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GLUCOSYLCERAMIDE AND RELATED COMPOUNDS IN NORMAL TISSUES AND IN GAUCHER DISEASE

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INTRODUCTION

It has been well established that Gaucher disease is caused by genetically determined defect in degradation of glucosylceramide by glucosylceramidase (E.C. 3.2.1.45). Such a defect can readily explain the enormous accumulation of glucosylceramide in the liver, spleen, and other tissues of patients afflicted by the disease. However, more subtle compositional abnormalities are known in this disease in lipids chemically or metabolically related to glucosylceramide. In order to understand these abnormalities, as well as the functional implications of the underlying enzymatic defect, knowledge of the normal distribution and metabolism of glucosylceramide and related compounds would be essential. This chapter attempts to provide a highly selective overview of the vast literature on this subject with special emphasis on areas relevant to Gaucher disease.

CHEMISTRY

Glucosylceramide consists of three major moieties; sphingosine, a long-chain fatty acid, and glucose (Fig. 1). Sphingosine is a long-chain amino diol which in nature is almost always acylated with a long-chain fatty acid (for a potentially important exception, see below). N-acylsphingosine is generically termed, ceramide. The reducing terminal of glucose in the β-anomeric configuration is linked

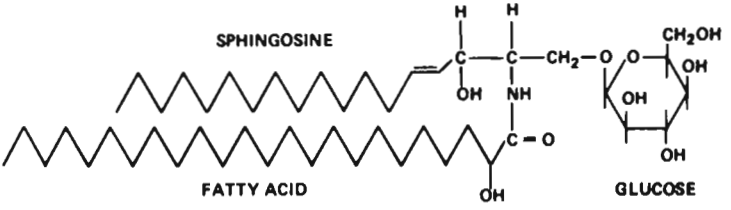

Fig. 1. Structure of glucosylceramide.

to C-1 of sphingosine. Glucosylceramide is also called glucocerebroside, the term, cerebroside, being a generic name for monohexosylceramide. There are many lipids that are chemically and/or metabolically related to glucosylceramide. They are depicted in simplified notations in Fig. 2.

BIOSYNTHESIS AND DEGRADATION

The long-chain base, sphingosine, and its saturated analogue, dihydrosphingosine, are synthesized from serine and palmitic acid (Brady and Koval, 1958; Brady et al.,1958; Braun et al., 1970). Sphingosine in turn is acylated through the acyl-CoA system to ceramide (Sribny, 1966; Morell and Radin, 1970). Formation of ceramide from sphingosine and free fatty acid through the reverse reaction of ceramidase was demonstrated in vitro (Yavin and Gatt, 1969). However, ceramidase is localized in the lysosome with an acidic pH optimum and its specificity with respect to the chain length of fatty acids does not correspond to the chain length distribution of natural sphingolipids. It is generally accepted now that synthesis of ceramide in vivo occurs through sphingosine and fatty acyl-CoA. The final step of glucosylceramide biosynthesis is achieved by glucosylation of ceramide by UDP-glucose:ceramide glucosyltransferase. The reaction was demonstrated in the particulate fraction of embryonic chick brain (Basu et al., 1968) and then in mammalian tissues (Morell et al., 1970; Brenkert and Radin, 1972; Costantino-Ceccarini and Morell, 1973). Glucosylceramide is a key intermediate for biosynthesis of numerous complex sphingoglycolipids. Biosynthesis generally proceeds by sequential

