

P019 Studies of Munc18 Proteins Mediating Insulin Exocytosis
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Our aim is to identify novel Cyclin-dependent kinase 5, Cdk5, phosphorylation substrates by which Cdk5 together with its activators p35 or p39 promotes insulin exocytosis. Currently we are focusing on the Sec1/Munc18 (SM) protein family that appears to be essential for both membrane trafficking and insulin exocytosis in pancreatic β -cells. RT-PCR and semi-quantitative RT-PCR demonstrated that both splice variants of Munc18-1, Munc18-1a and Munc18-1b, as well as Munc18-2 are expressed in mouse β -cells. Glucose stimulation of MIN6 cells recruited cytosolic wt Munc18-1 protein to the plasma membrane whereas Munc18-2 protein remained in soluble fractions. During unstimulated conditions, phosphorylation mutants of Munc18-1 sequestered in the plasma membrane, differently from the wt Munc18-1 protein. Transient overexpression of wt Munc18-1 or wt Munc18-2 into INS-1E cells resulted in increased glucose-stimulated secretion, as measured with the human growth hormone (hGH) assay. Thus, our results suggest that different Munc18 proteins localize to different cellular compartments in β -cells, phosphorylation of the Munc18 proteins regulate their subcellular distribution and transient overexpression of both wt Munc18-1 or wt Munc18-2 protein augments glucose-stimulated secretion.