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A slow endocytosis pathway in hippocampal synapses

Various modes of vesicle recycling have been proposed for central nervous synapses. In the classical endocytotic pathway (Heuser and Reese, 1973), vesicles collapse completely into the surface membrane, internalize slowly (>20 s) in a clathrin-dependent manner, and then recycle through the endosome. In a faster, local endocytotic pathway, vesicles are rapidly retrieved after short-lived fusion pore opening, so called ‘kiss-and-run’ (Ceccarelli and Hurlbut, 1980; Fesce et al., 1994). Recently Pyle et al. (2000) suggested a pathway of rapid vesicle recycling, where vesicle docked at the active zone are retrieved by kiss and run upon fusion, and then preferentially recycles back to the active zone for a second round of exocytosis (rapid reuse). They used styryl dyes as experimental tools. Styryl dyes are amphiphilic and therefore can reversibly insert into the outer leaflet of cell membranes. The dye can be internalized by endocytosis and then released by subsequent rounds of exocytosis. The two styryl dyes FM 2-10 and FM 1-43 differ in hydrocarbon chain length, and thus exhibit different rates of membrane dissociation. This can be used to reveal the existence of localized rapid endocytosis, if the dyes are differentially retained in the vesicle membrane depending on the competition between departamentoing and the rate of rapid endocytosis (Pyle et al., 2000).

Richards et al. (2000) also found in frog motor nerve terminals that FM 2-10 can be destained more efficiently than FM 1-43, but for a very different reason. After a strong electric tetanus large infoldings are formed, from which FM 2-10 can be rinsed, but not FM 1-43. These are pinched off after several minutes and become cisternae, from which vesicles are slowly regenerated.

The motivation of this study was to test the hypothesis that a similar pathway is responsible for differential destaining of the two styryl dyes FM 1-43 and FM 2-10 also in hippocampal synapses. I compared total recycling pool (TRP) and readily-releasable pool (RRP) sizes using weak (150 action potentials) or strong stimulation (45mM K+) for dye loading. I found that losses of absolute fluorescence for brief hypertonic sucrose pulses, i.e. RRP sizes, are similar for both protocols and dyes, while the relative contributions (RRP/TRP) are not (%±SEM; FM 2-10 K+ :14.0±0.6; FM 1-43 K+ :7.1±0.4; FM 2-10 AP:13.0±0.7; FM 1-43 AP:14.7±0.5). Comparing absolute TRP values for both protocols by normalisation with respect to the RRPs reveals a significantly larger TRP size for FM 1-43 after K+ loading. This points to an additional slow endocytotic pathway after strong stimulation that retrieves ~30% of the exocytosed membrane, probably via infoldings from which FM 2-10, but not FM 1-43 can be rinsed. However, when leaving FM 2-10 for an additional 15 min after K+ stimulation, the absolute TRP size rises dramatically by 106% with little change in the RRP. Varying systematically the washout time, I found that the endocytotic compartments are pinched off from the plasma membrane with a half time of 5-10 min.