Subsequently we could determine the structures of three Chol-1β gangliosides to be GD1aα, GT1bα and GM1α by using α2-3 linkage specific sialidase (kindly supplied by Dr. Yu-Teh Li). They possessed *N*-acetylneuraminic acid attached to *N*-acetylgalactosamine in α2-6 linkage. From the result of immunohistochemical staining of rat brain with monoclonal antibody, GGR41 [3], recognizing Chol-1α gangliosides, it was demonstrated that they were stained intensely the neuropile of dorsal horn on spinal cord, being presumably expressed on the cholinergic nerve terminals. This staining pattern, which is different from that with anti-Chol-1, suggests that Chol-1α gangliosides are expressed on the different region from Chol-1β gangliosides in the cholinergic neuron.

(1) Richardson, P. J. *et al.* (1982) *J. Neurochem.*, **38**, 1605 – 1614. (2) Hirabayashi, Y. *et al.* (1992) *J. Biol. Chem.*, **267**, 12973 – 12978. (3) Kusunoki, S. *et al.* submitted for publication.

S11.4

Immunohistochemical Localization of Major Gangliosides in Rat Central Nervous System

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We investigated the localization of major gangliosides in adult rat brain by immunofluorescence technique with mouse monoclonal antibodies (MAbs). Five MAbs (GMB16, GMR17, GGR12, GMR5, and GMR13) that specifically recognize gangliosides GM1, GD1a, GD1b, GT1b, and GQ1b, respectively, were used (1, 2). We have found that there is a cell type-specific expression of the ganglioside in the rat central nervous system (3, 4). In cerebellar cortex, GM1 was expressed in myelin and some glial cells. GD1a was detected exclusively in the molecular layer. GD1b and GQ1b were present restrictedly on the granular layer; GD1b was detected on the surface of the granular cell bodies, whereas GQ1b was present in the cerebellar glomerulus. GT1b was distributed intensely in both the molecular layer and the granular layer. In cerebral cortex, GM1 was detected in some glial cells. Dense staining was limited to the white matter. GD1a was distributed in the layers I, II/III, and Va and the upper part of the layer VI, whereas GQ1b was localized in the layers IV and Vb, and the lower part of the layer VI. GDlb was detected beneath the layer III. GT1b appeared to be distributed throughout all layers. In other regions such as hippocampal formation and spinal cord, the expression of the ganglioside was also highly localized to specific cell-type and layer.

1. Ozawa, H. *et al.* (1992) *Biochim. Biophys. Acta.*, **1123**: 184 – 190.
2. Kotani, M. *et al.* (1992) *ibid.*, **1117**: 97 – 103.
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4. Kotani, M. *et al.* (1993) *Glycobiol.* in press.

S11.5

Region-Specific Expression of α-Series Gangliosides in CNS of Rat and Frog

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α-Series gangliosides, GD1α and GM1b, were shown to be extremely minor components of mammalian brain gangliosides [1]. In order to understand their biological functions, we established specific monoclonal antibodies against GD1α and GM1b termed KA-17 and NA-6, respectively, and have investigated their histological locations in rat brain and central nervous system (CNS) of lower vertebrates using the monoclonal antibodies. Immunofluorescence staining of frozen sections of adult rat brain showed that both GD1α and GM1b were expressed mainly in the cerebellum. The most intense staining of GD1α was observed in Purkinje cells. Small vesicular structures in granular cell layer were also noticed with KA-17. In spite of the structural relation between GD1α and GM1b, the expression of GM1b was detected in the cell surface of granular cells. These observations seem to reflect the functional differences between the two α-series gangliosides in the neural network of the cerebellum. The differential distribution of major gangliosides in the cerebellum was also reported by Kotani *et al.* [2]. Phylogenetic significance of α-series gangliosides in CNS was also studied using *Xenopus laevis* embryos and its mature brain.

[1] Hirabayashi, Y. *et al. J. Biol. Chem.*, **256**, 8114 (1990); [2] Kotani, M. *et al. Pro. Japan. Acad.*, **68**, Ser. B (1992).

S11.6

Purification and Characterization of Ganglioside-Hydrolyzing Sialidase from Bovine Brain

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We previously demonstrated that rat brain contains three types of ganglioside-hydrolyzing sialidase that are mainly located in synaptosomal membrane, lysosomal membrane and cytosol, respectively. The synaptosomal membrane sialidase could be distinguished immunologically and catalytically from the other two sialidases. Immunoprecipitation study revealed that bovine brain is enriched in the ganglioside-hydrolyzing sialidase cross-reacting with the antiserum specific to rat synaptosomal sialidase.

The ganglioside-hydrolyzing sialidase was solubilized from bovine brain particulate fraction by using TritonX-100 and sodium deoxycholate. The solubilized sialidase was purified by using sequential chromatographies on DEAE-cellulose, Octyl-Sepharose, heparin-Sepharose, Sephacryl S-200, RCA-lectin agarose, MonoQ-and Superose 12-FPLC. Overall purification was about 20,000-fold from the particulate fraction. The sialidase was found to be a glycoprotein which possesses galactose residues in the non-reducing ends of the carbolydrate group, because of the adsorption to RCA-lectin column. The purified sialidase exhibited catalytic properties similar to the rat synaptosomal sialidase. The enzyme was