Presynaptic Regulation of Quantal Size: K^+/H^+ Exchange Stimulates Glutamate Storage by Increasing Membrane Potential

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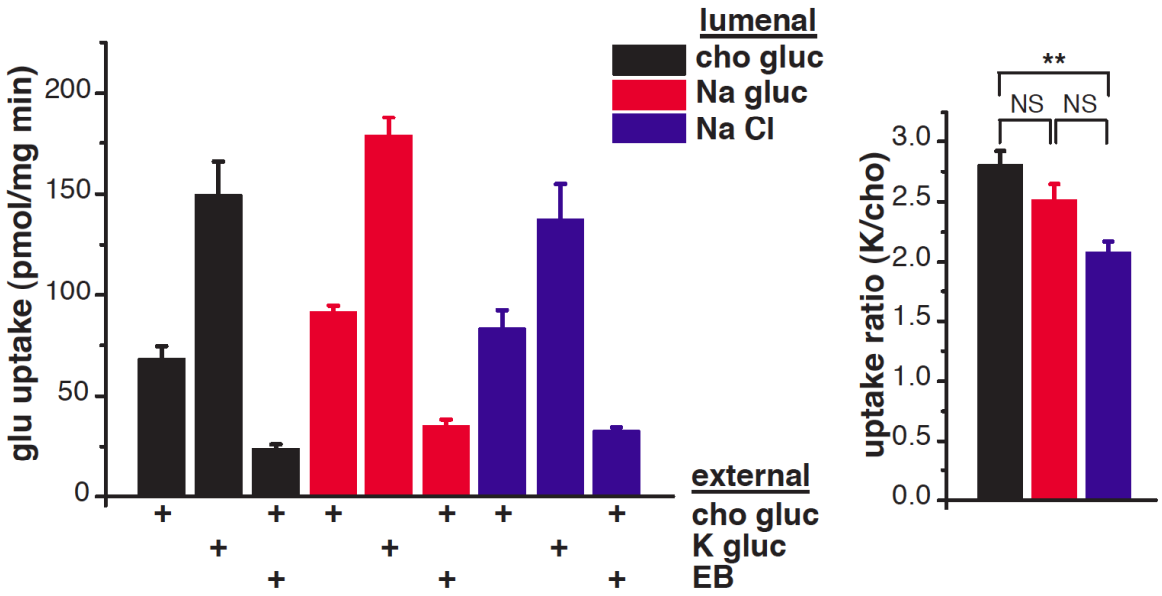
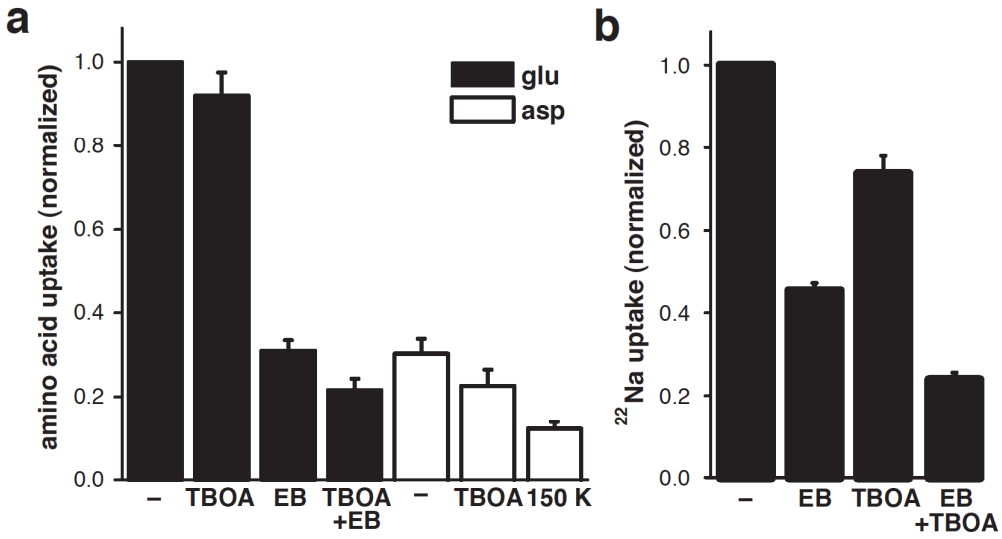


