[S4-2] [10/19/2001(Fri) 14:40-15:20 /Hall B]

The Role of Sphingolipids in Cell Signalling: Studies with Synthetic Analogues

Gerhild van Echten-Deckert and Sven Nätzker

Kekulé-Institut für Organische Chemie und Biochemie der Universität Bonn, Bonn, Germany

Glycosphingolipids are amphiphatic molecules consisting of a ceramide lipid moiety, embedded in the outer leaflet of the plasma membrane, linked to one of hundreds of different externally oriented oligosaccharide structures (1, Fig. 1). They form cell type specific profiles that characteristically change in development, differentiation and oncogenic transformation, suggesting their implication in fundamental cellular processes including growth, differentiation, morphogenesis, cell to matrix interaction and cell - cell communication (Fig. 2). Glycosphingolipids are believed to be integral components of the plasma membrane microdomains, known as rafts and caveolae. Furthermore, their biosynthesis has been shown to have a vital role for embryogenesis of mammals (2). In the last decade sphingolipid metabolites were recognized as bioactive molecules; whereas sphingosine and sphingosine-1-phosphate were found to be primarily mitogenic signals (3), ceramide appears to provide the breaks for unrestrained cell growth, being involved in apoptosis, differentiation and senescence (4) For a better understanding of the relations between sphingolipid metabolism and intracellular signalling we analysed the identity, intracellular localization and topology as well as the regulation of individual biosynthetic steps. Our work contributed substantially to the now accepted model for the biosynthesis of complex gangliosides (5). We also identified the potential key enzyme for the biosynthesis of ceramide, the dihydroceramide-desaturase (6). The fact that ceramide in contrast to its saturated counterpart dihydroceramide, is implicated as a mediator of apoptosis during early neural differentiation (7), argues in favour of a key physiological function of the desaturase. Experiments with synthetic sphingolipid analogues resulted in two interesting compounds that turned out to be valuable tools for studies of both: the regulation of sphingolipid biosynthesis and the role of sphingolipid metabolites in cell signalling. i.) 1-Methylthiodihydroceramide, a metabolically stable dihydroceramide analog uncouples *de novo* sphingolipid biosynthesis, by up-regulating sphinganine-kinase and thus depleting the cells of sphinganine, a vital biosynthetic precursor (8). ii.) *cis*-4- Methylsphingosine is a membrane permeable prodrug which is phosphorylated by the cells to *cis*-4- methylsphingosine-1-phosphate, a metabolically stable mimetic of the short living signalling molecule sphingosine-1-phosphate (Fig. 3). Like the latter, it triggers cell proliferation and mobilization of intracellular calcium in quiescent

Swiss 3T3 cells (9). *cis*-4- Methylsphingosine is, however, apoptotic in postmitotic neurons as well as in neuroblastoma cells (10, 11).

The presentation will focus on sphingosine-1-phosphate (SPP), an important lipid messenger acting both inside and outside cells (Fig. 4). Many external stimuli, particularly growth and survival factors activate sphingosine kinase the enzyme that forms SPP from sphingosine. Intracellular SPP in turn mobilizes calcium from intracellular stores and elicits diverse signalling pathways leading to proliferation and suppression of apoptosis. But SPP is also a ligand for the endothelial differentiation gene receptor family of proteins through which it mediates its biological effects, including processes like angiogenesis and blood flow.

References

1. ***van Echten-Deckert, G. & Sandhoff, K.*** **(1999).** Organization and topology of sphingolipid metabolism. In: ***Comprehensive Natural Products Chemistry*** (B.M. Pinto, ed.) Elsevier Science Amsterdam-Oxford, Vol. 3, p.87-106.
2. ***Yamashita T., Wada R., Sasaki T., Deng C., Bierfreund U., Sandhoff K. & Proia R.L.*** (1999) A vital role of glycosphingolipid synthesis during development and differentiation. ***Proc Natl. Acad. Sci. USA*** 96, 9142-9147.
3. ***Spiegel S. & Merrill A.H.Jr.*** (1996) Sphingolipid metabolism and cell growth regulation. ***FASEB J.*** *10, 1388-1397.*
4. ***Hannun Y.A. & Luberto C.*** (2000) Ceramide in the eukaryotic stress. ***TIBS*** *10, 73-80.*
5. ***van Echten, G. & Sandhoff, K.*** (1993) Ganglioside Metabolism. Enzymology, topology, and regulation. ***J. Biol. Chem.*** *268, 5341-5344.*
6. ***Schulze, H., Michel, C. & van Echten-Deckert, G.*** (2000) Dihydroceramide desaturase. ***Meth. Enzymol.,*** *311, 22-30.*
7. ***Herget T, Esdar C, Oehrlein S, Heinrich M, Schütze S, Maelicke A & van Echten-Deckert G.*** (2000) Production of ceramides causes apoptosis during early neural differentiation *in vitro*. ***J. Biol. Chem.*** 275, 30344-30354.
8. ***van Echten-Deckert, G., Giannis, A., Schwarz, A., Futerman, A. & Sandhoff, K.*** (1998) 1-Methylthiodihydroceramide, a novel analog of dihydroceramide, stimulates sphinganine degradation resulting in a decreased *de novo* sphingolipid biosynthesis. ***J. Biol. Chem.*** *273, 1184-1191.*

