various glycolipid acceptors and sugar nucleotides led to a new model of ganglioside biosynthesis: starting from lactosylceramide, ganglioside GM3 and GD3, formation of the various asialo-, a-, b- and c-series gangliosides in the Golgi involves only one GalNAc-transferase, one Gal-transferase II, one sialyltransferase IV and one sialyltransferase V[7-9]. Ganglioside biosynthesis of cultured neurons can be modulated by pH-changes in their culture medium[10]. Inhibition of exocytotic membrane flow in cultured murine cerebellar cells with monensin, Brefeldin A and other agents uncouples biosynthesis of a- and b-series gangliosides at specific sites[11]. Feeding of sphingosine analogues to cultured neurons results in their incorporation into newly synthesized glycolipids as well as in an inhibition of endogenous sphinganine biosynthesis[12-13].

After endocytosis catabolism of membrane-bound gangliosides and their derivatives is catalyzed by exohydrolases in the lysosomal compartment. Degradation of glycolipids with a short oligosacchanide moiety needs the assistance of glycolipid binding proteins to attack their membrane-bound lipid substrates[14]. Four of these sphingolipid activator proteins (SAPs) are homologous to each other and originate from a common precursor protein. Its mRNA and genomic structure[15] and that of another unrelated binding protein, the GM2-activator[16], have been analyzed. Data on the function and lipid specificity of two of these proteins, *sap*-B and *sap*-C, and of another unrelated protein, the GM2-activator, will be presented[17-18]. Analysis of atypical forms of lipid storage diseases revealed 7 different mutations in the activator proteins, 3 in MLD like patients, 2 in patients with GM2-gangliosidosis, 1 in a Gaucher like patient and 1 in a patient with the storage of several lipids[14,19-22].

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S7.2

## Synthesis of Glycosyl-Enkephalin Analogues which Rapidly Cross the Blood-Brain Barrier to Produce Analgesia in Mice. An Entirely New Class of "Designer Drugs"

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The use of D-serine and D-threonine Schiff base esters *1* and *2* permits the selective construction of either α- or β-linked glycosides, as desired.† Use of standard Fmoc-based solid-phase peptide synthesis provided a series of *O*-linked glycopeptide enkephalin analogues *3*. Interperitoneal (i.p.) injection of these compounds into mice produces profound and long-lasting analgesia comparable to morphine as demonstrated by both the tail-flick and hot-plate assays. Control experiments show that the drugs are acting at opiod receptors in the brain, not peripherally or at the spinal column. It is believed that the glucose transporter enzyme is responsible for the transport process.

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S7.3

## Biological Roles of HIV-1 Glycoproteins Glycans

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Human immunodeficiency virus (HIV) envelope precursor glycoprotein (gp160) is cleaved into gp120 and gp41 (4). gp120 and gp41 are responsible for HIV tropism to CD4+ cells and for viral and cell membrane fusion leading to virus entry, respectively. We have studied the functional role of gp160 *N*-linked glycans (CHO) which represent 50% of its MW.

Using an inhibitor of α-glucosidases (dNJ) which induces abnormal high mannose type synthesis, we showed that CHO clusters of gpl60 contribute, **during biosynthesis**, to the folding and bioactive conformation of **gp120**, i.e.: i) the interaction between dNJ-abnormally glycosylated gp160 and its CD4 receptor was altered ii) the accessibility of the V3 loop, a region that plays a key role in membrane fusion, was diminished (3). Such modified properties could account for the impairment of HIV-1 infectivity by dNJ, a promising anti-HIV drug.

Mutation of the **gp41** cluster of glycosylation sites (i) inhibited gp160 cleavage (ii) modified gp160 routing from RER to cis Golgi (EndoH resistance acquisition) and impaired