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研究課題：AMPA 型グルタミン酸受容体の N 型糖鎖修飾によるシナプス可塑性と学習・記憶の制御

Regulation of synaptic plasticity and learning and memory by N-glycosylation of AMPA-type glutamate receptor

研究要旨 (Research summary)

Glutamate is the main excitatory neurotransmitter in the central nervous system, and its postsynaptic glutamate receptors play an important role in many neural activities, including learning and memory. Among them, AMPA-type glutamate receptors (AMPA-R) are not only the main fast excitatory neurotransmitter receptors, but also play a central role in the development of synaptic plasticity and are considerably involved in higher brain functions such as learning and memory. AMPA-R functions by forming tetramers of four each subunit in various combinations to form channels.

It has been suggested that all four subunits of AMPA-R contain 4-6 consensus extracellular N-glycosylation sites and most of them are actually glycosylated. However, only one of the N-glycosylation sites of the GluA1 subunit (401st asparagine: N401) was found to be un-glycosylated in approximately half of the cases.

The purpose of this study was to clarify the question whether these variable N-type glycosylation at GluA1 N401 site is involved in higher brain functions such as learning and memory, including its mechanism.

In this study, we combined *in vivo* and *in vitro* approaches to figure out the importance of GluA1 N-glycosylation in neuronal functions. un-glycosylated GluA1 showed the loss of de-sensitization that is characteristic feature of AMPA receptor, following increasing of ion influx including calcium. Additional studies provide dynamic functions of glycosylation that un-glycosylated GluA1 tetra-homomer is essential for synaptic plasticity; chemical LTP (long term potentiation) using primary culture neurons.

Based on these *in vitro* results, we generated GluA1 N401Q knock in mice, in which glycosylation of GluA1 N401 is blocked in mice. After confirming the correct mutation of knock in mice, we analyzed them using biochemistry, cell biology, and electrophysiological technique. Almost all results were compatible results with one of *in vitro* experiments. In particular LTP of GluA1 N401Q knock in mice show deterioration.

We also performed comprehensive behavior studies. Basic behavior functions including motor- and sensory- functions are comparable in wild and mutant mice. Next focusing on learning and memory functions in mutant mice, we adopted contextual fear conditioning test and Barns maze test. In both behavior studies, mutant mice showed the impairment of memory retention, although the acquisition of memory in mutant mice are comparable with one of wild type mice. In addition, these mutant mice displayed behaviors, suggesting GluA1 glycosylation may affect depression and schizophrenia.

These studies demonstrated the importance of GluA1 glycosylation in learning and memory and psychotic disorders. In addition, these results provide the idea “the switching of glycosylation/unglycosylation affects on neuronal functions”, although the structure of sugar chain has been focused.