The modulation of vesicular volume and its effects on neurotransmitter secretion through the fusion pore in PC12 Cells.

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Understanding vesicular exocytosis is fundamental to developing insight into the biology, chemistry and physics of neurotransmission. Through the direct 'presynaptic' observation of quantal release using amperometry it becomes strikingly evident that the exocytotic release event is regulated by multiple mechanisms[1]. For instance, many transients in amperometric records of exocytosis events exhibit a pre-spike feature, or foot, which represents a steady-state flux of neurotransmitter through a stable fusion pore connecting the vesicle lumen to the extracellular fluid[2]. This flux is thought to be driven by diffusion along a gradient of chemical potential. Recent work in our laboratory indicates that vesicular volume prior to secretion is strongly correlated with the characteristics of amperometric foot events, and furthermore, suggests that the protein dense core in many vesicles acts to pressurize the inner solution and drive catecholamine through the fusion pore[3].

RESULTS AND DISCUSSION

In order to alter vesicular volume, we have used pharmacological manipulations that directly affect the VMAT-mediated transport of catecholamines into PC12 cell vesicles. Cells were incubated in 100 μM L-3,4-dihydroxyphenylalanine or 100 nM reserpine for 90 minutes in order to increase and decrease, respectively, the volume of single pheochromocytoma (PC12) cell vesicles[4]. Amperometry experiments utilizing a same-cell paradigm were used in conjunction with transmission electron microscopy to determine that as vesicle size is decreased the frequency with which foot events are observed increases. Consistent with this, fewer events with feet are observed as vesicle size is increased. Additionally, both the amount and duration of neurotransmitter released in the foot portion of the event decrease as vesicle size decreases, whereas both of these characteristics increase as vesicle size increases. Finally, smaller vesicles release a greater percentage of their total contents in the foot portion of the event. These trends provide evidence for a nanotube-like intermediate structure for the exocytotic fusion pore. This is shown in Figure 1B, where a model is presented to describe the effect of vesicle volume on membrane tension and fusion pore

dynamics. A brief description of the mechanisms regulating neurotransmitter release via the fusion pore is presented in the figure legend.

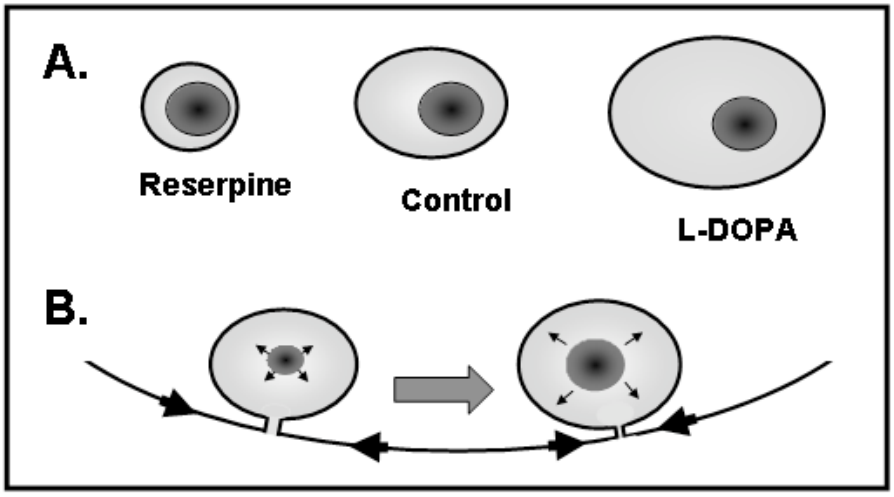

Figure 1: A) Pharmacological manipulation of vesicle volume alters the dense core to vesicular halo volume ratio. Treatment with reserpine diminishes the vesicular halo; however the volume of the dense core changes relatively little. In contrast, treatment with L-DOPA swells the vesicular halo, and again, the dense core volume remains relatively constant. **B)** Upon opening of the fusion pore, the dense core expands resulting in enhanced pressure-driven mass transport through the fusion pore and fluidic pressure on the interior vesicle walls. As the dense core to vesicular halo volume ratio is greatest for reserpine treated vesicles, it appears that the intravesicular pressure is greatest for these cells. An increase in intravesicular pressure increases vesicular membrane tension. If the fusion pore is of a nanotube geometry, this will lead to an immediate constriction of the fusion pore, as shown[3]. Subsequently, the increase in the difference in membrane tension from the cell to the vesicle should result in increased lipid flow through the fusion pore, decreasing its overall stability and resulting in an abrupt opening of the pore.

These studies are noteworthy because the dynamics of the exocytotic fusion pore have recently been proposed to play a role in synaptic plasticity[5]. Additionally, physiological[6-8], genetic[8] and pharmacological[3,10] conditions can affect vesicle size, and in several instances vesicle size has been found to be inversely related to levels of synaptic activity[9]. This work provides new insight into the biological consequences of such vesicular volume modification. It

provides evidence that cells may modulate the rate of neurotransmitter release, or the duration of that release, in the synaptic cleft by manipulating vesicle volume. This appears to be true in the case of release via the fusion pore that is formed in the early stages of exocytosis from PC12 cells.

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