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Synaptotagmin 5 regulates Ca^{2+}-dependent Weibel-Palade body exocytosis in human endothelial cells.

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Vascular endothelial cells secrete the adhesive and procoagulant protein Von Willebrand factor (VWF) from specialised secretory organelles called Weibel-Palade bodies (WPB). A potent trigger for WPB exocytosis is an elevation of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), that can be mediated by hormone action, mechanical stresses or cell damage. Although several cytosolic Ca^{2+}-binding proteins including calmodulin and the Annexin A2:S100A10 complex have been implicated in WPB exocytosis, the existence of WPB-associated Ca^{2+}-sensors involved in detecting and transducing acute increases in $[Ca^{2+}]_i$ into exocytosis remains unclear. Here we show that synaptotagmin 5 (SYT5) is recruited to WPBs and regulates Ca^{2+}-driven WPB exocytosis in human endothelial cells. qPCR analysis of human endothelial cells revealed the presence of mRNA for 10 of the 17 known SYT isoforms; five Ca^{2+}-dependent (1, 2, 3, 5 and 9) and five Ca^{2+}-independent (11, 14, 15, 16 and 17). Analysis of the subcellular distribution of epitope-tagged constructs of each of these 10 SYTs showed that SYT5 localized almost exclusively to WPBs. SYT17 showed partial localization to WPBs in some cells while all other SYTs localized to other subcellular compartments. Live-cell imaging of fluorescent WPB exocytosis in cells either depleted of SYT5 by shRNA or overexpressing SYT5-EGFP showed an inhibition or enhancement of histamine-evoked WPB exocytosis respectively. Similar results were obtained in biochemical studies of histamine-evoked VWF propeptide (VWFpp) secretion. Overexpression of a SYT5 Ca^{2+}-binding deficient mutant, Asp197Ser SYT5-EGFP, inhibited histamine-evoked WPB exocytosis. Depletion of SYT17 produced a very small reduction in histamine-evoked VWFpp secretion, while overexpression of SYT17 had no effect. We propose that SYT5 is a WPB-associated Ca^{2+}-sensor for regulated secretion of VWF from vascular endothelial cells.