Involvement of vH + −ATPase in synaptic vesicle swelling

Article *in* Journal of Neuroscience Research · January 2010

DOI: 10.1002/jnr.22180 · Source: PubMed

9 authors, including:

Aleksandar Jeremic
George Washington University
35 PUBLICATIONS **1,270** CITATIONS

SEE PROFILE

Jin-Sook Lee
Wayne State University
21 PUBLICATIONS **313** CITATIONS

SEE PROFILE

Won Jin Cho
Wayne State University
39 PUBLICATIONS **970** CITATIONS

SEE PROFILE

Published in final edited form as:
J Neurosci Res. 2010 January ; 88(1): 95–101. doi:10.1002/jnr.22180.

Involvement of vH+-ATPase in synaptic vesicle swelling

Leah Shin[*], **Nirukti Basi**[*], **Aleksandar Jeremic**, **Jin-Sook Lee**, **Won Jin Cho**, **ZhiHui Chen**, **Rania Abu-Hamdah**, **David Oupicky**[¶], and **Bhanu P Jena**[†]
Department of Physiology, Wayne State University School of Medicine, Detroit, MI, USA 48201

[¶]Department of Pharmaceutical Science, Wayne State University, Detroit, MI, USA 48202

Abstract

Secretory vesicle swelling is central to cell secretion, however the underlying mechanism of vesicle swelling, particularly synaptic vesicles, is not completely understood. The $G_{\alpha i3}$-PLA2-mediated involvement of water channel AQP-1 in the regulation of secretory vesicle swelling in exocrine pancreas, and the $G_{\alpha o}$-mediated AQP-6 involvement in synaptic vesicle swelling in neurons, has previously been reported. Furthermore, the role of vH^+-ATPase in neurotransmitter transport into synaptic vesicles, has also been shown. Using nanometer scale precision measurements of isolated synaptic vesicles, the present study reports for the first time, the involvement of vH^+-ATPase in GTP-$G_{\alpha o}$-mediated synaptic vesicle swelling. Results from this study, demonstrate that the GTP-$G_{\alpha o}$-mediated vesicle swelling is vH^+-ATPase–dependent, and pH sensitive. Zeta potential measurements of isolated synaptic vesicles further demonstrate, a bafilomycin-sensitive vesicle acidification, following the GTP-$G_{\alpha o}$-induced swelling stimulus. Since water channels are bidirectional, and the vH^+-ATPase inhibitor bafilomycin decreases both the volume of isolated synaptic vesicles and GTP-mastoparan stimulated swelling, suggests vH^+-ATPase to be upstream of AQP-6, in the pathway leading from $G_{\alpha o}$-stimulated swelling of synaptic vesicles. Vesicle acidification is therefore a prerequisite for AQP-6 mediated gating of water into synaptic vesicles.

Keywords

Synaptic vesicle swelling; vH^+-ATPase; AQP6; Photon correlation spectroscopy; Atomic Force Microscopy

INTRODUCTION

Earlier studies demonstrate the requirement of secretory vesicle swelling in cell secretion (Kelly et al., 2004). Although recent studies (Jeremic et al., 2005) provide much progress in our understanding of synaptic vesicle swelling, molecular details underlying the process, remain to be fully elucidated. The first direct measurement of secretory vesicle swelling at nanometer resolution within live cells was reported in studies on pancreatic acinar cells, using atomic force microscopy (AFM) (Cho et al., 2002). In neurons, the water channel AQP-6 and the heterotrimeric GTP-binding protein $G_{\alpha o}$ at the synaptic vesicle membrane, participate in rapid water gating and vesicle swelling (Jeremic et al., 2005). It has previously been shown that vH^+-ATPases are present at the synaptic vesicle membrane (Stadler and

[†]To whom correspondence should be addressed: Department of Physiology Wayne State University School of Medicine 5245 Scott Hall, 540 E. Canfield, Detroit, MI 48201 Telephone: 313-577-1532 Fax: 313-993-4177 bjena@med.wayne.edu .
[*]Equal contribution
Aleksandar Jeremic's current address: Department of Biological Sciences, George Washington University, 340 Lisner Hall, 2023 G Street, NW Washington, DC 20052, USA

