Gangliosides and Other Glycolipids of Human Placenta

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Gangliosides are lipids characteristic for the neurons. It has been suggested that gangliosides are involved in the selective cation transport at the nerve cell membrane.[1] In extraneural organs gangliosides occur in much smaller amounts but relatively large amounts occur in spleen [2] and red cells.[3] A similar function — participation in the cation transport at the red cell membrane — has been ascribed to them. In order to test the validity of the assumed correlation between selective membrane transport and ganglioside concentration a study of the gangliosides and other glycolipids of placenta was undertaken. The other lipids were not analysed as a thorough study of simple lipids and phospholipids has recently been reported by Eberhagen.[4]

Experimental. Two human placentae were obtained from the delivery room within two hours after the birth of the infants. They were placed immediately in 4 l of physiological saline and excess blood and the umbilical cords were removed. Blood clots were removed by squeezing with the hands. After this preliminary washing the placentae weighed together 725 g (percentage of dry weight = 13 %). They were then cut into hazel nut size pieces and placed into fresh four litres of physiological saline for 1 h under continuous stirring. The rinsing solution was removed by suction filtration and the placentar tissue was homogenized in a Turmix blender. The lipids were extracted with 3 l of chloroform-methanol 1:1, v/v, (C-M 1:1) over night at room temperature. After filtration the residue was reextracted twice with 1 l of C-M 1:1 at 50°C. The combined extracts were evaporated *in vacuo*, reextracted with C-M 2:1, filtered through a sintered glass filter evaporated and dried to constant weight over P_2O_5 in a desiccator. The total lipid weight was 11.78 g, 1.62 % of fresh weight or 12.5 % of dry weight.

From the total lipid extract the major part of the gangliosides was isolated by chromatography on 120 g of cellulose (Schleicher & Schüll No. 123) with chloroform-alcohol-water mixtures.[5] The other lipids, eluted from the cellulose column with chloroform-ethanol-water 16:4:1 v/v/v, (lower phase), were hydrolysed with 100 ml of aqueous 0.5 N KOH for 16 h at 37°C. After neutralisation with 2 N hydrochloric acid to pH 5—6 the water phase was extracted with 6 volumes of C-M 2:1 and then reextracted with 1 volume of chloroform twice. The completeness of the extraction was tested by thin-layer chromatography (TLC). The lipid extract was evaporated to dryness, and divided in two equal parts, and then the glycolipids were separated by chromatography on silicic acid columns and by preparative TLC as described for the isolation of serum glycolipids and spleen gangliosides.[2,6]

The amounts of isolated glycolipids were too small and contaminated with chromatographic adsorbents for accurate gravimetric determination. Instead hexose, hexosamine and sialic acid were analysed in the fractions and the glycolipid amounts calculated from the figures for the carbohydrate content of serum glycolipids [6] and spleen gangliosides.[2,3] The identity of the glycolipids was established from their R_F-values at TLC and degradation by acid hydrolysis and identification of the liberated sugars by paper chromatography and borate electrophoresis.[6]

Results and discussion. The yield of neutral glycolipids was calculated from the two separate runs while that of gangliosides only from one run, as the major part of them was isolated by cellulose chromatography. In Table 1 the distribution of the glycolipids from two human placentae is given. The four neutral glycolipids had the same composition as those of serum.

Table 1. The distribution of glycolipids in normal human placenta.

Glycolipid	mg/100 g fresh weight	mg/100 g dry weight
Ceramide monohexoside	1.5	11
dihexoside	2.9	22
trihexoside	3.6	28
N-tetrose (aminoglycolipid)	7.2	55
Gangliosides	15.5	120
Total glycolipids	30.7	236

The major ganglioside (about 90 %) was the same as that in spleen,[2] N-acylsphingosine-glucose-galactose-N-acetylneuraminic acid, termed ganglioside G_{M3} or monosialogangliolactose. There were also small amounts of monosialoganglio-triose and -tetrose. Of the disialogangliosides, the largest one was the disialoganglio-lactose (G_{D3}) but a disialogangliotetrose also occurred. Small amounts of sulphatides were indicated by paper chromatography [7] but it was not possible to get a reliable quantitative estimation.

In this study the glycolipids represent only about 2 % of total placenta lipids. From the same source Pratt *et al.*[8] found 4 % of cerebrosides, calculated from hexose analyses of a total lipid extract. There is no true discrepancy between the results but the figures for lipid bound hexose are about the same in the two studies. As most placenta glycolipids contain two or three moles of hexose one will arrive at too high figures when one assumes all glycolipids to be monohexosides. If the glycolipid pattern is not analysed it would be better only to report figures for lipid bound hexose.

In comparison to kidney [9] and spleen,[10] placenta may be regarded as rather poor in total glycolipids. Calculated on dry weight basis the former two organs contain 2—3 times more glycolipids than placenta. In spleen and kidney the ratio neutral to acidic glycolipids is about 5:1 but in placenta the acidic fraction is at least as large as the neutral one.

The pattern of neutral glycolipids is very similar to that of kidney and distinctly different from that of spleen and liver, in which the ceramide dihexosides constitute at least 50 % of total neutral glycolipids.[11] It may seem astonishing that the ceramide dihexoside is a rather small glycolipid fraction in placenta while the corresponding sialic acid containing lipid, sialogangliolactose, is the largest of them all. This ganglioside is completely split to ceramide dihexoside and free sialic acid by neuraminidase, which occurs in most tissues. The present results suggest that the neuraminidase activity is low in placenta.

The aim of the present study was to determine if an organ with an intense membrane function as placenta contained a high concentration of gangliosides. The present result indicates a certain correlation. The concentration of gangliosides, calculated as percentage of tissue dry weight, is larger in placenta than in all other extraneural organs studied — kidney, liver, lung, lymph node, and muscle — except in the spleen which contains about 50 % more. And when the concentration of gangliosides is expressed in percentage of total lipids or glycolipids, placenta contains more gangliosides than all the other organs.

Summary. The glycolipids of human placenta have been isolated in a nearly quantitative yield. Placenta contains the same glycolipids as serum and the other parenchymatous organs studied. The concentration of glycolipids is rather low but the pattern has some relevant features. Gangliosides constitute about 50 %, and of the neutral glycolipids the most complex ceramide saccharides dominate.

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1. McIlwain, H. *Chemical Exploration of the Brain*, Elsevier Publishing Co., Amsterdam 1963.
2. Svennerholm, L. *Acta Chem. Scand.* **17** (1963) 860.
3. Klenk, E. and Padberg, G. *Z. physiol. Chem.* **327** (1962) 249.
4. Eberhagen, D. *Z. physiol. Chem.* **333** (1963) 179.
5. Svennerholm, L. *J. Neurochem.* **10** (1963) 613.
6. Svennerholm, E. and Svennerholm, L. *Biochim. Biophys. Acta.* **70** (1963) 688.
7. Svennerholm, L. *Acta Chem. Scand.* **17** (1963) 1170.
8. Pratt, J. P., Kaucher, M., Moyer, E., Richards, A. J. and Williams, H. H. *Am. J. Obstet. Gynecol.* **52** (1946) 665.
9. Mårtensson, E. *Acta Chem. Scand.* **17** (1963) 2356.
10. Svennerholm, E. and Svennerholm, L. *In preparation.*
11. Svennerholm, E. and Svennerholm, L. *Nature* **198** (1963) 688.

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