Drebrin regulates sub-spine localization of Calcium/calmodulin-dependent kinase II β

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Dendritic spines are actin-rich small protrusions that contain postsynaptic components of excitatory synapse. Many actin-binding proteins have been identified as spine-resident protein, and they regulate actin-cytoskeleton through diverse processes. Drebrin is a major F-actin binding protein in neurons, and is localized in the center of dendritic spines. In this study, we isolated CaMKII β as a drebrin-binding protein by yeast two-hybrid screen and investigated drebrin-CaMKII β relationship in dendritic spines. CaMKII β is localized in dendritic spines more than in dendritic shaft. However, drebrin knockdown (KD) caused diffuse localization of CaMKII β in dendrites, suggesting that drebrin anchors CaMKII β in dendritic spines. To analyze drebrin-dependence of CaMKII β stability in dendritic spine more directly, we performed fluorescence recovery after photobleaching (FRAP) experiments on individual dendritic spines. We found that the stable fraction of CaMKII β in drebrin-KD neurons was greater than that of control neurons. This result seems to be inconsistent with the results that CaMKII β diffused in dendrites of drebrin-KD neurons. We consequently think that drebrin-independent stable pool became dominant in drebrin-KD neurons. To test the hypothesis, we used stochastic optical reconstruction microscopy (STORM) to elucidate the localization of drebrin and CaMKII β in a dendritic spine on the nanometer scale. Our super resolution data showed that CaMKII β is partially co-localize with drebrin in the inner side of dendritic spines. In addition, the co-localization of CaMKII β and drebrin was separated by NMDA receptor activation, suggesting that active form of CaMKII β is free from drebrin. Furthermore, FRAP analysis showed that NMDA receptor activation markedly increased the stable fraction of CaMKII β . Together, these results suggest that CaMKII β is localized in dendritic spines as both drebrin-dependent pool and drebrin-independent more stable pool.