Tsukita, 1984; Hicks and Parsons, 1992), and are responsible for the generation of electrochemical H^+ gradient (pH 5.2-5.5) within vesicles (Michaelson and Angel, 1980; Füldner and Stadler, 1982), required for transport of neurotransmitters into the vesicle lumen. In addition to the established role of vH^+-ATPase in neurotransmitter transport into synaptic vesicles, vH^+-ATPase has been suggested to participate in the secretion of the stored neurotransmitters (Morel et al., 2001; Peters et al., 2001). Furthermore, guanine nucleosides have been reported to influence the glutamate-induced cellular response via diverse trophic, proliferative, and modulatory effects of the nucleotide on neurons (Santos et al., 2005). Since synaptic vesicle swelling is $G_{\alpha o}$-mediated, and is required for cell secretion (Kelly et al., 2004), the vH^+-ATPase in synaptic vesicle membrane (Stadler and Tsukita, 1984; Hicks and Parsons, 1992), may participate in $G_{\alpha o}$-mediated water gating through the AQP-6 channels at the synaptic vesicle membrane, resulting in vesicle swelling. This hypothesis was tested in the present study. In agreement, our results demonstrate that the synaptic vesicle-associated vH^+-ATPase, is required for GTP-$G_{\alpha o}$-mediated swelling of synaptic vesicles.

Mastoparan, the amphiphilic tetradecapeptide from wasp venom, has been demonstrated to activate the GTPase activity of $G_{\alpha o/i}$ proteins (Konrad et al., 1995; Higashijima et al., 1988; Vitale et al., 1993). Stimulation of G proteins is believed to occur by the peptide inserting into the phospholipid membrane and mimicking a highly structured-helix that resembles the intracellular loops of G protein-coupled receptors. Analogous to receptor activation, mastoparan is thought to interact with the COOH-terminal domain of the G protein subunit (Weingarten et al., 1990). In the current study, GTP-mastoparan stimulated synaptic vesicle swelling, which was abrogated in the presence of vesicular proton pump inhibitor bafilomycin. Isolated synaptic vesicles exposed to various concentrations of ATP had no influence on GTP-mastoparan stimulated activity (data not shown). The possible reason for this may be that ATP being a neurotransmitter, and present in synaptic vesicles, is there to be utilized by the vH^+-ATPase following stimulation. Our study further demonstrates that the GTP-mastoparan stimulated swelling of synaptic vesicle is pH-sensitive. Since water channels are bidirectional, and bafilomycin exposure decreases the volume of isolated synaptic vesicles, this finding suggests that vH^+-ATPase function is upstream of the water channel AQP-6, in the GTP-$G_{\alpha o}$-mediated pathway. Hence it is concurred from these results, that synaptic vesicle acidification is required for AQP-6 mediated gating of water.

MATERIALS AND METHODS

Synaptosome and synaptic vesicle isolation

Synaptosomes and synaptic vesicles were prepared from rat brains using published procedures (Kelly et al., 2004; Jeremic et al., 2005). Whole brain from Sprague-Dawley rats, weighing 100-150 g, was isolated and placed in ice-cold buffered sucrose solution (5 mM Hepes pH 7.5, 0.32 M sucrose), supplemented with protease inhibitor cocktail (Sigma, St. Louis, MO). The brain tissue was homogenized using 8-10 strokes in a Teflon-glass homogenizer. The total homogenate was centrifuged for 3 min at 2,500 × *g*, and the supernatant fraction was further centrifuged for 15 min at 14,500 × *g*, to obtain a pellet. The resultant pellet was resuspended in buffered sucrose solution, and loaded onto a 3-10-23 % Percoll gradient. After centrifugation at 28,000 × *g* for 6 min, the enriched synaptosome fraction was collected at the 10-23 % Percoll gradient interface. To isolate synaptic vesicles, the synaptosome preparation was diluted using 9 vol. of ice-cold water, resulting in the lysis of synaptosomes to release synaptic vesicles, followed by 30 min incubation on ice. The homogenate was then centrifuged for 20 min at 25,500 × *g*, and the resultant supernatant enriched in synaptic vesicles was obtained.

Transmission electron microscopy

Isolated synaptic vesicle preparations were fixed in 2.5% buffered paraformaldehyde for 30 min, followed by dehydration and embedding in Unicryl resin. The resin-embedded tissue was sectioned at 40-70 nm. Thin sections were transferred to coated specimen TEM grids, dried in the presence of uranylacetate and methylcellulose, and examined in a JOEL transmission electron microscope.