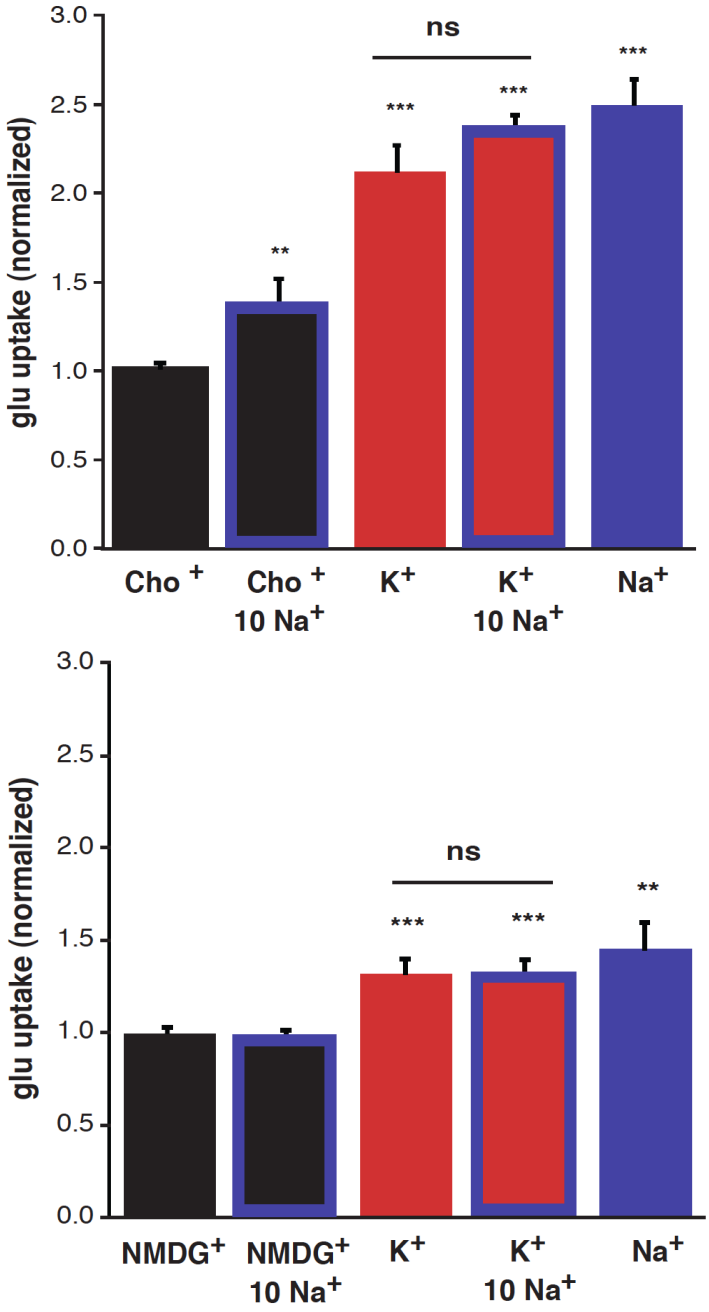
Supplemental Figure S1. Effect of lumenal ions on stimulation of vesicular glutamate transport by K^+.

The uptake of ^{3}H-glutamate by synaptic vesicles pre-loaded with either 150 mM choline gluconate (cho gluc; black bars), 150 mM Na gluconate (Na gluc; red bars), or 150 mM NaCl (NaCl; blue bars) was measured for 10 minutes at 30° C in assay buffer containing either 150 mM choline gluconate with or without Evans Blue (EB), or 150 mM K gluconate. The left panel indicates total uptake, and the right indicates the fold-stimulation by external K gluconate relative to choline gluconate after subtraction of the background (EB condition) from both. **, $p < 0.01$ by two-tailed paired t test (n=3). Data are presented as mean ± SEM.



