GLYCOSPHINGOLIPID BIOSYNTHESIS IN KIDNEYS OF NORMAL C3H/He MICE AND OF THOSE WITH BP8 ASCITES TUMOURS

J.B. Hay and G.M. Gray

The Lister Institute, Chelsea Bridge Road, London, S.W.1.

Received December 16, 1969

The ability to synthesise lactosylceramide is higher in the kidney of the male C3H/He mouse than in that of the female. The presence of a BP8 ascites tumour in the host depresses the synthesis of lactosylceramide and galactosylgalactosylglucosylceramide in the kidneys of both male and female mice.

Previous studies (1) showed that the glycosphingolipid composition of the kidneys in C3H/He mice with BP8 ascites (sarcoma) tumours was different from that in the kidneys of normal mice. It was also shown (2) that the glycosphingolipid composition of the kidneys in the normal male C3H/He mouse was different from that in the female and that the effect of the BP8 tumour on the kidney glycosphingolipids appeared to be confined to the female (2). The kidneys of these female mice contained only traces of galactosylgalactosylglucosylceramide compared with the significant amounts in normal female kidney.

This deficiency could arise from either (a) an increase in catabolism or (b) an increase in the synthesis of N-acetylgalactosaminylgalactosylgalactosylglucosylceramide (aminoglycolipid), for which the triglycosylceramide is the likely precursor, or (c) a decrease in its own synthesis from the precursor, lactosylceramide (3, 4). Studies on the degradation of triglycosylceramide in kidneys of C3H/He mice, so far, have been unrewarding. A deficiency resulting from an increased

requirement of the triglycosylceramide for the synthesis of aminoglycolipid appeared unlikely because the amounts of the latter compound in kidneys of both normal female mice and those with tumours were similar (2). It was relevant, therefore, to study the biosynthesis of the diglycosyl- and triglycosyl-ceramides in mouse kidneys and the results are presented in this communication.

Materials. Uniformly labelled UDP-^{14}C-galactose, 240 mCi/mM (Radiochemical Centre, Amersham, England). Glucosyl-(1→1)-ceramide and galactosyl-(1→4)-galactosyl-(1→4)-glucosyl-(1→1)-ceramide were obtained from pig lung (5, 6). Lactosylceramide was synthesised by the method of Hay and Gray (7).

Male and female C3H/He mice, 3-4 months old, were used. A tumour was grown from an intraperitoneal injection of 8 x 10^5 BP8 ascites cells. The mouse was killed 10-12 days later and the kidneys immediately removed. When required the BP8 cells were harvested at the same time and washed free from red cells and ascites fluid with phosphate-buffered saline (pH 7).

Methods. Protein was estimated by the method of Lowry et al. (8). Homogenates of whole mouse kidney (or BP8 cells) in 0.25M sucrose (0.25 g kidney/ml or 0.25 ml packed BP8 cells/ml) were used as the source of enzymes for glycosphingolipid biosynthesis.

The incubation mixture was similar to that described by Hauser (9) except that the pH was 6.1 and the detergent, Triton-X-100, was used instead of Cutscum. The glycosphingolipid acceptor (500 µg glucosylceramide or 250 µg lactosylceramide) in $CHCl_3$-CH_3OH (2:1, v/v) was pipetted into a test tube (5 ml) and the solvent was completely removed by evaporation. 0.25 ml tris maleate buffer, pH 6.1, (0.1M), 0.1 ml $MnCl_2$ (0.1M) and 0.04 ml Triton-X-100 (1% aqueous solution) were added. The lipid was

dispersed by warming the solution in boiling water. The solution was cooled and 0.06 ml UDP-^{14}C-galactose (0.75 μCi) and 0.2 ml tissue homogenate in 0.25M sucrose was added. The solutions were incubated for 1 hr at 37° after which the reactions were stopped by the addition of 10 ml $CHCl_3/CH_3OH$ (2:1, v/v).

Lactosylceramide (500 μg) and galactosylgalactosylglucosylceramide (500 μg) were added as carriers for the labelled products and the reaction mixture was washed twice with 2 ml 0.1M KCl and twice with 2 ml Folch theoretical upper-phase (10). The lower $CHCl_3$ phase, which contained the lipids, was evaporated to dryness and the residue was treated with mild alkali (11) to hydrolyse most of the phospholipids and neutral lipids. The solution was neutralised and washed with 0.1M KCl. The $CHCl_3$ solvent was evaporated and the residue was redissolved in $CHCl_3$-CH_3OH (2:1, v/v) and chromatographed on a thin layer plate of silicic acid (Merck precoated plate, 20 x 20 cm) with solvent $CHCl_3$-CH_3OH-H_2O (65:25:4, by vol.). The components were made visible with iodine vapour and radioactive compounds located with a thin-layer radiochromatogram scanner (Panax Ltd.). The radioactivity was measured, after transferring the relevant area of silicic acid into a vial of scintillation fluid, by a Nuclear-Chicago liquid-scintillation spectrometer. Unequivocal identification of the labelled products as glycosphingolipids was made as described by Coles and Gray (12).

