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Structure and Function of Sphingolipids

2. Differences in Sphingolipid Concentration, Especially Concerning Sulfatides, between Some Regions of Bovine Kidney

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In an attempt to correlate sphingolipid structure with tissue function, kidney was chosen as an object of study, as almost all known animal sphingolipids have been detected in this organ. The first paper in this series [1] reports qualitative as well as quantitative differences in the total long-chain base fractions from different regions of kidney. As a development of this, different classes of intact sphingolipids have been prepared from the same regions.

Fresh bovine kidneys were macroscopically dissected into cortex, medulla, and papillae. To obtain pure preparations of cortex and medulla, the transition zone was collected separately. Only small apices were taken as papillae in this dissection. From another number of kidneys, the apices were cut somewhat larger.

The homogenized and lyophilized tissue was extracted in hot chloroform-methanol (2/1, v/v), once with 20 ml/g dry tissue, and four times with 10 ml/g. This procedure was found to leave less than 2 % of the total long-chain bases, measured gravimetrically on dinitrophenyl (DNP) base fractions,[1] isolated after acid hydrolysis of remaining substance. The combined extracts were evaporated to dryness and submitted to alkaline degradation for 24 h at 37°C, using 1 M KOH in water, 10 ml/g lipid. After acidification to pH 2 with HCl, partition was done (chloroform-methanol-water 8:4:3, v/v/v). The upper phase from a total kidney preparation contained only 0.4 % of the total long-chain bases, measured as DNP derivatives, why the lower, lipid phase was taken alone for the preparations.

Fractionation by column chromatography was performed principally according to Rouser *et al.*[2], using silicic acid, magnesium silicate (Florisil) and diethylaminoethyl (DEAE) cellulose. Purity of fractions was tested by thin-layer chromatography. For identification, references of human brain and kidney sphingolipids were used, as well as infrared spectroscopy and analysis of hydrolysis products (carbohydrates,[3] long-chain bases,[1] and fatty acids). The figures given in Table 1 were determined gravimetrically.

The most prominent difference found concerns the sulfatides, which have a far higher concentration in medulla than in cortex. In medulla, the papillary part has a somewhat lower concentration. The total neutral glycolipids as well as the purified cerebrosides increase in concentration from cortex to papillae. On the other hand, the sphingomyelins, the dominating fraction, decrease in concentration from cortex to papillae. The composition of the transition zone is generally intermediary, compared to those of cortex and medulla. Cerebrosides are the dominating glycolipids, in contrast to human kidney cerebrosides, which are small components (0.22 mg/g dry tissue).[4] Tetraglycosyl ceramides are only trace components in bovine kidney, while they are the main glycolipids in human kidney (1.77 mg/g dry tissue).[4] Sulfatides with two hexose residues [5] have not been

Table 1. Sphingolipid composition of different bovine kidney regions. Figures are expressed as mg/g dry weight tissue.

	Cortex	Transition zone	Medulla	Papillae	
				Large apices	Small apices
Ceramides[a]	1.3	1.3	1.1	0.9	1.0
Cerebrosides	0.8	1.1	1.9	2.1	2.0
Oligoglycosylceramides	0.6	0.6	1.0	1.6	2.1
Sphingomyelins	17.8	16.4	9.8	5.2	5.1
Sulfatides	0.1	0.3	0.9	0.4	—

[a] A minor part of the ceramide fractions was identified as *N*-acyl ethanolamine (after isolation, hydrolysis, and comparison with synthetic *N*-stearoyl ethanolamine, a gift from I. Pascher of this Institute). This lipid may be a by-product [8] or a catabolite of ceramides, as long-chain bases recently were shown to be degraded to ethanolamine.[9,10]

detected. Acid glycolipids, behaving like gangliosides on thin layer chromatography, have been noticed in small amounts.

Preliminary studies on human kidney sulfatides have shown, that sulfatides containing one hexose residue are more concentrated in medulla than in cortex, while the opposite is true for the sulfatides containing two hexose residues (to be published).

The detailed composition of the lipids isolated will be reported in later communications. The sulfatides from bovine kidney cortex and medulla were almost identical in composition. Galactose was the only sugar found, mainly long-chain (C_{22}, C_{23}, C_{24}) 2-hydroxy fatty acids were present and phytosphingosine made up one third to one half of the total long-chain bases. A partial characterization of sphingomyelins has been reported.[6]

The localization of sulfatides to the outer zone of medulla, where the corticosteroid dependent sodium ion transport is present in the thick ascending part of the loop of Henle,[7] has directed our interest towards sulfatides as possible carriers or receptors in sodium ion transport. Therefore, a systematic study has been initiated to correlate sulfatide characteristics with variables in sodium ion transport.

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