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Mapping of FcεRI immunoglobulin E receptor in activated mast cells by scanning near-field optical microscopy

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Introduction. The cell membrane has a dynamic role that enables the reorganization of receptors and signal molecules in response to signaling processes. To visualize these phenomena we can nowadays benefit from techniques that allow subdiffraction optical resolution.(1) Among these, scanning near-field optical microscopy (SNOM) exploits the evanescent field exiting at the probe fibre apex. The lateral resolution depends essentially on the subwavelength aperture size of the optical fibre (typically better than 100 nm). This makes SNOM particularly suited for nanoscale study on intact biological membrane. Recently, we demonstrated that SNOM combined with immunolabelling and diaminobenzidine (DAB) staining is a valuable non-invasive approach for investigating nanostructures components within intact oligodendrocites.(2) Here we extend this approach to the study of reorganization of FcεRI immunoglobulin E (IgE) receptor in intact mast cells upon antigen-induced degranulation by IgE cross-linking.

Materials and Methods. Rat basophilic leukemia RBL-2H3 cells were grown on coverslips, incubated overnight with anti-2,4-Dinitrophenol (DNP) IgE and degranulated by adding DNP. After 30' cells were fixed and immunolabelled with anti-FcεRI monoclonal antibody. DAB staining was performed with VECTASTAIN® UNIVERSAL Elite ABC kit. TriA-SNOM microscope (A.P.E. Research, Trieste) was used for near-field measurements.

Results and discussions Resting and activated cells with and without DAB staining immunolabelling for the internal portion of FcεRI are analyzed by SNOM. Topography of activated cells shows membrane ridges over surface and in some cases a considerable cell flattening, in general accordance with the morphology observed as result of the degranulation process. The optical transmission images of DAB stained activated mast cell display numerous very dark circular areas, not observed in the unlabelled activated cells. Such features are likely due to a strong light adsorption for the presence of localized DAB reaction. These areas (lateral size about 300 nm) appear to be in agreement with IgE receptor FcεRI patches observed on cytoplasmatic side of membrane sheets of activated mast cells.(3) In conclusion these results demonstrate that SNOM combined with immunolabelling and DAB staining holds great potential for investigating the organization of proteins into micro- or nanodomains in cell membrane.

1. Bioch. Biophys. Acta 2010, 1798: 77.
2. Neuroimage 2010, 49: 517.
3. Biophys. J. 2006, 90: 2404.

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