Muscarinic agonist-induced recruitment of plasma membrane Ca^{2+} ATPase (PMCA) to the membrane involves the PSD95/Dlg/ZO-1 (PDZ) scaffold Na^{+} H^{+} exchanger regulatory factor 2 (NHERF-2)

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Despite the ubiquitous distribution of PMCA little is known about the protein-protein interactions that regulate its activity. The neuronal isoform of PMCA(2b) is known to interact with NHERF-2. The current study investigated the molecular basis and physiological role of the PMCA/NHERF-2 interaction during muscarinic-induced Ca^{2+} signalling in epithelial cells. HT-29 epithelial cells expressed both PMCA1 and 4 as shown by RT-PCR and Western blotting. GST/MBP-pulldowns demonstrated that PMCA bound NHERF-2 in HT29 cell lysates and mutation analysis confirmed that the interaction was mediated by the C-terminal PDZ binding motif of PMCA. Co-immunoprecipitations confirmed that PMCA and NHERF-2 were normally associated in HT29 cells. Cell surface biotinylations were used to show that cell surface PMCA increased in response to carbachol (CCh) to 154±12%; ($p<0.01$; $n=4$) of control levels within 60s. Interestingly, the recruitment of NHERF-2 to the membrane preceded PMCA, with membrane associated NHERF-2 increasing within 30s to 145.3±4.9% of control ($p<0.01$, $n=4$). In contrast, silencing NHERF-2 abolished the CCh evoked trafficking of PMCA, and reduced the levels of PMCA at the membrane to 54±5% ($p<0.01$; $n=3$) of control. Treatment with CCh also resulted in co-localisation of PMCA/NHERF-2 at the plasma membrane as determined by confocal microscopy. These data show rapid agonist-induced translocation of PMCA in a native cell model and suggest that NHERF-2 plays a key role in scaffolding and maintaining PMCA at the cell membrane.