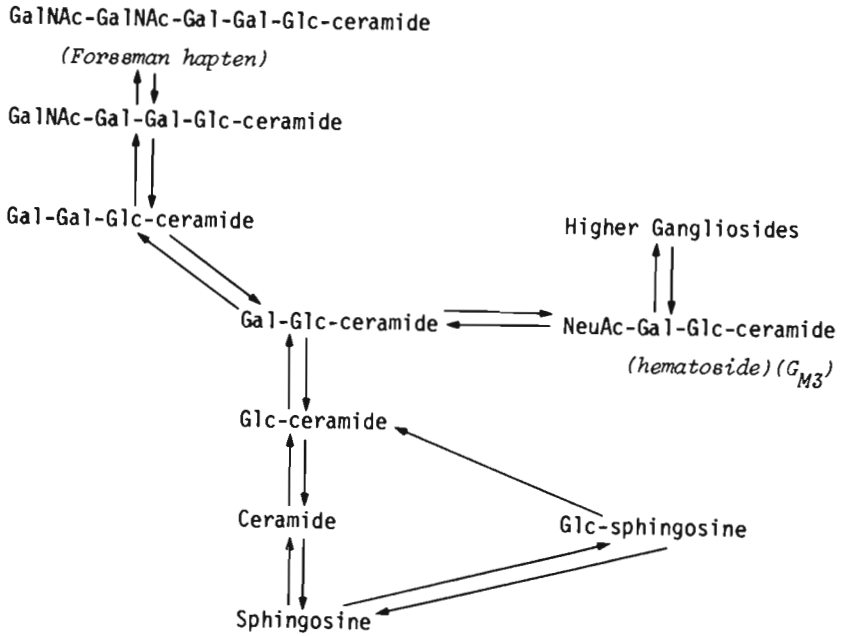

Fig. 2. Chemical and metabolic relationship among glucosylceramide and related compounds.

addition of a sugar moiety following the pathways depicted in Fig. 2. Synthesis of the carbohydrate chain on a lipid intermediate and its subsequent transfer as a whole to the final products, well established for glycoprotein synthesis, are not known to occur for biosynthesis of complex glycosphingolipids.

Degradation of glucosylceramide is catalyzed by an acid lysosomal hydrolase, glucosylceramidase. The enzyme hydrolyzes glucosylceramide to glucose and ceramide. A genetic deficiency of this enzyme causes Gaucher disease (Brady et al., 1965; Patrick, 1965). Since a major emphasis of this entire symposium will be on this particular enzyme, it will not be dealt with any further in this overview. As already been touched upon, there is a catabolic enzyme, ceramidase,

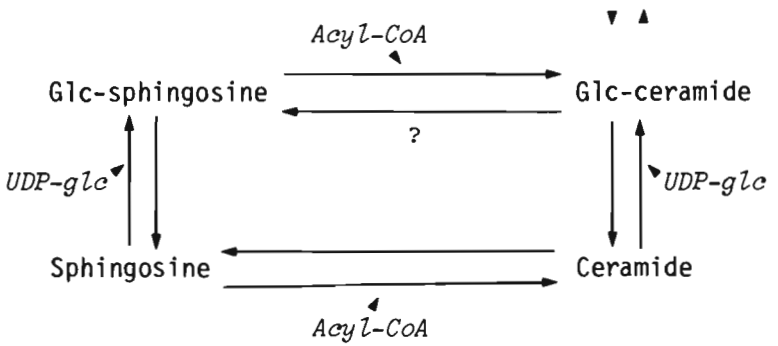

Fig. 3. Demonstrated and potential synthetic and degradative pathways for glucosylsphingosine (glucosylpsychosine)

which degrades ceramide further to sphingosine and fatty acid. The more complex glycosphingolipids that have glucosylceramide as the basic structure (Fig. 2) are catabolized by stepwise removal of the terminal sugars to glucosylceramide.

Potential Importance of Glucosylsphingosine

In the above discussion, one compound in Fig. 2 was left out, glucosylsphingosine. Since glucosylceramide consists of the three components, sphingosine, fatty acid, and glucose, a logically feasible alternative pathway for synthesis and degradation through glucosylsphingosine, rather than ceramide, exists (Fig. 3). The first step of this alternative synthetic pathway, glucosylation of sphingosine, was reported by Curtino and Caputto (1972) with rat brain microsomes. The evidence for the second step, acylation of glucosylsphingosine, was also presented by the same authors with an embryonic chick brain preparation (Curtino and Caputto, 1974). The proof for the enzymatic nature of the second reaction was somewhat tedious; there was no synthesis in a mixture containing boiled microsomes but relatively high conversion was observed in the absence of either intact or boiled microsomes. This finding was explained by inhibition of nonenzymatic conversion in the presence of microsomes. As for the reversal

of this alternative pathway, enzymatic de-acylation of glucosylceramide to glucosylsphingosine has not been demonstrated. However, glucosylsphingosine can be degraded to glucose and sphingosine by a β-glucosidase which is almost certainly identical with glucosylceramidase, because hydrolysis of glucosylsphingosine is also defective in Gaucher disease (Raghavan et al., 1973). Although glucosylsphingosine is absent in normal tissues, the presence of a metabolic machinery for its synthesis and the presence of detectable amounts of glucosylsphingosine in some tissues of patients with Gaucher disease (Raghavan et al., 1974) make it a potentially important compound with respect to the pathophysiology of the disease (more of this later).