Synaptic vesicle size measurements using photon correlation spectroscopy

Changes in synaptic vesicle size were determined using photon correlation spectroscopy (PCS). PCS is a well-known technique for the measurement of size of micron to nm size particles and macromolecules. PCS measurements were performed in a Zetasizer Nano ZS, (Malvern Instruments, UK). In a typical experiment, the size distribution of isolated synaptic vesicles was determined using built-in software provided by Malvern Instruments. Prior to determination of the vesicle hydrodynamic radius, calibration of the instrument was performed using latex spheres of known size. In PCS, subtle fluctuations in the sample scattering intensity are correlated across microsecond time scales. The correlation function was calculated, from which the diffusion coefficient was determined. Using Stokes-Einstein equation, hydrodynamics radius can be acquired from the diffusion coefficient (Higashijima et al., 1988). The intensity size distribution, which is obtained as a plot of the relative intensity of light scattered by particles in various size classes, is then calculated from a correlation function using built-in software. The particle scattering intensity is proportional to the molecular weight squared. Volume distribution can be derived from the intensity distribution using Mie theory (Vitale et al., 1993; Weingarten et al., 1990). The transforms of the PCS intensity distribution to volume distributions can be obtained using the provided software by Malvern Instruments. In experiments, isolated synaptic vesicles were suspended in isotonic buffer containing 0.3 M Sucrose, 10 mM Hepes pH 7.5, and 20 mM KCl, and changes in vesicle size monitored prior to and after addition of 40 μM GTP-mastoparan, and or 1 nM bafilomycin. Student's *t*-test was performed for comparison between groups (n=5) with significance established at $p<0.05$(*).

Measurements of synaptic vesicle size using right angle light scattering

Similar to PCS, isolated synaptic vesicles were suspended in isotonic buffer (0.3 M Sucrose; 10 mM Hepes pH 7.5; and 20 mM KCl) and changes in vesicle size monitored prior to and following the addition of 40 μM GTP-mastoparan, and or 1 nM bafilomycin. Synaptic vesicle size dynamics were determined using real time right angle light scattering, in a Hitachi F-2000 spectrofluorimeter. Scattered light intensities at 600 nm were measured as a function of vesicle radius (Cho et al., 2004). Values are expressed in arbitrary units and as percent light scattered over controls. Student's *t*-test was performed for comparison between groups (n=5) with significance established at $p<0.05$(*).

Synaptic vesicle acidification determined from zeta potential measurements

Zeta potential is the overall surface charge a particle acquires in a certain medium. Hence in the case of liposomes or isolated synaptic vesicles in aqueous media, the zeta potential is a direct reflection of both the internal and external pH of vesicles. If more alkali buffer is added to the vesicle suspension, then the vesicles acquire more negative charge. In contrast, if acid is added to the suspension, the vesicles acquire less negative charge. This implies that the pH of the buffer both within and outside the vesicle, dictates the zeta potential or the net surface charge of the vesicle. Experiments were performed on isolated synaptic vesicles suspended in isotonic buffer containing 0.3 M sucrose, 10 mM Hepes pH 7.5, and 20 mM KCl, and changes in vesicle zeta potential monitored prior to and after addition of 40 μM GTP-mastoparan, and or 1 nM bafilomycin. Zeta potential was determined using the

Zetasizer Nano ZS, from Malvern Instruments, UK. Student's *t*-test was performed for comparison between groups (n=8) with significance established at $p<0.001$(*).

Atomic force microscopy

Isolated synaptosome membrane or synaptic vesicles in buffer were plated on freshly cleaved mica, to be imaged using the atomic force microscope (AFM). Ten minutes after plating, the mica disk was placed in a fluid chamber and washed with the incubation buffer to remove unattached membrane and or synaptic vesicles, prior to imaging in the presence or absence of 40 μM GTP-mastoparan, and or 1 nM bafilomycin. Isolated synaptosome membrane and synaptic vesicles were imaged using the Nanoscope IIIa, Digital Instruments (Santa Barbara, CA). All images presented in this study were obtained in the "tapping" mode in fluid, using silicon nitride tips with a spring constant of 0.06 Nm^{-1} and an imaging force of less than 200 pN. Images were obtained at line frequencies of 2.523 Hz, with 512 lines per image and constant image gains. Topographical dimensions of synaptic vesicles were analyzed using the NANOSCOPE (R) IIIA 4.43r8 software, supplied by Digital Instruments.

