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Cerebroside Sulfatase Activator Deficiency Induced Metachromatic Leukodystrophy

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SUMMARY

Two siblings of consanguineous parents had presented with a variety of findings indicative of juvenile metachromatic leukodystrophy (MLD). However, instead of the expected profound deficiency of arylsulfatase A (ARS A), their enzyme levels were about half-normal, and enzyme from fibroblasts had properties identical with the properties of enzyme from normal fibroblasts. Nevertheless, the hydrolysis of cerebroside sulfate by growing fibroblasts was markedly attenuated. Supplementation of the fibroblasts with cerebroside sulfatase activator normalized the response in the loading test. These results imply that the fibroblasts, and by extension the patients, are deficient in activator. Although the defective catabolism of cerebroside sulfate and the clinical manifestations in these patients mimic MLD, the molecular basis is distinct from the classical forms of the disorder.

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INTRODUCTION

The primary biochemical defect in MLD is a deficiency of cerebroside sulfatase commonly known as ARS A [1]. By the usual enzyme assays, the classical forms of MLD—late infantile, juvenile, and adult—and the benign trait pseudo ARS A deficiency are deficient in ARS A to a similar degree, but these forms were differentiated by applying the cerebroside sulfate (sulfatide) loading test to growing fibroblasts [2–4]. By this test, late infantile MLD cells showed no hydrolysis of the sulfatide, juvenile MLD cells showed a finite but very small amount of hydrolysis, adult MLD cells showed about half-normal hydrolysis, and pseudodeficient cells showed hydrolysis identical with the hydrolysis by normal fibroblasts. This test system provides a highly sensitive measure of functional cerebroside sulfatase activity with close correlation to clinical observations.

We recently described two siblings of consanguineous Mexican-American parents with atypical MLD [5]. They presented with a variety of clinical and biochemical features of juvenile MLD, but their ARS A activities in leukocyte and fibroblast extracts were about half-normal, a level comparable to the levels in symptomless heterozygous carriers of MLD. Enzyme derived from cultured fibroblasts was indistinguishable from enzyme of normal fibroblasts. Nevertheless, the response of growing fibroblasts in the cerebroside sulfate loading test was markedly attenuated. A dysfunction of cerebroside sulfatase was clearly indicated.

The enzymatic degradation of cerebroside sulfate requires the presence of either a bile salt, such as sodium taurodeoxycholate [6, 7] or sodium cholate [8], or an activator [9]. The cerebroside sulfatase activator has been purified and characterized as a heat stable, low molecular weight, anionic, lysosomal protein that exerts its effect by interacting with and dispersing the hydrophobic sulfatide [10–14]. We had postulated that a possible cause of MLD in the two siblings was a deficiency of the activator, and this communication provides data in support of this view.

MATERIALS AND METHODS

Materials

Cerebroside sulfate (bovine sulfatide) was obtained from Supelco (Bellafonte, Pa.). [^{35}S]Cerebroside sulfate was biosynthesized and isolated from rat brain [15]. ARS A was purified from human urine [16]. Its specific activity was 3,520 U/mg, a unit being defined as the amount that catalyzes the hydrolysis of 1 μmol of nitrocatechol sulfate/hr. Cerebroside sulfatase activator was purified from human liver [14]. It had a specific activity of 35 U/mg, a unit being defined as the amount that promotes the hydrolysis of 1 nmol of cerebroside sulfate/hr per unit of enzyme. In some of the cerebroside sulfate loading experiments, partially purified activator carried through the acetone precipitation, heat treatment and ion exchange chromatography steps was used. Pure and partially purified activators yielded identical results.

Cerebroside Sulfatase Assay

The activator-promoted cerebroside sulfatase assay procedure was adapted from the procedure of Fischer and Jatzkewitz [11]. Reaction mixtures contained 200 mM sodium acetate and 12.4 mM Tris at pH 4.5, 200 μM [^{35}S]cerebroside sulfate (750 cpm/nmol), 0.4 U of ARS A, and activator in a total volume of 50 μl. Reactions were incubated at 37°C for 4 hrs and analyzed by extracting and counting the released [^{35}S]inorganic sulfate [6].

Cerebroside Sulfate Loading Test

The [^{35}S]cerebroside sulfate loading test on growing fibroblasts was carried out as described [4] except that MEM-HEPES medium [17] was used. The response of fibroblasts with impaired cerebroside sulfate metabolism shows more pronounced attenuation in the MEM-HEPES medium than in the normally used loading medium 199-CO_2 (our unpublished results, 1980). Hydrolysis of the sulfatide was estimated by counting the [^{35}S]inorganic sulfate released into the medium. The unhydrolyzed intracellularly accumulated sulfatide was determined by counting the chloroform:methanol extractable fraction in the cells at the end of the experiment [4]. The sum of the hydrolyzed and unhydrolyzed material was taken as the total amount of sulfatide incorporated.

