

Gangliosides and Neutral Glycosphingolipids of Human Pancreas

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The gangliosides and neutral glycosphingolipids composition of adult human pancreas were quantitatively isolated and characterized in autopsy material obtained from persons who died in accidents. The concentrations of proteins were 34,9 and 37,1 mg/g fresh tissue, whereas the values for DNA were 3,2 and 2,7 mg per g wet weight. The total gangliosides (lipid-bound sialic acid) in pancreas were 286,1 and 230,4 nmol NeuAc per g fresh tissue. Gangliosides G_{M3} and G_{D3} were most abundant, but G_{M2} , G_{M1} , G_{D1a} , G_{D1b} , and G_{T1b} were also present. The molar ratio of protein - and lipid-bound sialic acid were 2,1 and 1,8. The concentrations of neutral glycosphingolipids were 38,3 and 44,4 nmol/g wet weight. The neutral glycosphingolipids consisted of ceramide mono-, di-, tri-, tetrahexosides, asialo G_{M1} and two compounds having more than four monosaccharides.

INTRODUCTION

Glycosphingolipids are present in the plasma membrane of a great number of different cell types. Their location and their extensive variability suggest prominent role for these compounds in the membrane-mediated cellular phenomena. Indeed, there is a great number of evidence indicating that these compounds play an important role in the cell behavior during differentiation, development, growth control and oncogenic transformation (3). The complex glycolipids serve as cell surface receptors for various hormones (4 - 9), but the involvement of the cell surface glycosphingolipids in the hormone action is complex. Saltiel et al. (10) demonstrated involvement of a target cell glycolipid in the action of insulin. These authors suggested that stimulation of an endogenous phospholipase C activity results in formation of a complex carbohydrate effector which may mediate some of insulins' actions. Insulin has important medical implications. Five percent of the population in developed countries have diabetes mellitus, and an equal number are likely to develop the disease. Diabetes mellitus is due to the insufficient action of insulin, owing either to its absence or to resistance to its action (11).

Whereas glycosphingolipids have been extensively studied in other organs (12), little information is available on these structures in the pancreas. For this reason we report the results from our examinations of the glycosphingolipids in human pancreas.

Key words: Gangliosides, glycosphingolipids (human pancreas)

MATERIALS AND METHODS

Tissue material. The pancreas were obtained from two subjects, who had died in accidents. The pancreas were checked by a trained pathologist and were found to be without macroscopical signs of disorder. Surrounding connective tissue and large blood vessels were removed, and the pancreas was rinsed several times with saline.

Abbreviations: NeuAc, N-acetylneuraminic acid; CMH, ceramide monohexoside; CDH, ceramide dihexoside; CTH, ceramide trihexoside; CTetH, ceramide tetrahexoside; G_{M1} , $GgOse_4Cer^+$; G_{M3} , $II^3NeuAc-LacCer^+$; L_{M1} , $IV^3NeuAc-LcOse_4Cer^+$; G_{M1} , $II^3NeuAc-GgOse_4Cer^+$; G_{D3} , $II^3(NeuAc)_2-LacCer^+$; G_{D1a} , $IV^3NeuAc-GgOse_4Cer^+$; G_{D1b} , $II^3(NeuAc)_2-GgOse_4Cer^+$; HPTLC, high performance thin-layer chromatography.

^{*} Nomenclature according to Svennerholm (1)

^{*} Nomenclature according to IUPAC-IUB recommendations (2).

Chemicals. Sephadex LH 20 and DEAE Sephadex A 25 were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. HPTLC plates were obtained from Merck A.G. Darmstadt, F.R.G. *Vibrio cholerae* neuraminidase E.C.3.2.1.18. was obtained from Behringwerke A.G., Marburg, F.R.G. All other chemicals and organic solvents used in these experiments were of the highest purity grade available, and were purchased from Kemika, Zagreb, Croatia. Standard gangliosides were a gift from Dr.I.Kračun. Neutral glycosphingolipids and sphingosine base were isolated in this laboratory and were obtained from Prof.M.Proštenik.