9. ***van Echten-Deckert, G., Schick, A., Heinemann, T. & Schnieders, B.*** (1998) Phosphorylated *cis*-4-methylsphingosine mimics the mitogenic effect of sphingosine-1-phosphate in Swiss 3T3 fibroblasts. ***J. Biol. Chem.*** *273, 23585-23589.*

10. ***van Echten-Deckert, G., Zschoche, A., Bär, T., Schmidt, R.R., Raths, A., Heinemann, T. & Sandhoff, K.*** (1997) *cis*-4-Methylsphingosine decreases sphingolipid biosynthesis by specifically interfering with serine palmitoyltransferase activity in primary cultured neurons. ***J. Biol. Chem.*** *272, 15825-15833.*

11. ***Nätzker S., Heinemann T., Niederhausen L., Figueroa-Perez S., Schnieders B, Schmidt RR, Sandhoff K & van Echten-Deckert G.*** Phosphorylated cis-4-methylsphingosine induces apoptosis in neuroblastoma cells by stimulation of p38 and simultaneous suppression of ERK mitogen-activated protein kinases (submitted)

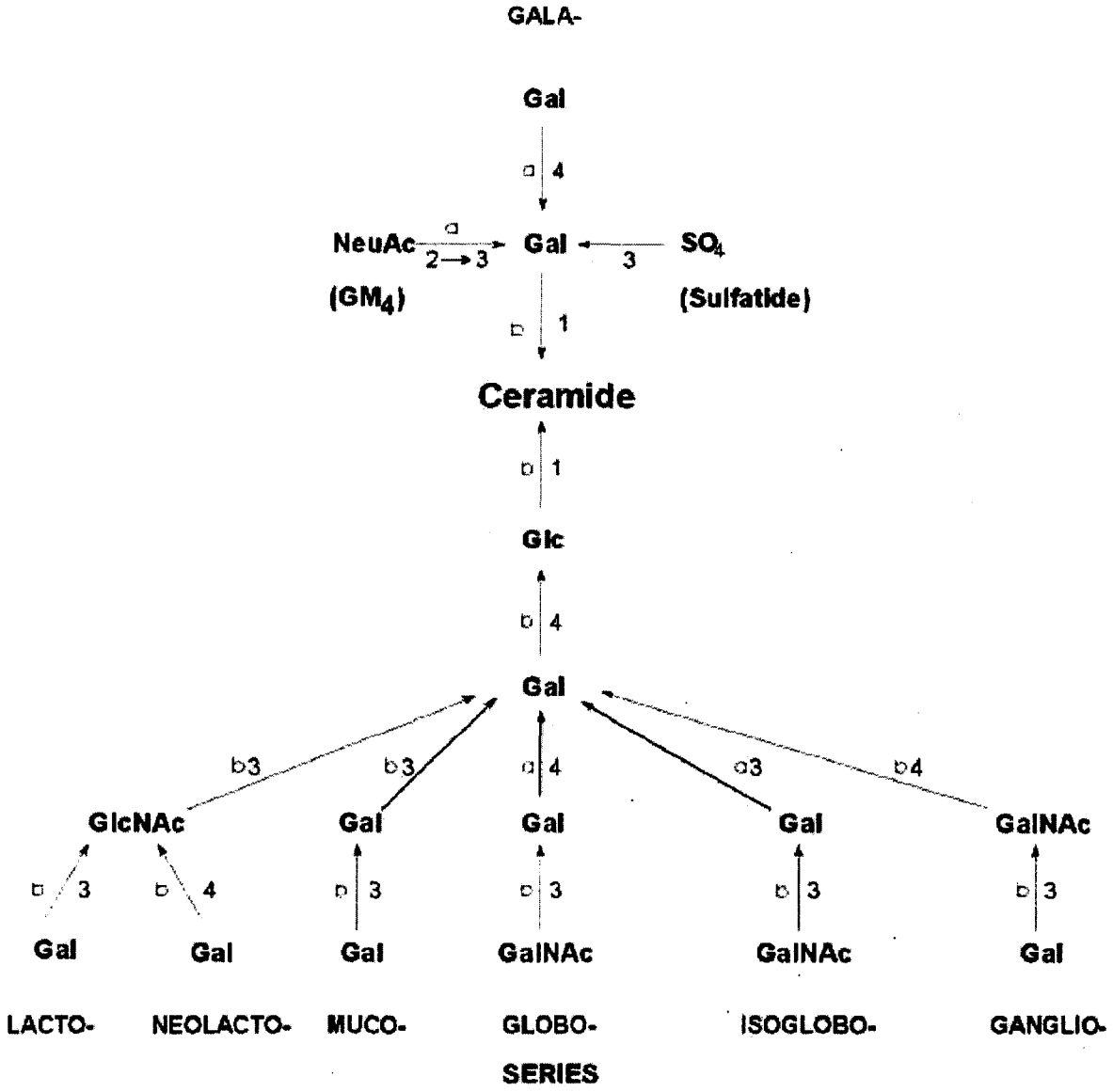

Figure 1 Scheme of glycosidic linkages and nomenclature of different glycosphingolipid series. Ceramide, N-Acylsphingosine; Gal, D-galactose; GalNAc, N-Acetyl-D-galactosamine; Glc, D-glucose; GlcNAc, N-Acetyl-glucosamine; NeuAc, N-Acetylneuraminic acid.

