SPHINGOMYELINASE ISOZYMES OF HUMAN TISSUES: A HYPOTHESIS ON ENZYMATIC DIFFERENTIATION OF THE NEUROPATHIC AND NON-NEUROPATHIC FORMS OF NIEMANN-PICK DISEASE

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SUMMARY

Sphingomyelinase of normal human brain can be separated into two discrete fractions (sphingomyelinase A and B) by Sephadex G-200 gel filtration. Available evidence suggests that these two fractions probably represent two different enzyme proteins. In normal human brain, sphingomyelinase A constitutes 30-40% of the total sphingomyelinase activity, while in the liver, almost all sphingomyelinase is present in the B form with only a trace of sphingomyelinase A. A plausible hypothesis is proposed regarding the enzymatic difference between the neuropathic and non-neuropathic forms of Niemann-Pick disease.

Sphingomyelinase (sphingomyelin phosphodiesterase, E.C. 3.1.4.12) is a phosphodiesterase which specifically hydrolyzes sphingomyelin to sphingosine and phosphorylcholine. The enzyme is genetically deficient in certain forms of Niemann-Pick disease (1, 2). Patients with the neuropathic form of the disease (Crocker's Type A) show severe neurological involvement, in addition to hepatosplenomegaly with an abnormal accumulation of sphingomyelin (3). On the other hand, patients with the non-neuropathic form (Type B) are completely intact neurologically, while the sphingomyelin accumulation in the liver or spleen is as severe as that in Type A patients (3). Although the degree of the sphingomyelinase deficiency often appears to be slightly more severe in Type A, the difference seems to be too small to account for the dramatic difference in the cerebral involvement. Another possibility would be that sphingomyelinase in the brain might, in itself or in the isozyme distribution,

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be different from the hepatic or splenic enzyme and that certain mutations can cause differential inactivation of sphingomyelinase in different organs. Normal human brain sphingomyelinase can be separated into two discrete fractions by Sephadex G-200 gel filtration (4). This report presents a finding that human liver sphingomyelinase consists almost exclusively of only one of the two sphingomyelinase fractions found in the brain. A plausible hypothesis is proposed to explain the phenotypic difference between the two types of Niemann-Pick disease. Unavailability of appropriate pathological specimens make it impossible to test the hypothesis at the present time.

MATERIALS AND METHODS

Postmortem human brain and liver tissues were obtained from patients without neurological or hepatic disorders and were kept frozen at -90°. Sphingomyelinase was solubilized and purified exactly as described previously (4). Briefly, the tissue was homogenized at a 10% concentration (w/v) in 10 mM Tris-HCl buffer, pH 7.2, containing 0.1% Triton X-100 and 10 mM 2-mercaptoethanol. After freeze-thawing and sonication, the homogenate was centrifuged at 100,000g for 60 min. Liver homogenate were filtered through a few layer of gauze before centrifugation. The pellets were re-extracted twice more, and all supernatants were combined. The combined supernatant was applied to a concanavalin A-Sepharose 4B column (Pharmacia Fine Chemicals, Piscataway, N.J.), recycled for 40-45 hours, washed, and the sphingomyelinase activity eluted with additional 0.75 M α-methyl-mannoside. The fraction was dialyzed against 10 mM Tris-HCl buffer, pH 7.2, containing 0.1% Triton X-100 and 10 mM 2-mercaptoethanol and concentrated in an Amicon ultrafiltration apparatus (Amicon Corp., Lexington, Mass.). At this stage, the enzyme had a purification factor of 75 with a recovery of approximately 75% of the total tissue activity. This enzyme preparation was fractionated by Sephadex G-200, pre-equilibrated with 10 mM Tris-HCl buffer, pH 7.2, containing 0.1% Triton X-100 and 10 mM 2-mercaptoethanol. Activities of sphingomyelinase were assayed, as described previously, using sphingomyelin labelled at the methyl group of choline as the substrate, at pH 5.0 in the absence of magnesium ions.

RESULTS

Three brains and three livers were examined in this study for the Sephadex G-200 gel filtration patterns of sphingomyelinase. Representative Sephadex G-200 patterns of normal human cerebral and hepatic sphingomyelinase are shown in Fig. 1. As described in our previous report (4), normal human brain sphingomyelinase consistently gave two distinct fractions by Sephadex G-200 gel filtration. However, with the identical procedure, all of the three normal human livers gave only a trace of sphingomyelinase activities corresponding to the sphingomyelinase A of normal brain. Almost all hepatic sphingomyelinase

