Form 2-4-2

Japan-US Brain Research Cooperation Program
The Group Joint Study Report [field: 1]

1. The Representative of Group Joint Study:
Affiliation/ Title/ Name
Kohji Takei / Professor / Department of Neuroscience, Okayama University Graduate School of Medicine and Dentistry, phone: 086-235-7120

2. The Project Title:
Molecular mechanisms of AMPA receptor endocytosis

3. Japanese Investigator's Name, Title, Affiliation and Phone Number:
Chief:
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Collaborator:
Masahiro Kinuta, Assistant Professor, Department of Neuroscience, Okayama University Graduate School of Medicine and Dentistry, phone: 086-235-7125
Hiroshi Yamada, Assistant Professor, Department of Neuroscience, Okayama University Graduate School of Medicine and Dentistry, phone: 086-235-7125

4. U.S. Investigator's Name, Title, and Affiliation:
Chief:
Pietro De Camilli, Professor, Department of Cell Biology, Howard Hughes Medical Institute, Yale University
Collaborator:
Gilbert Di Paolo, Postdoctoral Fellow, Department of Cell Biology, Yale University School of Medicine

5.

6. The Term of Research: From 2002. 4. 1 To 2005. 3. 31 (3 Years)

7. The Abstract, the Result and the Significance of Research (300 Words):

Endocytosis of AMPA receptor: AMPA receptors were expressed in a neuroendocrine cell, MIN6 (pancreatic β cell). The expression of AMPA receptor was revealed by Western blot. By Immunofluorescent microscopy and immunoelectron microscopy, AMPA receptors were observed not only at the cell surface but also at the endosomes. In order to examine whether AMPA receptors are internalized in MIN6, the cell surface was labeled with biotin, then stimulated with AMPA. After inactivation of biotin remaining at the cell surface, the cells were lysed and internalized biotinilated AMPA receptors were collected with avidin beads. The internalized AMPA receptors were detected by western blotting. Antagonist of AMPA inhibited the internalization of AMPA receptors. As, AMPA stimulation leads to depolarization of the cell, It was tested whether AMPA receptors were internalized by depolarization by 30 mM KCl. As expected, AMPA receptors were internalized by the depolarization. (Yamada et al. in preparation)
Functional analysis of Amphiphysin 1: To elucidate function of Amphiphysin 1, we utilized brain cytosol from Amphiphysin 1 knockout mouse for in vitro reconstitution of endocytic vesicles. Activity of endocytic vesicle formation was drastically reduced with Amphiphysin 1knockout brain cytosol, demonstrating that amphiphysin 1 is essential for endocytic vesicle formation. Furthermore, Amphiphysin 1 greatly stimulated dynamin GTP activity. Functional model for Amphiphysin 1 was proposed.(Yoshida et al EMBO J.2004)

These results shed light on mechanism of AMPA receptor endocytosis, which is considered as basis of Long Term Depression in the synapse.

8. The Others (Practical Issues, Special Mention Matters):
Great program!!