Immunoblot analysis

Protein content in the various brain fractions, were determined by the Bradford method (Bradford, 1976). Sample aliquots solubilized in Laemmli (Laemmli, 1970) sample preparation buffer, were resolved using 12.5% SDS-PAGE. Five micrograms protein, each from total brain homogenate, synaptosome, and synaptic vesicle fractions, were resolved using SDS-PAGE. Resolved proteins were electrotransferred to nitrocellulose membrane for immunoblot analysis using specific antibodies to VAMP-2 (Alomone Labs, Jerusalem, Israel), vH^+-ATPase and AQP-6 from the Aquaporins1-9 kit (α–Diagnostic, San Antonio, TX). The nitrocellulose membranes electrotransferred with the resolved proteins were incubated for 1 h at 4°C in blocking buffer (5% non-fat milk in PBS containing 0.1% Triton X-100 and 0.02% NaN_3), and immunoblotted for 1 h at room temperature with the specific antibody. Primary antibodies were used at a dilution of 1:3,000 (VAMP-2); 1:1000 AQP-6 and vH^+-ATPase) in blocking buffer. The immunoblotted nitrocellulose sheets were washed in PBS containing 0.1% Triton X-100 and 0.02% NaN_3 and incubated for 1 h at room temperature in horseradish peroxidase-conjugated secondary antibody at a dilution of 1:3,000 in blocking buffer. The immunoblots were then washed in PBS buffer, processed for enhanced chemiluminescence (GE Healthcare Life Sciences, Piscataway, NJ) and developed using a Kodak 440 image station.

RESULTS AND DISCUSSION

Electron microscopy (EM) (Fig. 1A), atomic force microscopy (AFM) (Fig. 1B), immunoblot analysis (Fig. 1C), and photon correlation spectroscopy (PCS) (Fig. 2A), demonstrated a highly enriched synaptic vesicle (SV) preparation. Synaptic vesicles were found to have a mean diameter of 35 nm. Immunoblot analysis further demonstrated the SV preparation to be enriched in VAMP-2 and AQP-6 (Fig. 1C), both SV-specific proteins (Jeremic et al., 2005). Collectively, these studies demonstrate the isolation of a highly enriched SV preparation from brain tissue, for our SV swelling assays.

To determine the relative concentration of vH^+-ATPase in SV, immunoblot analysis was performed using 5 μg each of total brain homogenate (BH), isolated synaptosome (S), and SV fractions (Fig. 1C). In conformation with earlier findings (Stadler and Tsukita, 1984;Hicks and Parsons, 1992), vH^+-ATPase was present both in the S and the SV fraction, but was enriched in SV (Fig. 1C). To determine the role of vH^+-ATPase on SV swelling, the size of isolated SV was monitored prior to and following addition of the vH^+-ATPase

inhibitor bafilomycin (Fig. 2A-C, 2E). Exposure of SV to a bafilomycin concentration of as low as 0.5 nM, was found to significantly (~25%, $p<0.001$) inhibit the GTP-mastoparan-stimulated SV swelling. Surprisingly, exposure of unstimulated SV to bafilomycin, resulted in 18-20% ($p<0.001$) decrease in SV size (Fig. 2B). Since bafilomycin directly inhibits vH^+-ATPase, bypassing the SV membrane-associated $G_{\alpha o}$ protein, the participation of vH^+-ATPase is upstream to AQP-6, in the GTP-$G_{\alpha o}$ stimulated pathway of SV swelling. These results further demonstrate that in the resting state (unstimulated or steady state), SV volume is in part regulated by the SV membrane-associated water channel AQP-6, and the proton pump vH^+-ATPase. It is highly likely, that at the SV membrane, facilitated water transport into the vesicle via AQP-6 is a consequence of vH^+-ATPase-induced intravesicular acidification.

