda, 1983). In addition, Na^+ -dependent uptakes of aspartate and Cl^- residing in the synaptic plasma membrane were minimal in our vesicle fraction (J. Shioi, L. Sichel & T. Ueda unpublished work). (2) The $\Delta\psi$ measured in the vesicle fraction could not result from mitochondrial H^+ translocation since it possessed the opposite sign (positive inside) from that expected in mitochondria (negative inside). (3) Oligomycin, vanadate and ouabain did not inhibit MgATP-induced $\Delta\psi$. These compounds are specific inhibitors of mitochondria-type H^+ -ATPase, plasma membrane H^+ -ATPase and Na^+/K^+ -ATPase respectively (Goffeau & Slayman, 1981; Amzel & Pedersen, 1983; Serrano, 1984).

Although the amounts of the synaptic vesicle preparation used were comparable to or larger than that of other endomembrane preparations in similar 9-aminoacridine measurements (Salama *et al.*, 1980; Russell, 1984), the quenching was quite small. It is likely that our purified synaptic vesicle preparation produced a ΔpH smaller than that of other endomembrane systems. Another possibility not excluded is that a small proportion of sealed vesicles could lead to the small quenching.

In contrast, the magnitude of the ATP-generated $\Delta\psi$ (+125 mV) in the synaptic vesicle is larger than that in chromaffin granules (+50 to +70 mV; see the review by Njus *et al.*, 1981), platelet dense granules (+14 to +40 mV; Carty *et al.*, 1981; Fishkes & Rudnick, 1982) and serotonin-containing insulinoma vesicles (+45 to +70 mV; Hutton, 1982). It is an interesting possibility that the difference in a partition of each component of the protonmotive force may have evolved to fit the transporter mechanism for either weak base or weak acid.

A small amount of Cl^- (4 mM) enhanced the ATP-dependent uptake 5-fold (Naito & Ueda, 1985); but in this study it increased ATPase activity by only 9% (see the Results section), did not affect ΔpH and rather decreased the ATP-induced $\Delta\psi$ by 18% (results not shown). If Cl^- directly affected the H^+ -pump, one could expect a concomitant increase in ATPase activity, ΔpH and $\Delta\psi$. Thus, it is probable that Cl^- is involved in a

glutamate/H⁺ transport system rather than an ATP-driven H⁺-pump.

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