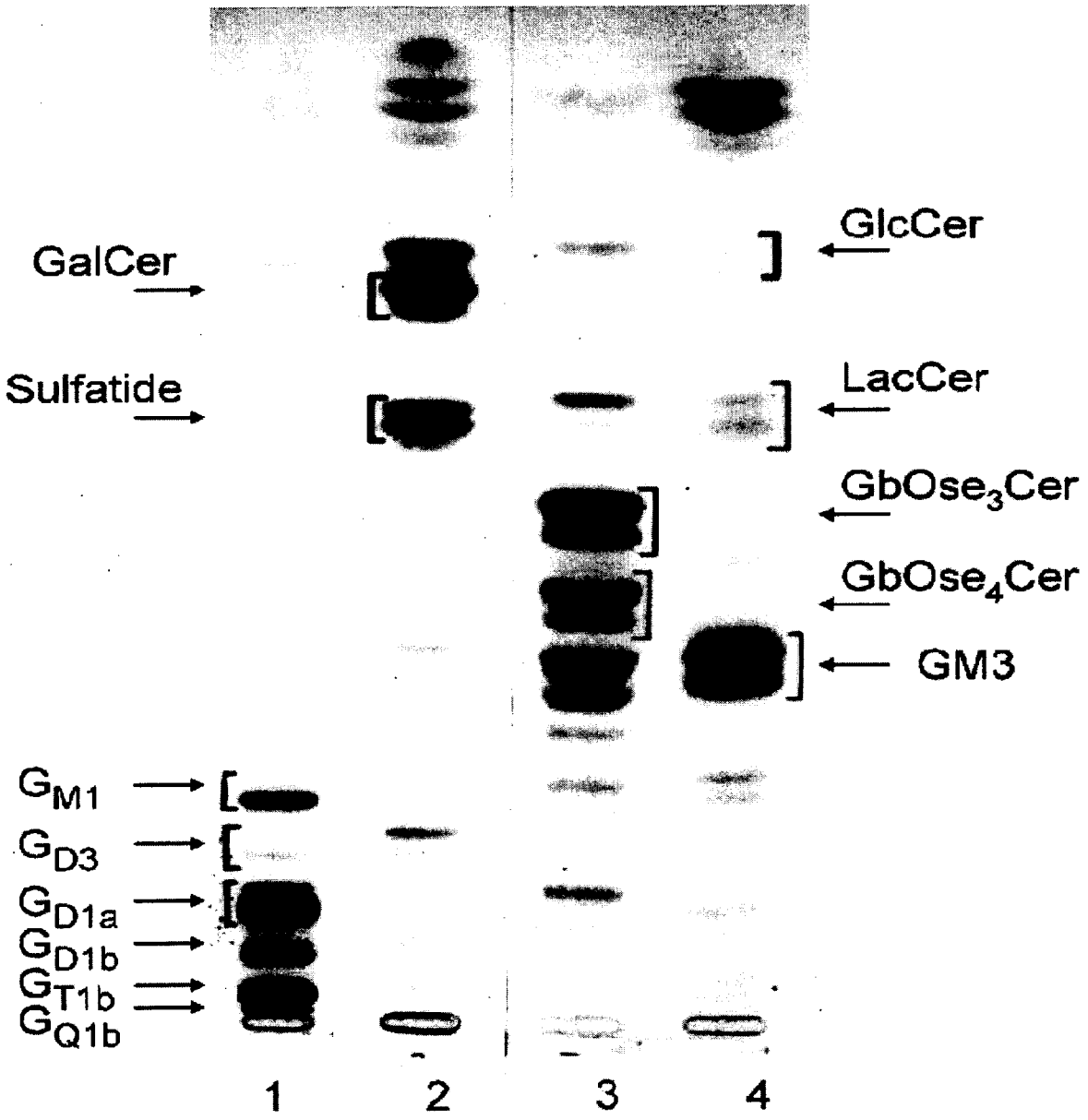

Figure 2 Biosynthetic labeling of glycosphingolipids of different cell types (1). Cells were incubated for 48 h in the presence of [^{14}C]galactose and then harvested. Glycolipids were extracted, desalted, separated by thin layer chromatography and visualized