THE BIOSYNTHESIS OF DIGALACTOSYLCERAMIDE IN THE KIDNEY OF THE C57/BL MOUSE

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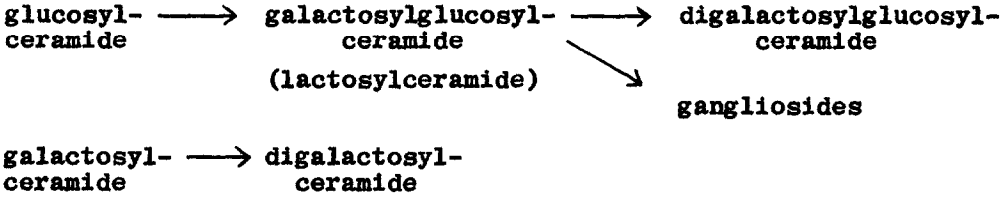
In the presence of UDP-^{14}C-galactose, homogenates of male or female mouse strain C57/BL kidneys catalysed the synthesis of lactosylceramide from added glucosylceramide, digalactosylglucosylceramide from lactosylceramide and digalactosylceramide from galactosylceramide. The synthesis of galactosylceramide was stimulated by ceramide containing hydroxy fatty acids rather than that with nonhydroxy fatty acids. Differences in activity between male and female tissue are reported.

Lactosylceramide is the predominant diglycosylceramide in most mammalian tissues (1) and is the immediate precursor of the gangliosides (2). A second compound, digalactosylceramide, is also known, (3) and is the predominant diglycosylceramide in the kidneys of several mouse strains (e.g. C57/BL, BALB/c,A) (4). Hauser (5, 6) showed that homogenates of several rat tissues, including kidney, were capable of synthesising lactosylceramide from added glucosylceramide and UDP-^{14}C-galactose, and digalactosylglucosylceramide from added lactosylceramide.



In a study of the biosynthesis of diglycosylceramides, with homogenates of mouse strain C57/BL kidney as the enzyme source, Hauser and Hildebrand (7) obtained a synthesis of lactosylceramide from added glucosylceramide and UDP-^{14}C-galactose, but

not of digalactosylceramide when galactosylceramide was added as acceptor.

It was recently shown (8) that whereas the kidneys of male C3H/He, C57/BL, C57xA, A and BALB/c mice contained substantial proportions of diglycosylceramides, those of the females contained at most only traces. These findings and those of Hauser and Hildebrand, prompted us to study the pathways of biosynthesis of the diglycosyl- and triglycosylceramides in the kidneys of male and female C57/BL mice, with the results presented in this paper.

MATERIALS Glucosylceramide, lactosylceramide and digalactosylglucosylceramide were obtained from pig-lung (9). Galactosylceramide from human brain was separated into nHFA[1]-galactosylceramide and HFA[1]-galactosylceramide by chromatography on silica gel H with the solvent system $CHCl_3/CH_3OH/H_2O$, (90:10:1 by vol.). Ceramide, obtained from pig-lung sphingomyelin treated with phospholipase C (10), was separated into nHFA-ceramide and HFA-ceramide (5% of total) by chromatography on silica gel (11) and the fatty acid composition checked by gas chromatography of the methyl esters (3% OV-1 on Gas Chrom Q). Uniformly labelled UDP-^{14}C-galactose, 240 mCi/mmole, was obtained from the Radiochemical Centre, Amersham, England.

Kidneys were obtained immediately after death from 10- to 12-week old C57/BL male or female mice and were homogenised with a Potter-Elvehjem homogeniser.

METHODS Protein was determined by the method of Lowry (12). Incubation mixtures (see Table 1) were vigorously stirred at 37° for 30 min. and the reaction stopped with 5 ml $CHCl_3/CH_3OH$, (2:1 v/v). The water-soluble components were removed by washing

[1] nHFA, non-hydroxy fatty acid; HFA, hydroxy fatty acid.

TABLE 1

Incorporation of ^{14}C-galactose into glycosphingolipids with male and female kidney homogenates

Incubation mixture: celite (50 mg) was added to a solution of glycosphingolipid substrate (500 µg) in $CHCl_3/CH_3OH$ (2:1 v/v) and the solvent removed by a stream of nitrogen. To this was added 0.05N Tris/HCl buffer, pH 7.2 (containing 0.8% mercaptoethanol), 0.1 ml; 0.15M manganese chloride, 0.02 ml; 0.1M ATP, 0.01 ml; UDP-^{14}C-galactose, 500,000 counts/min in 0.1 ml; and 0.1 ml (4-5 mg protein) whole homogenate of kidney, 0.4 g. wet weight in 1 ml 0.15N KCl containing 0.8% mercaptoethanol.

ADDED ACCEPTOR	MALE		FEMALE	
	diglycosyl-ceramide	triglycosyl-ceramide	diglycosyl-ceramide	triglycosyl-ceramide
none	415	350	70	110
glucosyl-ceramide	925	1,050	415	470
nHFA-galactosyl-ceramide	6,950	695	4,550	510
galactosyl-glucosyl-ceramide	565	4,150	210	5,050

Results expressed as counts/min per 5 mg protein.