Earlier studies report that exposure to moderately acidic extracellular pH (pH 6.5), augments plasmalemmal vH^+-ATPase activity in cultured osteoclasts (Nordstrom et al., 1997). In addition, studies (Bastani et al., 1991, 1994; Chambrey et al., 1994) report that the number of vH^+-ATPase in apical membrane of renal epithelial cells, are up regulated in animals exposed to acidosis (Bastani et al., 1991, 1994; Chambrey et al., 1994). In view of this, the role of pH on vH^+-ATPase activity at the SV membrane and on GTP-$G_{\alpha o}$-mediated SV swelling was explored (Figure 2C, 2D). Our studies demonstrate that GTP-$G_{\alpha o}$-mediated SV swelling is pH-sensitive, since exposure of isolated SV to both alkaline and acidic buffers, resulted in a complete abrogation of both GTP-mastoparan-stimulable SV swelling, and bafilomycin-induced SV deflation. The GTP-mastoparan-stimulable SV swelling was found to be rapid (Figure 2E), as opposed to SV swelling in hypotonic medium. No change in SV size is determined up to 105 seconds following exposure to a 50% hypotonic buffer (data not shown). Since bafilomycin is specific in its inhibition of vH^+-ATPase, this pH-sensitivity of SV swelling may be a reflection of the direct inhibition of vH^+-ATPase activity under both basic and acidic conditions (Figure 2B-D).

The role of vH^+-ATPase activity on the potency and efficacy of SV swelling was further evaluated by online real-time light scattering studies (Figure 2E). The kinetics of GTP-Mas-induced swelling of SV in presence or absence of bafilomycin, was therefore determined. GTP-Mas-induced SV swelling takes on logarithmic form that can be expressed by a first-order equation, with a rate constant $k=0.0106$ s-1 (Figure 2E). Bafilomycin was found to decrease the rate of GTP-Mas stimulated SV swelling by ~35% to $k=0.0068$ s-1. Results from the study demonstrate that bafilomycin exposure decrease both the potency and efficacy of GTP-Mas-induced SV swelling by ~20% and 35%, respectively, suggesting a strong correlation between AQP-6 and vH^+-ATPase activity at the SV membrane.

Further conformation of the inhibition of GTP-mastoparan stimulated increase in isolated SV volume following exposure to bafilomycin, was demonstrated using AFM (Figure 3). Exposure of SV to 40 μM GTP-mastoparan, demonstrated a robust increase in vesicle size (Figure 3A). However, prior exposure of SV's to 1 nM bafilomycin, resulted in a significant (*$p<0.001$) inhibition of the GTP-mastoparan-induced swelling of synaptic vesicles (Figure 3B, C).

Next, to determine if exposure to bafilomycin results in a decrease in vesicle acidification, and to further test the hypothesis that exposure of isolated SV's to both acidic and basic pH abrogated vH^+-ATPase function, zeta potential measurements were carried out on SV's exposed to bafilomycin, and to various pH conditions in the presence and absence of GTP-mastoparan. Isolated SV's are negatively charged, with a zeta potential of approximately −20 to −25 mV. Vesicle acidification was measured as a net loss of negativity of the SV membrane. Exposure of SV to GTP-Mas results in vesicle acidification, and a net loss in negative charges at the vesicle membrane (-24.4 ± 2.1 to -12.3 ± 1.0). Exposure of SV's to

the vH^+-ATPase inhibitor bafilomycin, abrogates the GTP-Mas induced net loss in negative charges at the vesicle membrane (Figure 4A). With the exception of pH 7.5, vesicles in either acidic or alkaline medium, fail to elicit any change in their zeta potential (Figure 4B), demonstrating that both acidic and basic suspension mediums inhibited vH^+-ATPase activity, thereby preventing the entry of protons into the vesicle lumen. Results from these studies support that luminal acidification of SV is a requirement for AQP-6-induced active water transport and vesicle swelling, and either low (pH 4) or high (pH 8) pH, inhibits the activity of SV-associated vH^+-ATPase (Figure 4). However, since $G_{\alpha o}$-stimulated SV acidification is completely abrogated (Figure 4A) and bafilomycin is able to inhibit only partially the $G_{\alpha o}$-stimulated SV swelling (Figure 2B), suggests that vH^+-ATPase is in part responsible for $G_{\alpha o}$-mediated SV swelling.