by fluorography. Lane 1, primary cultured cerebellar neurons; lane 2, oligodendrocytes; lane 3, fibroblasts; lane 4, neuroblastoma cells (B104). The mobility of standard lipids is indicated.

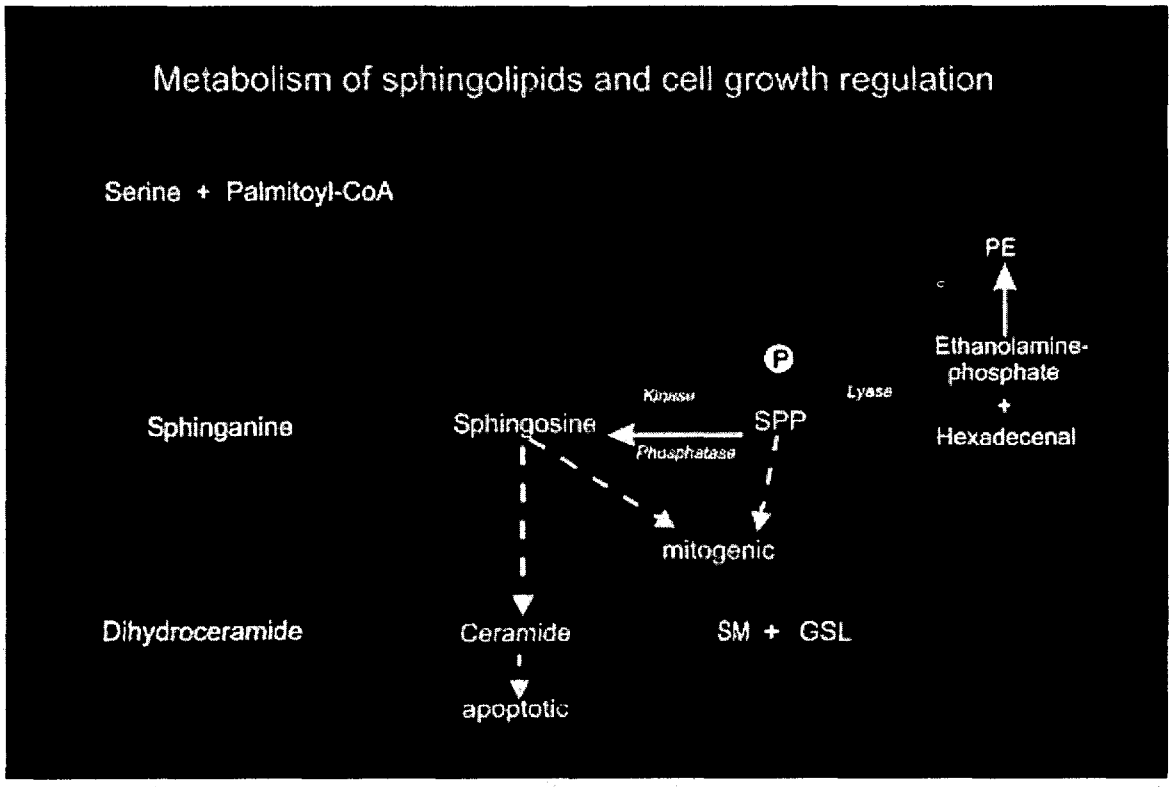

Figure 3 Metabolism of sphingolipid signalling molecules.