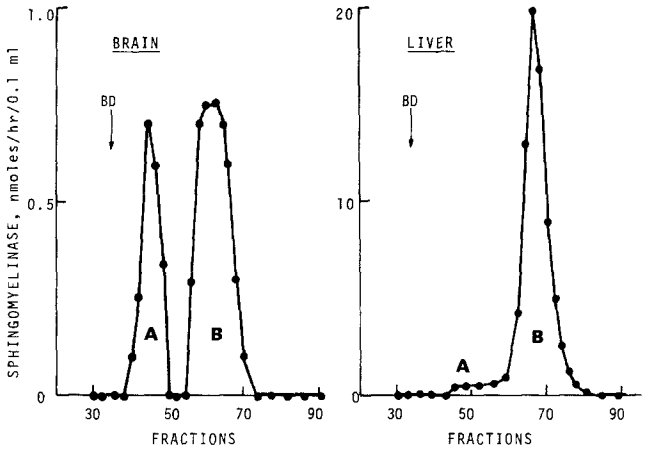

Fig. 1. Sephadex G-200 gel filtration of human cerebral and hepatic sphingomyelinase. The partially purified enzyme preparations after con A-Sepharose affinity fractionation were applied to a Sephadex G-200 column, 2.6X 80 cm, in 10 mM Tris-HCl buffer, pH 7.2, containing 0.1% Triton X-100 and 10 mM 2-mercaptoethanol, which was also used to pre-equilibrate the column. Elution was with the same buffer at a flow rate of 5 ml/hr, and 3.8-ml fractions were collected. BD indicates the position of the blue dextran peak.

activity was eluted from the Sephadex G-200 column corresponding to the B enzyme. This fraction gave, after further purification by DEAE-cellulose and carboxymethyl-cellulose, an isoelectric point identical with that of the brain sphingomyelinase B. When each peak of activity was pooled and the relative distribution of sphingomyelinase A and B was quantitatively estimated, sphingomyelinase A constituted 30-40% of the total in all of the three normal brains, while it was less than 3% of total in all liver specimens (Table 1).

DISCUSSION

The results presented above suggest that there are at least two sphingomyelinase isozymes in human tissues and that the brain and liver differ in their relative distributions. Crucial for this conclusion is the non-identity of sphingomyelinase A and B. In our previous report we could not conclusively exclude the possibility that sphingomyelinase A is merely aggregates of sphingomyelinase B. However, more recent data made it more likely that these two enzyme fractions in fact represent two different enzyme proteins. In the

Table 1. Relative Distribution of Sphingomyelinase A and B in Human Brain and Liver

Tissue		Sphingomyelinase A	Sphingomyelinase B
Brain	1	36%	64%
	2	39%	61%
	3	33%	67%
Liver	1	<3%	>97%
	2	<3%	>97%
	3	<3%	>97%

The Sephadex G-200 fractions corresponding to sphingomyelinase A and B activities were separately pooled. The relative distribution of the two isozymes are expressed in percent of the total activity recovered from the Sephadex column, which was approximately 60% of the total activity in the starting tissue.

previous study we noted some differences between the two enzymes. Sphingomyelinase A required a much higher concentration of NaCl to elute from DEAE-cellulose than sphingomyelinase B. The K_m for sphingomyelin was approximately three times greater for the A enzyme. Since then we have observed that sphingomyelinase A or B each gives a single sharp peak of activity in the electrofocusing procedure with approximate isoelectric points of 5.0 and 5.6, respectively. Once separated, we have never observed interconversion of the two enzymes during storage or in any of the subsequent manipulations, including DEAE-cellulose, carboxymethyl-cellulose, and electrofocusing. The identical extraction and fractionation procedures yield entirely different proportions of the two isozymes from different organs. Although circumstantial, these pieces of evidence suggest that sphingomyelinase A and B are distinct.

On the basis of the above findings, a plausible hypothesis can be constructed to explain the phenotypic difference between the neuropathic (Type A) and non-neuropathic (Type B) forms of Niemann-Pick disease. The neuropathic

form may lack both sphingomyelinase A and B, resulting in severe cerebral and hepatic involvement. The non-neuropathic form may be characterized by a specific deficiency of sphingomyelinase B. In such a condition, the liver would be as severely affected as in the neuropathic form, because almost all sphingomyelinase activity in normal liver is due to sphingomyelinase B. On the other hand, the brain may remain unaffected in sphingomyelinase B deficiency, because 30-40% of normal sphingomyelinase activity will remain due to the intact sphingomyelinase A. Such simultaneous or differential inactivation of the two sphingomyelinases can occur if, for example, the two enzymes share a common subunit in addition to possessing subunits specific for the respective enzymes.

This hypothesis could have been readily tested if appropriately preserved brain and liver specimens from patients with the non-neuropathic form of Niemann-Pick disease were available. Our extensive search for such specimens, however, has been unsuccessful. One of the purposes of this communication is to alert interested colleagues for the need for such specimens.

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