Abstract

Synaptic transmission is an essential process allowing neurons to communicate with each other and other cells within milliseconds. Depolarization of the membrane induces calcium influx and consequently a cascade of cellular processes such as exocytosis of neurotransmitter-filled synaptic vesicles, neurotransmitters binding to their respective receptors and endocytosis of plasma membrane for generation of new synaptic vesicles. Dynamic posttranslational modifications (PTMs), such as phosphorylation, are known to facilitate these ultrafast processes. However, synaptic proteins are also highly modified by other PTMs such as N-linked glycosylation and lysine acetylation. Sialylated N-linked glycosylation, adding a negative charge to the protein surface, has previously been reported to play a role in synapses and might be modified by neuronal activity. Similarly, lysine acetylation has been linked with protein activity in the synapse. However, none of these PTMs have been investigated for their dynamics in the nerve terminal after ultra-short depolarization and thus their potential role in synaptic transmission. Using state-of-the-art high-resolution mass spectrometry, we aimed to characterize lysine acetylation and (sialylated) N-linked glycosylation in isolated rat nerve terminals (synaptosomes) under naïve conditions and during KCl stimulated depolarization for five seconds. Investigations of the acetylome mapped 1594 acetylation sites in synaptosomes whereof 296 lysine acetylated peptides changed significantly during synaptic transmission of synaptosomes. Protein acetylation was highly dependent on protein localization, as proteins from the active zone enriched fraction (0.1 % sodium dodecyl sulphate (SDS)- insoluble proteins) showed a different acetylation pattern than proteins which were soluble in 0.1 % SDS. Furthermore, changes in the acetylome upon synaptic transmission predominantly involved metabolic enzymes suggesting a link between acetylation and the regulation of synaptic metabolism in neuronal signalling. In addition, we characterized N-linked glycosylation in synaptosomes by analysing released N-glycans, formerly sialylated N-linked glycopeptides and intact N-linked glycopeptides. N-glycan analysis revealed the structure of 42 N-glycans, 1965 glycosylation sites (detected by deglycoproteomics/sialiomics) and 3451 unique intact glycopeptides. Synaptic N-glycans were mainly neutral, including various oligomannose ix saccharides. Proteins from the SDS-insoluble and SDS-soluble fraction showed spatially resolved glycosylation patterns indicated by a pronounced glycan microheterogeneity that varied between the two fractions. By investigating changes in abundances of formerly sialylated N-linked glycopeptides (obtained by titanium dioxide enrichment of sialylated glycopeptides and subsequent deglycosylation), we observed that sialylation changed on 430 glycosylation sites within five seconds of depolarization. Changes in sialylation suggest a new mechanism of modulation of sialic acids at the plasma membrane since

synaptosomes are assumed to lack the Golgi apparatus. We discussed the glycosylation of ion channels, neurotransmitter receptors, synaptic vesicle proteins and cell adhesion molecules regarding the function of N-linked glycosylation in synapses. Nevertheless, the function of sialylated glycosylation needs further investigation as many questions could not be addressed with the currently available methods. Overall, the described large-scale data of the synaptic proteome, PTMome and glycome might reflect the largest global characterization of synaptosomes. We demonstrated for the first time that protein separation by SDS solubility can be advantageous in unravelling spatial differences in synaptosomes and detecting changes on proteins in the active zone. Furthermore, lysine acetylation and N-linked glycosylation represented different protein subsets likely to perform distinct functions during synaptic transmission. This indicates that various PTMs in the synapse are connected, spatially resolved and highly dynamic. The understanding of these is essential in order to interpret the function of the brain and the symptoms of neurodegenerative diseases especially in the synapse.