cis-4-MethylSo, *cis*-4-methylsphingosine; GSL, glycosphingolipids; P, phosphorylated *cis*-4-methylsphingosine; PE, phposphathidylethanolamine; SM, sphingomyelin;

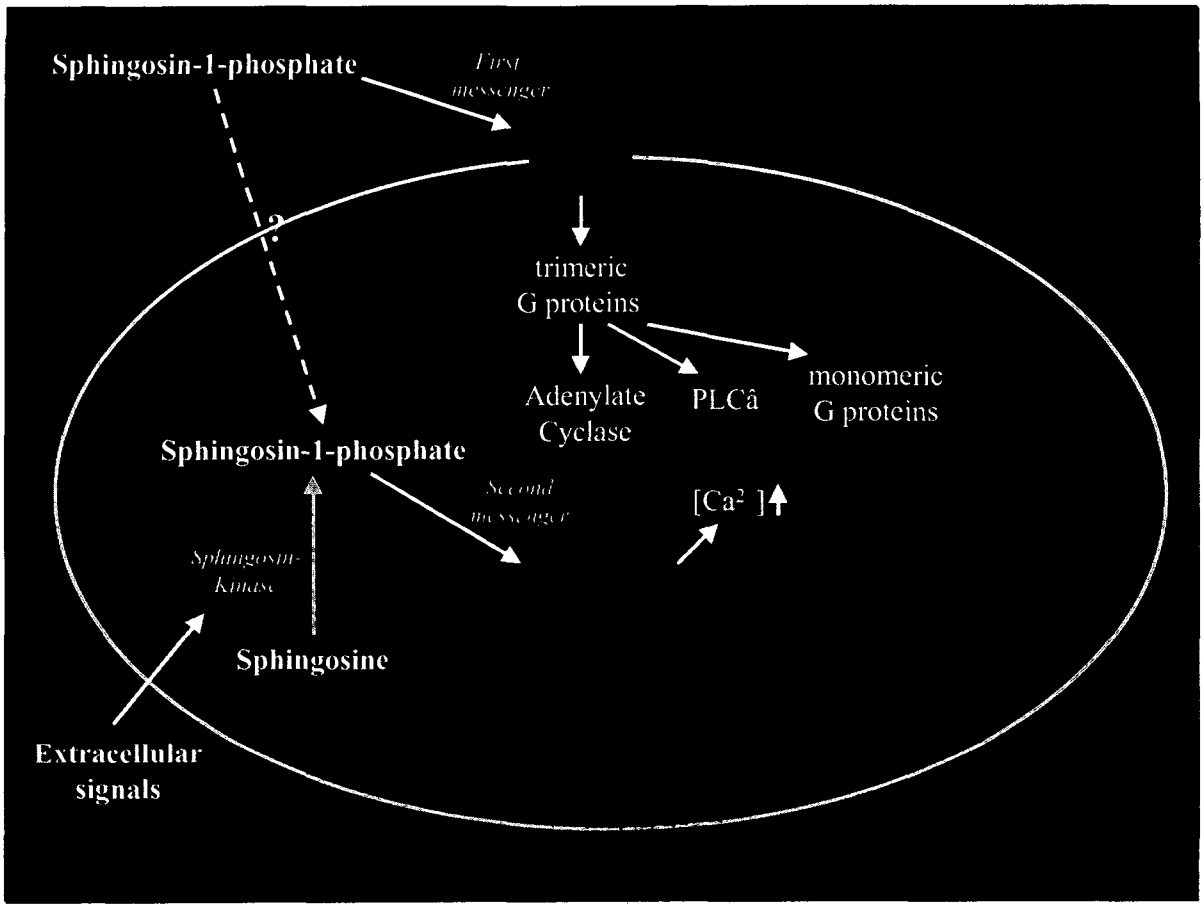

Figure 4 Proposed model for sphingosine-1-phosphate mechanism of action.