Pre-, hemi- and postfusion stages of lamellar body (LB) exocytosis in the rat lung: mechanisms of regulation and implications for surfactant release

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Exocytotic systems in biology are highly variable with regard to vesicle size, content solubility, dynamics of release and modes of stimulation. The alveolar type II cell is a paradigm for a "slow secreter", with vesicles (lamellar bodies = LBs) of about 100-fold diameter (i.e. ≈ 1 million-fold volume) of a synaptic vesicle, and a poorly soluble, lipoprotein-like secretory product (surfactant). Due to their large size and sequential (rather than simultaneous) mode of release, LBs in type II cells are an ideal model system to elucidate single vesicle-related events in the course of exocytosis using live-cell imaging techniques. We have developed several fluorescence techniques that enable a "dissection" of the exocytotic process into various stages, based on quantum yield, solubility, diffusion and accumulation of dyes in different compartments according to biophysical properties and modes of application. In combination with other techniques, these methods allow to estimate with high spatial and temporal resolution the hemifusion lifetime, the instance of fusion pore formation, dynamics and physical forces of fusion pore expansion, and postfusion events in and around single fused LBs. We found that an elevation of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$) above ≈ 300 nmol/l is a stimulus for LB fusion events, where the amount of fusion correlates with the integrated $[Ca^{2+}]_c$ over time. Each fusion event is initiated by a hemifusion phase, i.e. a period of lipid merger between plasma and LB membrane, which can be detected by a decay of light intensity (SLID = scattered light intensity decrease) of the limiting LB membrane in darkfield microscopy. After fusion pore formation, LB contents remain within the fused LB, because the fusion pore opens slowly and surfactant is a hydrophopic material that does not immediately disintegrate. Cells expressing actin-GFP form a dense "actin coat" around the fused and swollen LB, and this actin coat formation is necessary for surfactant release throught the pore. When actin coat formation is prevented by removal of Ca^{2+} or by pharmacological treatment with Ca^{2+} channel blockers, surfactant release is inhibited. The dependence of actin coat formation and contraction on extracellular Ca^{2+} prompted to investigate localized $[Ca^{2+}]_c$ changes at the site of fusion. Fluo-4-fluorescence measurements revealed transient $[Ca^{2+}]_c$ elevations around single fused LBs subsequent to fusion pore formation (FACE = fusion-activated Ca^{2+} entry). Current experiments aim at elucidating the molecular components of Ca^{2+} entry in type II cells. We conclude that the postfusion phase plays an important active role and is rate-limiting for the release of surfactant. Ca^{2+} channels, which are selectively activated and/or accessible to the extracellular space during this phase account for a yet undetected postfusion Ca^{2+} signal, boosting release of vesicle contents. This type of Ca^{2+}-secretion-coupling may exist in all cell types, where vesicle content release by diffusion through a slowly expanding pore is not sufficient or fast enough.