Mechanisms involved in the facilitation of phosphoinositide dynamics in PC12 cells overexpressing Neuronal calcium sensor-1

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Previous studies have shown that Neuronal Calcium Sensor-1 (NCS-1) regulates exocytosis by mechanisms dependent on phophoinositide metabolism. In the present study, we have used the pleckstrin homology (PH) domain of phospholipase C (PLC) in a fusion complex with EGFP (PHPLC-EGFP) to investigate the dynamics of phosphatidyl inositol 4,5-bisphosphate (PIP2) hidrolysis and 1,4,5-trisphosphate (InsP3) production in wild type (WT) PC12 cells, and PC12 cells overexpressing NCS-1 (NCS-1 cells). Furthermore we studied the influence of Ca2+ entry and release from intracellular stocks on PHPLC-EGFP dynamics by using an intracelular Ca2+ chelator (BAPTA-AM) or extracelular Ca2+ chelator (EGTA). We have also analysed the intracellular Ca2+ response to 300?M carbachol in both WT and NCS-1 cells using the fluorescent Ca2+ probe Indo-1. We found that overexpression of NCS-1 greatly increased the degree of PHPLC-EGFP translocation in response to stimulation with carbachol, which suggests a greater breakdown of PIP2 to InsP3 in NCS-1 cells. The cytosolic translocation of PHPLC-EGFP in response to carbachol was blocked by BAPTA whereas EGTA did not block the translocation. The Ca2+ transient in response to carbachol was different in the two cell types. In NCS-1 cells, Ca2+ levels reached a sharp peak and decayed rapidly. In contrast, WT cells exibited a plateau of intracellular Ca2+ levels. Our data indicated that NCS-1 facilitates the breakdown of PIP2 to InsP3 in response to receptor activation, and that this response seems to be more related to Ca2+ release from intracellular sources.