Membrane trafficking in neuroendocrine cells and neurons is essential for neurosecretion and involves protein trafficking and recruitment to specific target membranes. Fusion of synaptic vesicles with the presynaptic membrane is mediated by components of the SNARE family (soluble NSF attachment protein receptor), such as syntaxin 1 (syn1), SNAP-25 (SN-25) and VAMP-1.2 (vesicle-associated membrane protein), and synaptotagmin (Syt). In addition neurotransmitter release is triggered by the local entry of Ca2+ through voltage-gated calcium channels (VGCCs) (see Figure 1). The clustered accumulation of these proteins (t-SNAREs and P/Q calcium channels) in specific membrane regions is though to be important for their role in exocytosis. It has been proposed that the formation of protein-clusters in the active zone may involve cytoskeletal elements and/or multiprotein interactions with modular adaptors. A further mechanism may be based on the recruitment of the proteins in lipid microdomains.

Recent data have demonstrated that components of the exocytic machinery, the SNARE proteins, are localised in cholesterol-enriched domains of neuroendocrine cells. This finding suggests that lipid microdomains may define sites for synaptic vesicle release and may help to organize proteins that are involved in exocytosis including VGCC subtypes and signalling molecules. The aim of our studies is to investigate the role of lipids (cholesterol and sphingolipids) in controlling the assembly and function of SNARE proteins and functionally linked proteins. We have recently focused our attention on the P/Q channels, since these channels are mainly localized in the presynaptic terminals, and on signalling molecules converging on presynaptic channels and SNARE proteins.

Main results

P/Q-type calcium channels colocalize with SNARE proteins in lipid microdomains.

We have investigated whether P/Q and Lc calcium channel subtypes are localised in lipid microdomains isolated from nerve terminals (synaptosomes). These channels are particularly interesting because they both interact with SNARE proteins but show different intracellular distribution: whereas P/Q type channels are mainly clustered in presynaptic membranes, Lc type channels are preferentially located in dendrites and perikaria. Synaptosomes isolated from rat brain total homogenates were treated with non-ionic detergent at 4°C and the so-called detergent resistant membranes (DRMs, believed to represent lipid microdomains in intact cells) were separated from the soluble proteins on sucrose gradients by floatation. Immunochemical and biochemical analyses of the gradients demonstrate that P/Q, but not Lc, channels are largely concentrated in DRMs.

Furthermore, the immunoisolation of multiprotein complexes from the detergent-resistant or detergent-soluble fractions shows that the alpha1A subunits of P/Q channels co-localise and interact with the SNARE complexes localized in the same microdomains. The altered organisation of these domains by saponin and methyl-beta-cyclodextrin (MBCD) treatments (which reduce the level of cholesterol in the membranes) largely impairs the distribution of P/Q channels and SNAREs in DRMs. In addition, MBCD treatment alters the interaction of alpha1A subunits with Syn1 and SNAP-25. These results strongly suggest that lipid microdomains may play a role in organizing membrane sites specialised for synaptic vesicle exocytosis (Taverna et al, 2004).

Synaptic proteins involved in neurosecretion are localized in different lipid microdomains.

A number of proteins and signalling molecules are known to modulate VGCC and SNARE complexes. We focused our attention on the heterotrimeric G proteins and neuronal calcium sensor-1 (NCS-1). Several results have demonstrated that the activation of G protein coupled receptors (GPCRs) affects Calcium channel activity via different mechanisms that imply the direct binding of Gprotein βγ subunits to the alpha1A subunit or the modulation of the level of phosphatidylinositol-4,5-bisphosphate (PIP2), a lipid
that is known to interact with the channel and sustain its activity.
On the other hand NCS-1 is also known to increase the levels of polyphosphoinositide (PPIs) by modulating the membrane recruitment and activity of phosphatidylinositol 4-OH kinase (Taverna et al, 2002). In addition, it has been proposed to mediate the activity-dependent facilitation of P/Q-type calcium currents via direct interaction. Since lipid microdomains function as platforms for spatial organization of signalling molecules, we have investigated whether heterotrimeric G proteins and NCS1 were recruited in microdomains together with P/Q channels and/or SNAREs. Our results demonstrate that the exocytic machinery and NCS1 are recruited in distinct lipid microdomains.

**SELECTED PUBLICATIONS**


**COLLABORATIONS**

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**GRANTS**

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**Propects for the future**

1. We plan to analyze the role of lipids on synaptic vesicle exo-endocytosis in neurons after depletion of cholesterol and/or sphingolipids.

2. Next, we plan to investigate the trafficking and membrane recruitment of GPR17, a recently deorphanized GPCR (Ceruti et al, 2009; Lecca et al, 2008), in differentianting oligodendrocytes. This receptor might have differential role in response to injury. After brain damage, the expression of GPR17 is highly modulated in a population of oligodendrocyte precursor cells. Little information, however, is available on the trafficking, membrane distribution and role of this receptor in oligodendrocytes.