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the chloroform phase once with 0.8 M KCl (1.0 ml) and twice with 'theoretical upper phase' containing KCl (13). The chloroform phase was removed from the celite after centrifugation and evaporated to dryness. The residue was redissolved in a little $CHCl_3/CH_3OH$, (2:1 v/v), and applied to a thin-layer chromatography plate (Merck precoated silica gel) which was developed in $CHCl_3/CH_3OH/H_2O$ (65:25:4, by vol.). The components were made visible with iodine vapour, and the glycosphingolipids located by reference to known compounds chromatographed on the same plate. Radioactive compounds were located with a thin-layer radiochromatogram scanner (Panax Ltd.). The amount of radioactivity

was estimated, after scraping the band of silica gel containing the component into the vial containing scintillation fluid, by a liquid-scintillation spectrometer (Nuclear Chicago, Model 720).

Identification of Labelled Products. The silicic acid which retained the radioactive compounds while in the scintillation fluid was separated by filtration, and the compounds recovered by elution with $CHCl_3/CH_3OH$ (1:4, v/v). Phospholipids were removed by treating the fractions with 0.1N methanolic NaOH and glycosphingolipids were recovered by chromatography (14). Each labelled glycosphingolipid so obtained was mixed with a corresponding compound of known structure and chromatographed (15) on a thin layer plate, 20 cm x 20 cm, in two dimensions (solvent 1, $CHCl_3/CH_3OH/H_2O$, 65:25:4; solvent 2, tetrahydrofuran/methylal/methanol/2N ammonia, 50/30/20/5, v/v). In addition, the monoglycosylceramide was chromatographed on a plate impregnated with borate (16), with HFA-galactosylceramide, nHFA-galactosylceramide and glucosylceramide as reference compounds.

RESULTS The incubation system described, without detergent and with the substrate dispersed on celite (11), was preferred to that of Hauser (5) because there was less breakdown of added UDP-^{14}C-galactose by the homogenate and a greater stimulation of triglycosylceramide synthesis. With this system, and with either male or female kidney homogenates as the enzyme source, diglycosyl- and triglycosylceramides were synthesised from UDP-^{14}C-galactose and the added substrates glucosylceramide, nHFA-galactosylceramide and lactosylceramide, (Table 1). There was also synthesis of galactosylceramide with HFA-ceramide as substrate (Table 2).

The purified labelled diglycosyl- and triglycosylceramides formed in the incubations were indistinguishable from the

TABLE 2

Incorporation of ^{14}C-galactose into ceramides with male and female kidney homogenates

ADDED ACCEPTOR	MALE		FEMALE	
	monoglycosyl-ceramide	diglycosyl-ceramide	monoglycosyl-ceramide	diglycosyl-ceramide
none	610	415	80	70
HFA-ceramide	2,125	455	530	420
nHFA-ceramide	650	460	105	215

Results expressed as counts/min per 5 mg protein.

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authentic glycosphingolipid carriers on two dimensional chromatography. The monoglycosylceramide produced endogenously (Table 2) had the same R_f as HFA-galactosylceramide on a borate impregnated plate.

DISCUSSION In the absence of added substrate, UDP-^{14}C-galactose is incorporated into the endogenous glycosphingolipids of the kidney homogenate (Tables 1 and 2). The level of incorporation is higher in the male, possibly due to the greater amounts of glycosphingolipids in male tissue (male 0.53 μmoles/g. wet weight; female 0.26 μmoles/g. (8)).

In the presence of added glucosylceramide, lactosylceramide is actively synthesised by kidney homogenates from both sexes. However, as the synthesis of digalactosylglucosylceramide from lactosylceramide is more rapid (Table 1), accumulation of the latter compound in the tissue does not occur. The diglycosyl-ceramide in male C57/BL kidney is almost exclusively digalactosylceramide, and in contrast to those of Hauser and Hildebrand (7) our results showed that kidney homogenates are

able to synthesise this compound from UDP-^{14}C-galactose and galactosylceramide. Although female kidney contains only a small amount of diglycosylceramide (5% of the total glycosphingolipids) compared with male tissue (29%) (17) the rate of synthesis of this compound from added substrate is similar to that in the male. A possible explanation of this anomaly is a lack of the substrate, galactosylceramide, in vivo, and so the biosynthesis of this compound via ceramide (11) was investigated. The results (Table 2) show that the rate of the synthesis was indeed much higher in homogenates of male kidney than of female kidney.

It was noticed that the chromatographic properties of the monogalactosylceramide produced endogenously were similar to those of a compound containing hydroxy fatty acids (HFA) and so ceramides containing either HFA or nHFA were used as substrates. The results (Table 2) suggested that in our experimental conditions HFA-ceramide was preferred to nHFA-ceramide.

Another point for consideration is that monogalactosylceramide is also the likely precursor for sulphatide (galactosyl-(3-sulphate)-(1→1)-ceramide) (18), the major component of C57/BL mouse kidneys (male 27% of total (17), female 42%), and in conditions where there is only a limited amount of substrate, the synthesis of sulphatide may take precedence over that of digalactosylceramide.

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