In the current study, an assay system of monitoring nm scale changes in synaptic vesicle size was used in determining the role of vH^+-ATPase, in the regulation of SV volume. Unlike slow water entry by diffusion, GTP-mastoparan-induced rapid swelling of SV's via facilitated transport of water molecules through water channels or aquaporins at the SV membrane has been previously reported (Jeremic et al., 2005). In these earlier studies (Jeremic et al., 2005), AQP-6 at the SV membrane was demonstrated to be involved in GTP-mastoparan-induce SV swelling (Jeremic et al., 2005). Results from the current study, further demonstrate that the $G_{\alpha o}$-stimulated SV swelling is vH^+-ATPase–dependent, and pH sensitive. Since water channels including AQP-6 are bidirectional, and the vH^+-ATPase inhibitor bafilomycin decreases the volume of resting synaptic vesicles, suggests vH^+-ATPase to be upstream of AQP-6, in the pathway leading from $G_{\alpha o}$-stimulated swelling of synaptic vesicles (Figure 5). In Figure 5, the involvement of vH^+-ATPase in GTP-$G_{\alpha o}$-mediated synaptic vesicle swelling is summarized. Since our results demonstrate a bafilomycin (BM)-sensitive vesicle acidification following GTP-$G_{\alpha o}$ stimulus, and because water channels are bidirectional and the vH^+-ATPase inhibitor BM decreases both the volume of isolated synaptic vesicles and GTP-mastoparan stimulated swelling, suggests vH^+-ATPase to be upstream of AQP-6. Vesicle acidification is therefore a prerequisite for AQP-6 mediated rapid gating of water into synaptic vesicles. To our knowledge, this is the first direct demonstration of the involvement of synaptic vesicle membrane-associated vH^+-ATPase in $G_{\alpha o}$ –stimulated SV swelling.

Acknowledgments

This work was supported by NIH grant NS39918 and support from the Wayne State University Research Enhancement Program (B.P.J).

REFERENCES

Bastani B, McEnaney S, Yang L, Gluck S. Adaptation of inner medullary collecting duct vacuolar H-adenosine triphosphatase to chronic acid or alkali loads in the rat. Exp Nephrol. 1994; 2:171–175. [PubMed: 7922269]

Bastani B, Purcell H, Hemken P, Trigg D, Gluck S. Expression and distribution of renal vacuolar proton-translocating adenosine triphosphatase in response to chronic acid and alkali loads in the rat. J Clin Invest. 1991; 88:126–136. [PubMed: 1829094]

Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem. 1976; 72:248–254. [PubMed: 942051]

Chambrey R, Paillard M, Podevin RA. Enzymatic and functional evidence for adaptation of the vacuolar H-ATPase in proximal tubule apical membranes from rats with chronic metabolic acidosis. J Biol Chem. 1994; 269:3243–3250. [PubMed: 8106360]

Cho SJ, Cho J, Jena B. The number of secretory vesicles remains unchanged following exocytosis. Cell Biol Int. 2002; 26:29–33. [PubMed: 11779218]

Cho WJ, Jeremic A, Rognlien K, Zhvania M, Lazrishvili I, Tamar B, Jena B. Structure, isolation, composition and reconstitution of the neuronal fusion pore. Cell Biol Int. 2004; 28:699–708. [PubMed: 15516328]

Füldner H, Stadler H. 31P-NMR analysis of synaptic vesicles. Status of ATP and internal pH. Eur J Biochem. 1982; 121:519–524. [PubMed: 7056254]

Hicks B, Parsons S. Characterization of the P-type and V-type ATPases of cholinergic synaptic vesicles and coupling of nucleotide hydrolysis to acetylcholine transport. J Neurochem. 1992; 58:1211–1220. [PubMed: 1312570]

Higashijima T, Uzu S, Nakajima T, Ross E. Mastoparan, a peptide toxin from wasp venom, mimics receptors by activating GTP-binding regulatory proteins (G proteins). J Biol Chem. 1988; 263:6491–6494. [PubMed: 3129426]

Jeremic A, Cho WJ, Jena B. Involvement of water channels in synaptic vesicle swelling. Exp Biol Med. 2005; 230:674–680.

