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N-acetylglucosamine kinase in rat hippocampal neurons

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Distribution of N-acetyl glucosamine kinase (GlcNAc kinase) in rat hippocampal neurons was investigated by immunocytochemistry. In mature hippocampal neurons (DIV21) GlcNAc kinase was distributed throughout neuronal dendrites often protruding out of the long axis. These punctae-like protrusions did not overlap with synaptic markers such as NMDA receptor subunit 2 B (NR2B) and PSD95, but closely apposed each other. Such distribution profile is in good accordance to the finding that GlcNAc kinase is enriched in the synaptosome but absent from postsynaptic density (PSD) fractions. Throughout *in vitro* development GlcNAc kinase colocalized with tubulin. When neurotubule was dissociated by vincristine its distribution was much shifted toward dendritic membrane and formed more distinct punctae. To my best knowledge this is the first report on the expression of GlcNAc kinase in neuron, and imply that GlcNAc kinase has a fundamental role(s), in addition to the kinase activity, in neuron.

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SEPT6 in Rat Hippocampal Neurons

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Septins are members of a subfamily of GTPases whose expression and functions in mammals are not well characterized. In this study, we identified a 50-kDa protein in the rat forebrain postsynaptic density (PSD) fraction as SEPT6 by amino-acid sequencing and immunoblotting. A rabbit polyclonal antibody against SEPT6 peptide recognized a single protein band at 50 kDa in forebrain homogenate. This protein was only moderately enriched in the forebrain One-Triton PSD fraction, a protein fraction enriched in type I (excitatory) PSDs. Instead, this protein was highly enriched in the inhibitory PSD-enriched fractions. Immunocytochemical labeling of dissociated hippocampal cultures revealed that SEPT6 was expressed widely in neurons. However, relatively strong expression was associated with a subpopulation of neurons (~5% of all neurons) that exhibited a characteristic morphology including several long, straight dendrites. SETP6 expression was punctate in somatodendritic domains. In double staining experiments, the majority of synaptophysin (SNP, a presynaptic marker) and glutamic acid decarboxylase (GAD, a marker for GABAergic axon terminal) clusters were closely juxtaposed with SEPT6 ones. However, SEPT6 did not colocalize with PSD-95 or the ㅀ-subunit of type II Ca2+/calmodulin-dependent protein kinase (ㅀCaMKII, an excitatory postsynaptic marker). Interestingly, when microtubule is disrupted by vicristine, SEPT6 polymerized into a thread-like structure. Our results indicate that SEPT6 is expressed postsynaptically at GABAergic inhibitory synapses and its localization is influenced by microtubule.

Keywords : hippocampal neuron, immunocytochemistry, inhibitor synapse, microtubule, SEPT6, PSD