Isolation and separation of glycosphingolipids. Pancreas tissue (between 400 and 500 mg) was homogenized in a glass homogenizer with Teflon pestle in ice cold water. Glycosphingolipids were isolated with chloroform/methanol/ water mixture (13). After reextraction and centrifugation the quantity of protein-bound sialic acid was determined in the residue after hydrolysis with 0.1 M H_2SO_4 (14). Lipid extracts were applied to the column with 1.1 g DEAE Sephadex A 25 equilibrated with 100 ml chloroform /methanol/water 30:60:8 (15). After elution two fractions were collected: Fraction I which was eluted with 150 ml chloroform/methanol/water 30:60:8 contained neutral glycosphingolipids and Fraction II eluted with 100 ml chloroform/methanol/0.08 M NaOAc 30:60:8 which contained gangliosides. After mild alkaline hydrolysis and purification on Sephadex LH 20 column (13), the gangliosides were quantified with resorcinol/HCl reagent (16). Neutral glycosphingolipids were purified on magnesia-silica gel column (17). The amounts of neutral glycosphingolipids were measured by determination of the sphingosine content (18). The proportions of individual glycosphingolipids were quantified after separation on HPTLC plates at 515 nm (neutral glycosphingolipids) and 620 nm (gangliosides) using Camag densitometer. The developing systems were chloroform / methanol / 0.2% $CaCl_2$ 60:40:10 for gangliosides and chloroform/methanol/ water 65:25:4 for neutral glycosphingolipids. The spots were located with resorcinol reagent and with orcinol- H_2SO_4 reagent respectively. The gangliosides were also analysed by enzymatic hydrolysis (19).

A small amount of tissue (about 50 mg) was homogenized separately and quantity of proteins (20), and DNA (21) was determined.

RESULTS AND DISCUSSION

The concentrations of proteins in the samples of human pancreas were 34.9 (Case 1) and 37.1 (Case 2) mg per gram fresh tissue weight. The concentrations of DNA were 3.2 (Case 1) and 2.7 (Case 2) mg per g wet weight. The values for sialoglycoproteins were 477.7 (Case 1) and 509.3 nmol NeuAc/g tissue.

The total gangliosides determined in pancreas were 230.4 (Case 1) and 286.1 (Case 2) nmol per g fresh tissue. Gangliosides can be divided into several groups according to their biosynthetic pathway (22). Starting from the G_{M3} which is a key intermediate of ganglioside biosynthesis, the gangliosides G_{M2} , G_{M1} , and G_{D1a} are assigned to the a-series and G_{D3} , G_{D1b} and G_{T1b} are regarded as b-series products. The ganglioside pattern of human pancreas is shown in Fig. 1. For quantification, the bands were analysed by densitometry and the proportion of gangliosides was calculated. In Table 1 the percentage of gangliosides is presented for human pancreas. The a/b ratio of 0.7 which was calculated by dividing the percentage of sialic acid bound to G_{M2} , G_{M1} plus G_{D1a} by the percentage bound to G_{D3} , G_{D1b} plus

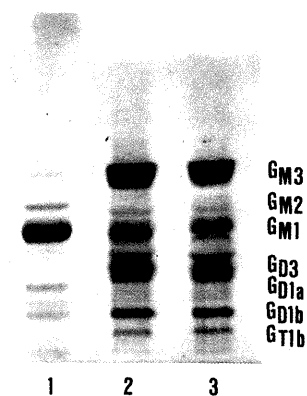


Figure 1:

Gangliosides from human pancreas.

Gangliosides from human pancreas were isolated and purified as described in Material and Methods, separated on HPTLC plates using chloroform/methanol/0.2% $CaCl_2$ 60:40:10, and visualised by spraying with resorcinol reagent. Lane 1: standard of gangliosides; Lane 2 and 3: gangliosides from human pancreas.

TABLE 1
Ganglioside composition in the human pancreas. (%)

Ganglioside	Case 1	Case 2
G _{M3}	43.2	61.7
G _{M2}	3.7	2.0
G _{M1}	16.7	9.2
G _{D3}	20.3	16.4
G _{D1a}	3.2	2.7
G _{D1b}	9.9	5.6
G _{T1b}	3.0	2.4
a/b ratio	0.7	0.7

The gangliosides are designated according to Svennerholm (1).
Results are expressed as percentage distribution of total lipid-bound sialic acid.

G_{T1b} showed preference for b-series gangliosides. It is well known that extraneural tissues contained predominantly lactose-containing gangliosides such as G_{M3} and G_{D3}, but they also contained polar gangliosides with ganglio-N-tetraose in significant concentrations (23).

The molar ratio of protein- and lipid-bound sialic acid which were 2.1 (Case 1) and 1.8 (Case 2) could point out the prominent role of sialoglycoproteins in the adult human pancreas.

