ROLE OF SNARE PROTEIN PHOSPHORYLATION IN THE MODULATION OF NEUROSECRETION

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Signal transduction is a very important intracellular event controlling eucaryotic cell functions in interfase and mitosis. At the presynaptic region, phosphorylation of various proteins involved in synaptic transmission has been very well documented. Synapsins for example, are phosphorylated at many different sites by diverse intracellular kinases. Phosphorylation of proteins directly implicated in synaptic vesicle and large dense-core vesicle release has been also reported. This class of proteins includes the SNAREs present in the target membrane (e.g. the t-SNAREs at the plasma membrane are syntaxin1 (Syn1) and SNAP-25) and in the vesicle membrane (the v-SNARE VAMP). Following stimulation, the t-SNAREs associate with v-SNAREs to form the "SNARE complex" which allows the vesicle to fuse with the membrane and release its contents. After fusion the complex is dissociated by the action of the ATPase NSF allowing the SNAREs to be recycled for another round of fusion. The association/disassociation of the SNARE complex is a highly regulated event and involves other intracellular proteins. Syn1, for example, binds to a cytoplasmic protein known as Munc-18 and the association of syn1 with Munc-18 regulates the availability of syn1 to participate in SNARE complex formation. Moreover the t-SNARE syntaxin and SNAP-25 are known to interact and modulate the function of different ion channels and transporters (e.g. Ca^{2+}-channels (VOCC), GABA-transporter). The molecular mechanisms involved in the control of SNARE-protein interactions are only partially known. It has been reported that Munc-18 and the SNARE proteins themselves can undergo phosphorylation thus suggesting that signal transduction events may play a role in controlling SNARE complex assembly and/or disassembly (Figure1).
Most of the experiments regarding the phosphorylation of the SNARE proteins and accessory proteins such as Munc-18 proteins have been done using cell free system and the physiological significances of these events still remains unclear.
Therefore we focused our attention to investigate the phosphorylation of SNAREs in vivo using as models the neuroendocrine cells PC12 and synaptosomes isolated from rat brains. Our recent results have demonstrated, using an antibodies directed against phosphoamino acids, that immunoprecipitated syn1 from

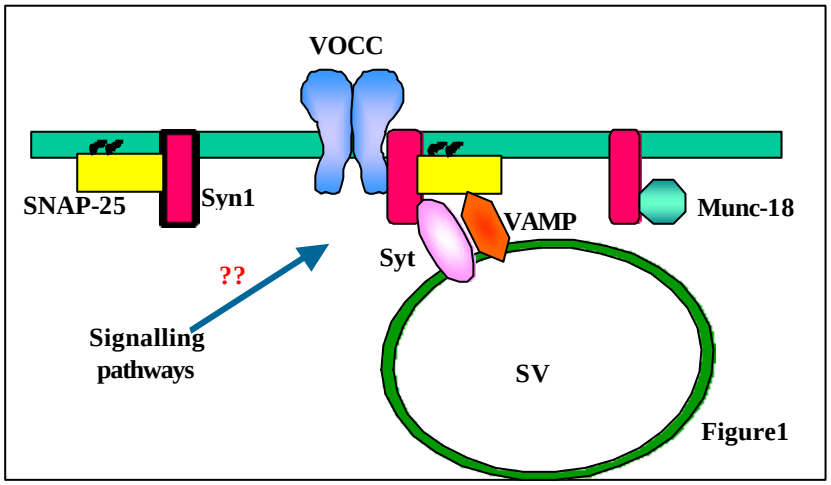

Figure1

rat brain total extracts and PC12 cells is phopshorylated on tyrosine residues revealing a new phosphorylation event which has never been described (Figure2). To further demonstrate that this phosphorylation is a physiological event, we radiolabelled PC12 cells with [^{32}P]-orthophosphate and performed phosphoamino acid analysis on the immunoprecipitates synt1. The characterization of the tyrosine-phosphorylation site of syn1 is under investigation. Phosphorylated syn1 has been purified by affinity chromatography followed by

gel electrophoresis and will be subjected to MALDI-analysis. We have analyzed the effect of syn1 phosphorylation on the assembly of the SNARE complexes. Our preliminary results indicate that tyrosine-phosphorylation does not inhibit the formation of these complexes at least at the steady-state. We will investigate whether manipulation of the phosphorylation status of syntaxin by protein-kinases may modify its interaction with SNAREs or other molecules (e.g. Ca^{2+}-channels) and modulate the neurotransmitter release.

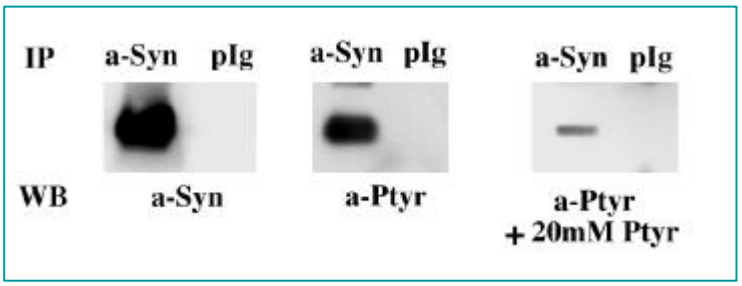

Figure 2: Rat brain extracts were incubated (IP) with α-syntaxin1 antibodie (α-syn) or pre-immune IgG (pIg). Immunoprecipitates were analyzed by western blotting (WB) using α-syn1 antibodie or α-P-Tyrosine antibodie (α-Ptyr) or α-P-Tyrosine antibodie pre-incubated with 20 nM P-Tyrosine.