CELL BIOLOGICAL CONSIDERATION

From Fig. 2 and the above descriptions, it is clear that synthesis and degradation of glucosylceramide and related compounds follow the same metabolic pathways in the opposite direction. However, the current concept is that, once a complex glycosphingolipid starts down the path of its degradation, it is degraded completely through the pathway and that re-utilization of degradative intermediates back up in the synthetic direction does not occur. This segregation of the synthetic and degradative pathways is presumably due to the topographic separation of the two events within the cell. All of the synthetic enzymes are primarily localized in the microsomal fraction, which is a non-entity cell biologically and is a highly heterogeneous mixture of the endoplasmic reticulum, ribosomes, plasma membranes, Golgi apparatus, and other subcellular components of diverse nature, depending on the source of the tissue. There is considerable evidence that glycosyltransferases are enriched in the Golgi apparatus. On the other hand, physiological degradation of complex glycosphingolipids takes place within the lysosome. All degrading enzymes, including glucosylceramidase, have acidic pH optima, are of glycoprotein in nature, and are localized almost exclusively in the lysosomes, the inside of which is presumably strictly segregated from the rest of the cellular constituents.

When the pathological accumulation of glucosylceramide and related compounds is considered, degradation of constituents of other cells by the cells of the reticuloendothelial system should be kept in mind. The most prominent

accumulation in and consequent enlargements of the liver and spleen in Gaucher disease patients are by this mechanism.

TISSUE DISTRIBUTION

Glucosylceramide is by no means a major constituents of any organs or tissues. Quantitatively it almost always constitutes a minor fraction of total lipids. On the other hand, its distribution among different organs is relatively uniform in contrast to galactosylceramide, which is highly concentrated in the nervous system (Mårtensson, 1969). With the exceptions of the nervous system and the kidney, monohexosylceramide in other tissues are predominantly glucosylceramide. Perhaps more important with respect to the pathogenesis of Gaucher disease is that the erythrocytes, leukocytes and plasma contain glucosylceramide and higher related glycosphingolipids. The erythrocytes contain GM3-ganglioside (hematoside), globoside, digalactosylglucosylceramide, and lactosylceramide in addition to a substantial amount of glucosylceramide. On the other hand, the major glycolipid in the leukocytes is lactosylceramide with glucosylceramide as the second most abundant. The plasma contains glucosylceramide and lactosylceramide as the most abundant glycosphingolipids and lesser amounts of digalactosylglucosylceramide and globoside, most of which are bound to the low and high density lipoprotein fractions with smaller amounts in the very low density lipoprotein fraction (van den Bergh and Tager, 1976). Defective disposition of glucosylceramide and related compounds in these blood components due to the deficiency of glucosylceramidase appears to be the primary cause of most of the systemic clinicopathological manifestation of the disease.

Glucosylceramide is a very minor constituent of normal cerebral lipids. It is present in easily detectable amount in fetal and newborn human brains (Svennerholm, 1964), but its concentration in adult brains is nearly undetectable. However, since the brain is relatively rich in gangliosides, constant synthesis and degradation of glucosylceramide must be required as a part of normal ganglioside turnover, and defective catabolism of glucosylceramide can be expected to result in brain dysfunction.

GLUCOSYLCERAMIDE AND RELATED COMPOUNDS IN GAUCHER DISEASE

Glucosylceramide

The gross enlargement of the spleen and liver, and their histological appearance suggested from early on that abnormal accumulation of certain tissue constituents was responsible for the clinicopathological manifestations of Gaucher disease. Since the first identification of glucosylceramide by Aghion (1934), the abnormal accumulation of glucosylceramide in the reticuloendothelial system of patients with Gaucher disease has been well established (for a recent review, see Brady, 1978).