Kelly M, Cho WJ, Jeremic A, Abu-Hamdah R, Jena B. Vesicle swelling regulates content expulsion during secretion. Cell Biol Int. 2004; 28:709–716. [PubMed: 15516329]

Konrad R, Young R, Record R, Smith R, Butkerait P, Manning D, Jarett L, Wolf B. The heterotrimeric G-protein Gi is localized to the insulin secretory granules of beta-cells and is involved in insulin exocytosis. J Biol Chem. 1995; 270:12869–12876. [PubMed: 7759545]

Laemmli U. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227:680–685. [PubMed: 5432063]

Michaelson D, Angel I. Determination of ΔpH in cholinergic synaptic vesicles: its effect on storage and release of acetylcholine. Life Sci. 1980; 27:39–44. [PubMed: 7401925]

Morel N, Dunant Y, Israël M. Neurotransmitter release through the V0 sector of V-ATPase. J Neurochem. 2001; 79:485–488. [PubMed: 11701751]

Nordstrom T, Shrode L, Rotstein O, Romanek R, Guto T, Heersche J, Manolson M, Brisseau G, Grinstein S. Chronic extracellular acidosis induces plasmalemma vacuolar type H+ ATPase activity in osteoclasts. J Biol Chem. 1997; 272:6354–6360. [PubMed: 9045656]

Peters C, Bayer M, Bühler S, Andersen J, Mann M, Mayer A. Trans-complex formation by proteolipid channels in the terminal phase of membrane fusion. Nature. 2001; 409:581–588. [PubMed: 11214310]

Santos T, Souza D, Tasca C. GTP uptake into rat synaptic vesicles. Brain Res. 2005; 1070:71–76. [PubMed: 16405924]

Stadler H, Tsukita S. Synaptic vesicles contain an ATP-dependent proton pump and show ‘knob-like’ protrusions on their surface. EMBO J. 1984; 3:3333–3337. [PubMed: 6151900]

Vitale N, Mukai H, Rouot B, Thierse D, Aunis D, Bader MF. Exocytosis in chromaffin cells. Possible involvement of the heterotrimeric GTP-binding protein G(o). J Biol Chem. 1993; 268:14715–14723. [PubMed: 7686903]

Weingarten R, Ransnas L, Mueller H, Sklar L, Bokoch G. Mastoparan interacts with the carboxyl terminus of the alpha subunit of Gi. J Biol Chem. 1990; 265:11044–11049. [PubMed: 2113529]

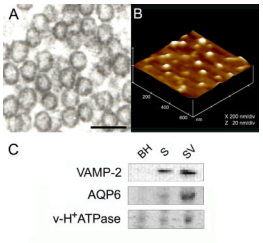

Figure 1.

Association of vH^+–ATPase with synaptic vesicles. Purity of synaptic vesicles was determined using transmission electron microscopy (A, Bar = 100 nm), atomic force microscopy (B), and immunoblot analysis (C) on isolated synaptic vesicles. Both electron, and atomic force micrographs demonstrate the average size of synaptic vesicles to be 40 nm, which is further confirmed by photon correlation spectroscopy in figure 2. Immunoblot analysis of 5 μg protein each, of total rat brain homogenate (BH), synaptosome (S) and synaptic vesicles (SV), demonstrate the enriched presence of SV proteins VAMP-2 and the water channel AQP6. Note the enriched presence of vH^+–ATPase in the SV fraction.

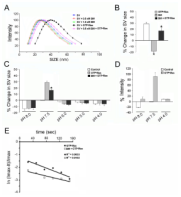

Figure 2.

Guanosine triphosphate-mastoparan (GTP-Mas) induced synaptic vesicle (SV) swelling is both pH and bafilomycin (BM) sensitive. Isolated SV swell in response to 40 μM GTP-Mas, as demonstrated using photon correlation spectroscopy (A-C). Similarly, right angle light scattering also demonstrates an increase in SV size following exposure to the GTP-Mas mixture. Exposure of SV to 0.5 or 1nM of the vH^+–ATPase inhibitor bafilomycin or low pH, significantly inhibits GTP-Mas induced vesicle swelling (A-D). Note the loss in SV size following exposure to bafilomycin alone (B). The GTP-Mas induced SV swelling is pH-sensitive (C), since besides the near physiological pH of 7.5, both alkaline and acidic environments, nearly abolish GTP-Mas induced swelling of SV, as determined both by photon correlation spectroscopy (C), and dynamic light scattering measurements (D). (B-D, n= 5, *p<0.001).