Results and Discussion. Under conditions similar to those described by Hauser (9) incorporation of radioactivity from UDP-^{14}C-galactose was obtained with homogenates of C3H/He mouse kidneys and BP8 ascites tumour cells (Tables I and II). Incorporation was stimulated in the presence of glucosylceramide and lactosylceramide and the amount varied in different homogenates.

Table I

Incorporation of ^{14}C-galactose into glycosphingolipids in homogenates of kidneys from normal C3H/He mice and those with BP8 tumours

	Normal C3H/He mouse		C3H/He mouse with BP8 ascites tumour	
	^{14}C-labelled product*			
Added acceptor	diglycosyl ceramide	triglycosyl ceramide	diglycosyl ceramide	triglycosyl ceramide
A. Male				
None	133	172	106	131
glucosylceramide	2000	412	365	152
lactosylceramide	73	970	244	480
B. Female				
None	152	110	105	96
glucosylceramide	365	331	132	48
lactosylceramide	49	1640	98	630

* results expressed as counts/min per 5 mg protein (homogenate)

The lactosylceramide synthesised from added glucosylceramide by normal female kidney homogenates was much less than that by male kidney homogenates (Table I). On the other hand there was a greater synthesis of galactosylgalactosylglucosylceramide from added lactosylceramide in the normal female than in the normal male. In kidney homogenates from mice with BP8 tumours the synthesis of both lactosylceramide and galactosylgalactosyl-glucosylceramide were depressed well below the levels observed in normal mice (Table I) and in the kidney homogenate from the female with tumour there was virtually no stimulation of lactosylceramide synthesis by added glucosylceramide. It was noteworthy that homogenates of BP8 cells from either male or female mice actively synthesised lactosylceramide and galactosyl-

Table II

Incorporation of ^{14}C-galactose into glycosphingolipids in homogenates of BP8 ascites tumour cells

	cells from male C3H/He mouse		cells from female C3H/He mouse	
	^{14}C-labelled product*			
Added acceptor	diglycosyl ceramide	triglycosyl ceramide	diglycosyl ceramide	triglycosyl ceramide
None	1325	845	1505	960
glucosylceramide	8810	1530	7590	2125
lactosylceramide	1505	2710	1685	4230

* as for Table I.

galactosylglucosylceramide from added acceptors (Table II).

These incorporation studies with the different kidney homogenates provide explanations for some of the earlier results (1, 2). For instance, the much higher level of lactosylceramide synthesis in the normal male C3H/He mouse may account for the significant amounts of this compound in male kidney compared with only traces in the female kidney. It is also of interest that the synthesis of lactosylceramide in the female kidney is stimulated by testosterone (Hay and Gray, unpublished results). Since in kidney homogenates from females with tumours, the synthesis of the triglycosylceramide was still stimulated by added lactosylceramide, a possible explanation for its apparent disappearance in the kidney might be a lack of precursor (lactosylceramide) from which to synthesise it in order to replace any used in the normal metabolic processes. There is some support for this by the fact that little or no synthesis of lactosylceramide occurred when glucosylceramide was added to the kidney homogenate (Table I).

Acknowledgement

The support of this work by the British Empire Cancer Campaign for Research is gratefully acknowledged.

References

1. Adams, E.P. and Gray, G.M. Nature, 216, 277 (1967).
2. Coles, L., Hay, J.B. and Gray, G.M. J. Lipid Res. To be published.
3. Hauser, G. and Hildebrand, J. Fed. Proc. 28, 595 (1969)
4. Hauser, G. and Hildebrand, J. J. Biol. Chem., 244, 5170 (1969)
5. Gallai-Hatchard, J.J. and Gray, G.M. Biochim. Biophys. Acta 116, 532 (1966)
6. Adams, E.P. and Gray, G.M. Chem. Phys. Lipids, 1, 368, (1967)
7. Hay, J.B. and Gray, G.M. Chem. Phys. Lipids, 3, 59 (1969)
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randell, R.J. J. Biol. Chem., 193, 265 (1951)
9. Hauser, G. Biochem. Biophys. Res. Comm., 28, 502 (1967)
10. Folch, J., Lees, M. and Sloane-Stanley, G.H. J. Biol. Chem. 226, 497 (1957)
11. Gray, G.M. Biochim. Biophys. Acta. 144, 511 (1967)
12. Coles, L. and Gray, G.M. Biochem. Biophys. Res. Comm. (See preceding article.)