The concentration of neutral glycosphingolipids was 38.3 (Case 1) and 44.4 (Case 2) nmol per g fresh tissue weight. The neutral glycosphingolipid fraction of human pancreas was significantly lower than the concentration of the ganglioside fraction. Thin-layer chromatography of neutral glycosphingolipids from human pancreas showed five orcinol positive double bands migrating at the position of standard ceramide monohexoside, ceramide dihexoside, ceramide trihexoside, ceramide tetrahexoside, asialo G_{M1} and two bands migrating at the position of more polar components (Fig. 2). After densitometry at 580 nm proportion of neutral glycosphingolipids was calculated. Table 2 presented the percentage distribution of neutral glycosphingolipids in human pancreas. The pattern of pancreatic neutral glycosphingolipids has not been previously described. In other extraneural tissues it ranged from predominantly glucosylceramide with only traces of more polar components (24), to a composition with predominantly polar glycosphingolipids (25). In the present study pancreatic neutral glycosphingolipids were found to consist of predominantly polar fractions.

Three systems, nervous, haematopoietic and digestive, are characterized by a wide molecular diversity of glycosphingolipids, that is, the molecular diversification of gangliosides in brain, gangliosides and neutral glycosphingolipids in haematopoietic system and neutral glycosphingolipids in intestinal tissues (26). Although we have only been able to determine the major glycosphingolipids of human pancreas, this study has shown the diversity in the composition of gangliosides and neutral glycosphingolipids.

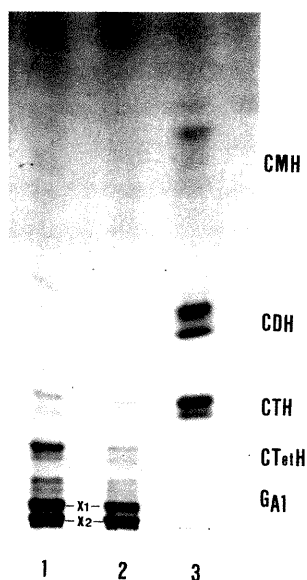


Figure 2:

Neutral glycosphingolipids from human pancreas.

Neutral glycosphingolipids from human pancreas were isolated and purified as described in Material and Methods. Samples were separated on HPTLC plates using chloroform/methanol/water 65:25:4, and the spots were located with orcinol- H_2SO_4 reagent.

Lane 1 and 2: neutral glycosphingolipids from human pancreas; Lane 3: standard of neutral glycosphingolipids.

TABLE 2.

Composition of neutral glycosphingolipids in human pancreas (%)

Glycosphingolipid	Case 1	Case 2
CMH	3.1	3.9
CDH	6.7	7.5
CTH	10.2	8.5
CTetH	21.1	13.5
G _{A1}	15.1	14.7
X1	22.3	26.0
X2	21.5	25.9

Results are expressed as percentage distribution of total sphingosine content.

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SAŽETAK

Gangliozidi i neutralni glikosfingolipidi pankreasa čovjeka

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Gangliozidi i neutralni glikosfingolipidi pankreasa čovjeka su kvantitativno izolirani i karakterizirani u materijalu dobivenim nakon autopsije osoba koje su poginule u nesrećama. Koncentracija proteina iznosila je 34.9 odnosno 37.1 mg/g svježeg tkiva, dok su vrijednosti za DNA bile 3.2 i 2.7 mg/g svježeg tkiva. Koncentracija ukupnih gangliozida (lipidno vezana sijalinska kiselina) iznosila je 286.1 odnosno 230.4 nmol NeuAc po gramu svježeg tkiva. Humani pankreas sadrži najviše G_{M3} i G_{D3} gangliozida, a prisutni su također G_{M2} , G_{M1} , G_{D1a} , G_{D1b} , G_{T1b} gangliozidi. Molarni odnos između proteinski i lipidno vezane sijalinske kiseline iznosio je 2.1 odnosno 1.8. Koncentracija neutralnih glikosfingolipida u pankreasu čovjeka bila je 38.3 odnosno 44.4 nmol/g svježeg tkiva. Metodom tankoslojne kromatografije ustanovljeno je da se neutralni glikosfingolipidi sastoje od ceramid mono-, di-, tri-, i tetrasaharida, od asialo G_{M1} i dvije frakcije koje sadrže više od četiri šećerna ostatka vezana na ceramidni dio.