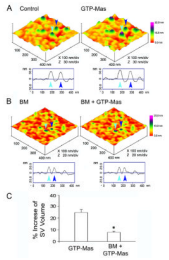

Figure 3.

Atomic force microscopy on synaptic vesicles demonstrating inhibition of GTP-Mas induced synaptic vesicle (SV) swelling by bafilomycin (BM). In conformation with photon correlation spectroscopy and dynamic light scattering studies (figure 2), isolated SV (A, left) swell when exposed to 40 μM GTP-Mas (A, right). Exposure of SV to 1nM of the vH^+–ATPase inhibitor bafilomycin (B, left), significantly inhibits GTP-Mas induced vesicle swelling (B, right). Note the loss in GTP-Mas induced SV volume increase following exposure to bafilomycin (C, n = 22, *$p<0.001$).

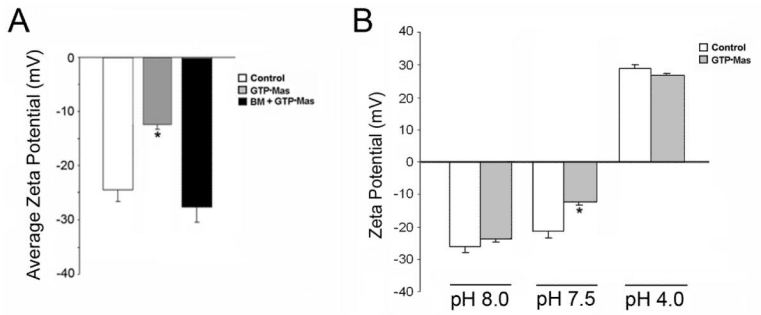

Figure 4.

Exposure of SV's to the vH^+-ATPase inhibitor bafilomycin, abrogates the GTP-Mas induced net loss in negative charges at the vesicle membrane by inhibiting vesicle acidification. (A) With the exception of pH 7.5, vesicles in either acidic or alkaline medium, fail to elicit any change in their zeta potential (B), demonstrating that both acidic and basic suspension mediums inhibited vH^+-ATPase activity, thereby preventing the entry of protons into the vesicle lumen.

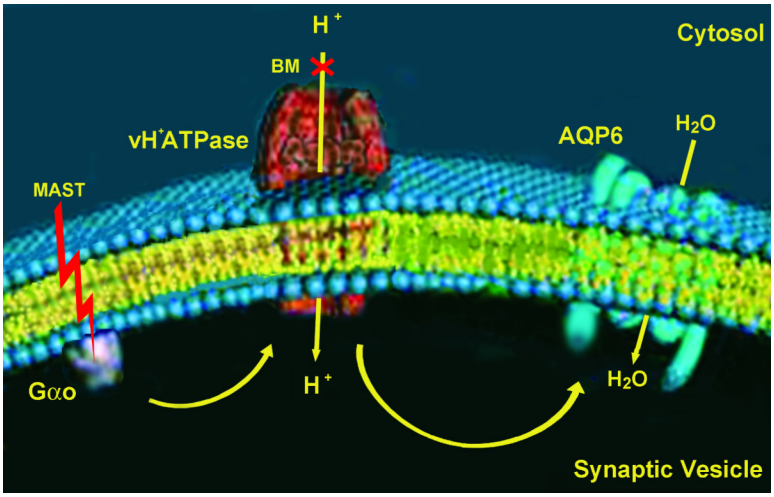

Figure 5.

Schematics of the synaptic vesicle membrane depicting the presence of G_{ao}, vH^+-ATPase, and the water channel AQP6. Mastoparan (MAST) stimulates GTP-$G_{\alpha o}$ protein. The study demonstrates involvement of vH^+-ATPase in GTP-$G_{\alpha o}$-mediated synaptic vesicle swelling. Since results demonstrate a bafilomycin (BM)-sensitive (red X) vesicle acidification following the GTP-$G_{\alpha o}$ stimulus, and because water channels are bidirectional and the vH^+-ATPase inhibitor BM decreases both the volume of isolated synaptic vesicles and GTP-mastoparan stimulated swelling, suggests vH^+-ATPase to be upstream of AQP-6. Vesicle acidification is therefore a prerequisite for AQP-6 mediated gating of water into synaptic vesicles.