Conotoxin, a selective *N*-type channel blocker did not alter the B subunit-induced influx. However, either of the dihydropyridine L-type channel antagonists, niguldipine or nicardipine, completely inhibited B subunit-induced influx. Thus, the B subunit-induced calcium influx is likely due to activation of an L-type voltage dependent calcium channel. The regulation of $Ca^{2+}$ fluxes by endogenous ganglioside GM1 has important implications not only for the function of ganglioside GM1 in cell growth but also for its potential function in the electrical excitability of neurons and for its role in neural development, differentiation and regeneration.

This work was supported by Research Grants 1RO1 GM43880 and 1R29 GM 39718 from the National Institutes of Health and 3018M from The Council For Tobacco Research.

S3.3

## Modulation of Calmodulin-Dependent Enzymes by Gangliosides Through Binding to their Specific Polypeptide Sequences

*H. Higashi*, S. Yoshida, K. Sato* and T. Yamagata
*Laboratory of Glycoconjugate Research and *Laboratory of Organic Chemistry, Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan.*

Gangliosides inhibit the activity of calmodulin (CaM)-stimulated enzymes. Without CaM, gangliosides stimulate CaM-dependent enzyme activity at low concentrations and inhibit the activity at higher concentrations. CaM-dependent enzymes self-suppress the activity through binding of their CaM-binding site (CBS) to CaM-like binding site (CLBS), and CaM releases them from the suppression by binding to the CBS. We recently found that gangliosides bound to CaM and proposed a hypothesis that gangliosides, as they have CaM-binding nature, modulate CaM-dependent enzymes through binding to CaM and CLBSs of the enzymes (1 – 3). In the present study, we have examined interaction between gangliosides and synthetic polypeptides of CaM-dependent enzymes to confirm the hypothesis. A peptide consisting of 17 amino acid residues of a CLBS of plasma membrane Ca2+, Mg2+-ATPase has eliminated ganglioside-mediated inhibition of CaM-independently stimulated cAMP phosphodiesterase activity, indicating direct interaction of the peptide with ganglioside GD1 b, GT1b, and GD1a. Unexpectedly, on the other hand, synthetic peptides of CBSs of phosphodiesterase, $Ca^{2+}$, $Mg^{2+}$-ATPase, and calcineurin have also shown the same effects, indicating that the interaction between CBS and gangliosides is also present. Thus we here have revised the model of ganglioside-mediated direct modulation of CaM-dependent enzymes as follows: Without CaM, gangliosides stimulate the enzyme activity by the same manner as CaM, binding to CBS; and at higher concentrations, inhibit the activity by the same manner as the CBS of the enzyme, binding to CLBS.

(1) Higashi, H. and Yamagata, T. (1992) *J. Biol. Chem.* **267**, 9839 – 9843.
(2) Higashi, H., Omori, A. and Yamagata, T. (1992) *J. Biol. Chem.* **267**, 9831 – 9838.
(3) Higashi, H. and Yamagata, T. (1992) *FEBS Lett.* **314**, 53 – 57.

S3.4

## Preliminary Studies on the Structure of the Putative Second Messenger of Insulin

*H. N. Caro*[1], A. Guadaño[2], M. Bernabe[1], M. Martin-Lomas[1], J. M. Mato[2], R. A. Dwek[3] and T. W. Rademacher[3]
[1]*Instituto de Quimica Organica General, C.S.I.C., Madrid, Spain.*
[2]*Instituto de Investigaciones Biomedicas, C.S.I.C., Madrid, Spain.*
[3]*Glycobiology Institute, Dept. of Biochemistry, University of Oxford, Oxford, UK.*

Several lines of evidence have shown that insulin stimulates the generation of at least two low molecular weight mediators which serve as intermediates in the intracellular insulin signaling process. Saltiel and Cuatrecasas[1,2] reported the purification and partial identification of two related substances, generated from liver plasma in response to insulin, containing carbohydrate glycosidically linked to inositol and phosphate. Mato *et al.*[3] reported on the chemical composition of an inositol glycan which inhibited cyclic AMP dependent protein kinase. The detailed structure of this glycosyl phosphatidylinositol which acts as a putative second messenger is not known, but it seems to contain *myo*- and *chiro*- inositol, non-*N*-acetylated glucosamine, galactosamine, galactose and mannose. The determination of the complete structure of these mediators has been hampered by the minute amounts which can be obtained from biological material which prevents the application of conventional chemical and spectroscopic methods with an acceptable degree of reliability. Starting from bovine liver, a workable amount of active material has been obtained which after treatment with PI-PLC, the aqueous phase extracts showed insulin-like activity. Further labelling and purification of this extract, yielded the material ready for its preliminary sequential analysis. The data obtained so far also indicate that this glycosyl-phosphatidylinositol presents a novel carbohydrate core, different to those structures which have been determined up to present.

(1) A. R. Saltiel and P. Cuatrecasas, *Proc. Natl. Acad. Sci. USA*, **83** (1986) 5793 – 5797.
(2) A. R. Saltiel, J. A. Fox, P. Sherline and P. Cuatrecasas, *Science,* **233** (1986) 967 – 972.
(3) J. M. Mato, K. L. Kelly, A. Abler, L. Jarrett, R. Corkey, J. A. Cashe and D. Zopf, *Biochem. Biophys. Res. Commun.* **146** (1987) 764 – 770.

S3.5

## Apoptosis of Burkitt's Lymphoma Cells via Gb3/CD77, a Neutral Glycolipid Antigen

M. Mangeney, C. Lingwood, S. Taga, B. Caillou, T. Tursz and *J. Wiels*
*Laboratoire de Biologie des Tumeurs Humaines, CNRS URA 1156, Institut G. Roussy, 94805 Villejuif Cedex, France.*

Gb3/CD77, a neutral glycolipid antigen is specifically expressed on two B cell populations: Burkitt's lymphoma (BL) and a subset of tonsillar B lymphocytes, which could be the normal counterparts of BL cells. We have recently demonstrated that the Gb3/CD77(+) normal B lymphocytes are highly susceptible to enter programmed cell death (or apoptosis) and can be rescued by a combination of anti-CD40 monoclonal antibodies and IL-4. Interestingly, the same results were also described for BL cells. To investigate the