In normal spleen, glucosylceramide is present in the range of 50-300 μg/g wet weight (Suomi and Agranoff, 1965; Makita et al., 1966; Kennaway and Woolf, 1968; Kuske and Rosenberg, 1972). The values reported in the literature for Gaucher spleen are up to 300 times normal on the basis of unit wet weight. Similarly hepatic glucosylceramide is increased up to 400-fold from the normal concentration of less than 50 μg/g wet weight (Kwiterovich et al., 1970). Since these organs are enormously enlarged, the total glucosylceramide accumulation per organ can easily be several hundred-fold.

Glucosylceramide in the plasma is also increased in all types of Gaucher disease patients (Vance et al., 1969; Philippart, 1972; Brady, 1978; Håkansson, 1979). The degree of the increase in the plasma is modest and rarely exceeds 2-3 times the normal level. The plasma level of glucosylceramide tends to increase after splenectomy. Slightly greater accumulations are usually observed in red cells of patients (Desnick et al., 1973; Brady, 1978; Håkansson, 1979). On the other hand, Klibansky et al. (1976) reported normal levels of glucosylceramide and increased levels of lactosylceramide in leukocytes from ten patients with the type I disease.

Glucosylceramide in the brain of patients with Gaucher disease has long been a subject of controversy. Svennerholm (1967) reported that in a patient with the neuropathic (type II) form of the disease, 70% of monohexosylceramide in the cerebral cortex was glucosylceramide. There are other reports in the literature describing increased levels of glucosylceramide, including those by Maloney and Cumings

(1960), Montreuil et al. (1953), Gonzales-Sastre et al. (1974) and Sudo (1977). On the other hand, there have been reports of normal glucosylceramide in the brain (Philippart et al., 1965; French et al., 1969). However, the data by French et al. were in fact suggestive of presence of a significant amount of glucosylceramide. Compounding the difficulty is that some reports did not make attempt to distinguish glucosyl- and galactosylceramides and that the phenotypic classification of the patients studied was not always clear. It is this author's impression that with the increasing sophistication in the knowledge of the disease and the analytical methodology, firmer documentations of increased glucosylceramide in the brain are forthcoming. Even then, it should be noted that the abnormal increase in the brain would not be anywhere near the level in the spleen or liver. Glucosylceramide in the brain of patients with Gaucher disease remains to be a very minor constituent in the total brain lipid even if the increase is 100-fold over the normal level.

Other Related Compounds

Other glycosphingolipids metabolically related to glucosylceramide have been often found to be moderately increased in organs of patients, such as GM3-ganglioside and lactosylceramide in the spleen (Philippart et al., 1965) or in the brain (Svennerholm, 1967), GM3-ganglioside but not lactosylceramide in the spleen (Kuske and Rosenberg, 1972), or fatty acid esters of glucosylceramide in the brain (Makita et al., 1966). The degrees of accumulation of these compounds, however, are much lower than that of glucosylceramide and their significance in the pathogenesis of Gaucher disease is uncertain.

On the other hand, the potential importance of glucosylsphingosine (gluco-psychosine) in the pathogenesis of Gaucher disease deserves attention. An analogous compound, galactosylsphingosine, is highly cytotoxic and is considered to be a crucial compound in the pathogenetic mechanism in globoid cell leukodystrophy (Krabbe disease), in which an analogous enzyme, galactosylceramidase, is genetically deficient (Suzuki and Suzuki, 1978). Glucosylsphingosine was detected in the spleen of a Gaucher patient by Raghavan et al. (1974). Oshima (1976) and Oshima et al. (1977) provided mass-spectrometric identification of glucosylsphingosine in the spleen of an adult patient. In view of its highly cytotoxic nature,

glucosylsphingosine may well be the key compound in the pathogenetic mechanism of Gaucher disease, particularly as the cause of brain dysfunction in the neuropathic form. Although no definite documentation of glucosylsphingosine in patients' brain is not yet available in the literature as of this writing, the "sulfated glucosylceramide" reported by Sudo (1977) might have been glucosylsphingosine since the identification appears to have been by thin-layer chromatographic mobility only. It is hoped that systematic information on glucosylsphingosine in organs of patients with Gaucher disease be forthcoming shortly.*

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* During the course of the symposium, such information was presented by Svennerholm. See pages 231-252.

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