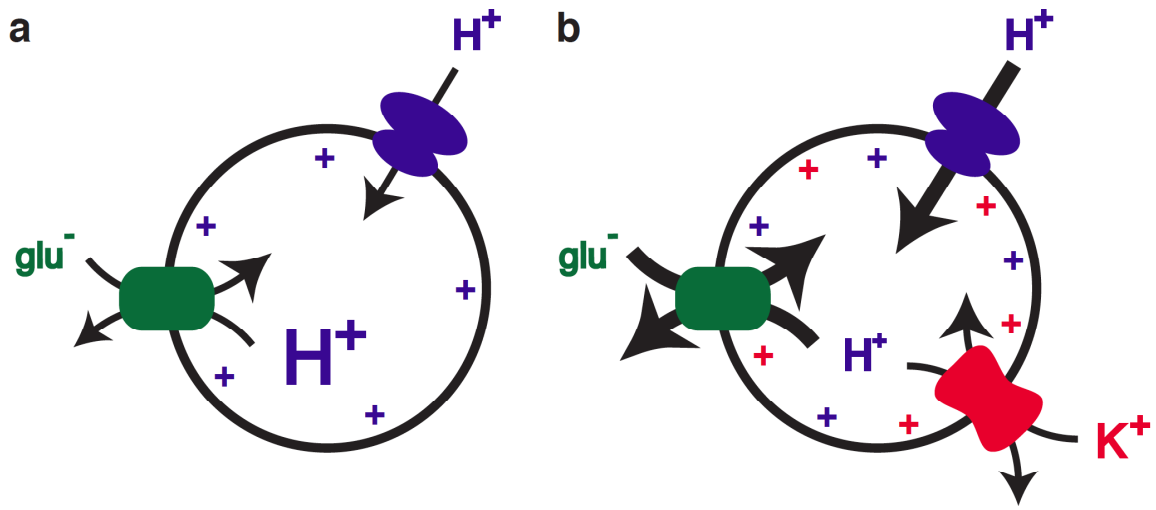
Supplemental Figure S2. TBOA inhibits contaminating plasma membrane glutamate transport activity observed in the presence of Na^+.

(**a**) The uptake of ^{3}H-glutamate (filled bars) or ^{3}H-aspartate (open bars) by synaptic vesicles in the presence or absence of excitatory amino acid transporter (EAAT) inhibitor TBOA (1 mM) or VGLUT inhibitor Evans Blue (100 μM, EB) was measured for 10 minutes at 30º C in assay buffer containing 148 mM Na gluconate, 2 mM NaCl and 1 mM glutamate (six bars on left). To assess background, Na^+-independent uptake, Na^+ was replaced by 150 mM K^+ (150 K), with no inhibitors in the assay buffer. The inhibition of glutamate uptake by Evans Blue and TBOA are both significant, but independent of each other by two-way ANOVA (interaction p = 0.85). (**b**) The uptake of ^{22}Na by synaptic vesicles in the presence or absence of 1 mM TBOA and 100 μM EB was measured for 10 minutes at 30º C in assay buffer containing choline salts of gluconate (148 mM), chloride (2 mM), and glutamate (10 mM). Similar to glutamate uptake, the inhibition of ^{22}Na uptake by Evans Blue and TBOA are both significant, but independent of each other by two-way ANOVA (interaction p = 0.3497). Data are presented as mean ± SEM.



Supplemental Figure S3. Cytosolic concentrations of Na gluconate (10 mM) do not occlude the effect of high K^+ on vesicular glutamate transport.

The uptake of ^{3}H-glutamate by synaptic vesicles was measured for 10 minutes at 30° C in assay buffer containing 4 mM MgATP, 2 mM choline Cl^-, 10 mM glutamate and 150 mM NMDG, choline, K or Na gluconate, with in addition, either 10 mM NMDG/choline or Na gluconate. Background determined in the presence of 100 μM Evans Blue was subtracted, and uptake normalized to that observed in either high choline (top panel) or high $NMDG^+$ (bottom panel). Both 150 mM K^+ (red) and 150 mM Na^+ (blue) significantly stimulate glutamate uptake (***, $p < 0.001$ by one-way ANOVA), although the magnitude of stimulation was smaller with $NMDG^+$ as control. The addition of 10 mM Na^+ does not affect the amount of glutamate taken up into synaptic vesicles in reactions containing 150 mM $NMDG^+$ or 150 mM K^+ ($p = 0.76$, top panel, and 0.12, bottom panel), but modestly stimulates glutamate transport in the presence of 150 mM choline. n = 9-15, and data are presented as mean ± SEM.



Supplemental Figure S4. Model for stimulation of vesicular glutamate transport by K^+/H^+ exchange.

(**a**) Vesicular glutamate transport (green) driven by the vacuolar H^+-ATPase (blue) increases ΔpH at the expense of Δψ. (**b**) Electroneutral K^+/H^+ exchange (red) selectively decreases ΔpH, enabling the accumulation of larger Δψ. As a result, K^+/H^+ exchange effectively converts ΔpH into Δψ, increasing the driving force for glutamate filling.