RESULTS

Cerebroside Sulfatase

We had previously observed that extracts of the atypical MLD fibroblasts were capable of catalyzing the hydrolysis of cerebroside sulfate in the taurodeoxycholate-promoted reaction system [5]. However, before examining the effect of the cerebroside sulfatase activator in the loading test, it was important to ascertain whether the atypical MLD ARS A was capable of carrying out sulfatide hydrolysis in the activator-promoted reaction system. Extraneous proteins are inhibitory to activator, so crude fibroblast extracts are unsuitable in this reaction system. Fibroblast extracts were, therefore, partially purified by chromatography on DEAE-cellulose and by concanavalin A-Sepharose adsorption. Activator promoted near identical stimulation of cerebroside sulfate hydrolysis with enzyme preparations from atypical MLD and normal fibroblasts (fig. 1). Pure ARS A with bovine serum albumin included to simulate total protein in fibroblast preparations showed a reasonably similar response. Hydrolysis was barely detectable in the absence of activator with each of the preparations. Concentration dependence on activator was indicated by a twofold increase in hydrolysis when activator concen-

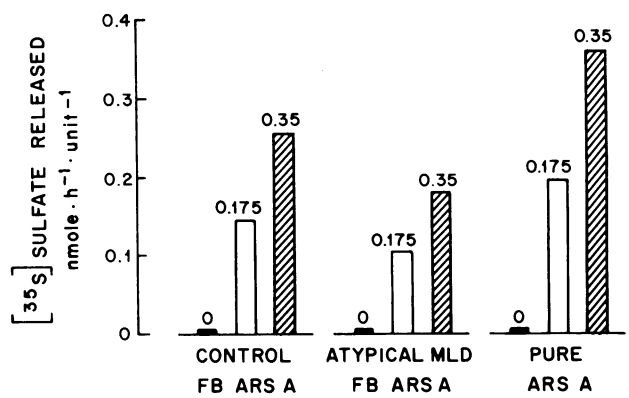

FIG. 1.—Effect of cerebroside sulfatase activator on cerebroside sulfatase reaction; 0.4 *ARS A* U of partially purified normal and atypical MLD fibroblast (*FB*) extracts and pure *ARS A* were used, and 36 μg bovine serum albumin added to pure *ARS A* so protein concentration would be comparable to fibroblast preparations. *Solid Bars*, 0; *open bars*, 0.175 U; and *hatched bars*, 0.35 U activator/reaction.

tration was doubled. The results implied that atypical MLD ARS A did not have impaired ability to interact with the activator-substrate complex.

Cerebroside Sulfate Loading Test

The attenuated response of atypical MLD fibroblasts in the cerebroside sulfate loading test was corrected in a dose-related fashion by supplementation with cerebroside sulfatase activator (fig. 2). Complete normalization was achieved by supplementation with excess activator (fig. 3). The response of normal fibroblasts was unaffected by activator. Late infantile MLD fibroblasts showed no hydrolysis in the absence or presence of activator.

DISCUSSION

Progressive neurological deterioration, delayed nerve conduction time, sulfatide accumulation in sural nerve, and massive urinary excretion of sulfatide were indicative of a dysfunction in cerebroside sulfate metabolism in the present patients [5]. Such dysfunctions are normally caused by a deficiency of ARS A, but the presence of the enzyme in the heterozygote range in leukocytes and fibroblasts was enigmatic [5]. Heterozygous carriers of MLD and individuals with much lower levels of enzyme activity as in pseudo ARS A deficiency [4] are phenotypically normal. Nevertheless, the attenuated response of fibroblasts in the cerebroside sulfate loading test was consistent with the clinical findings.

One possible explanation for the disparate observations was that the ARS A produced was defective. Various properties of the enzyme derived from the atypical MLD fibroblasts as assessed with the synthetic substrate, nitrocatechol sulfate, were, however, identical with the properties of the enzyme from normal fibroblasts [5]. Alternatively, it was possible that only its reaction with the natural

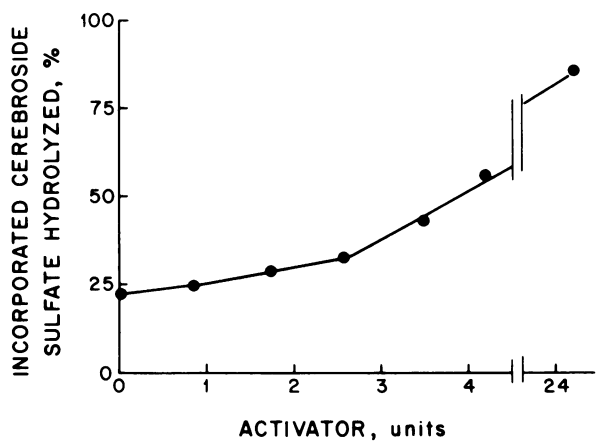

FIG. 2.—Effect of cerebroside sulfatase activator on response of atypical MLD fibroblasts in cerebroside sulfate loading test. Activator and sulfatide were provided simultaneously on day 0. Amount of sulfatide hydrolyzed was determined on day 4. [^{35}S]Inorganic sulfate released into medium was taken as amount of sulfatide hydrolyzed. The sum of hydrolyzed and intracellularly accumulated [^{35}S]cerebroside sulfate was taken as total sulfatide incorporated by fibroblasts.

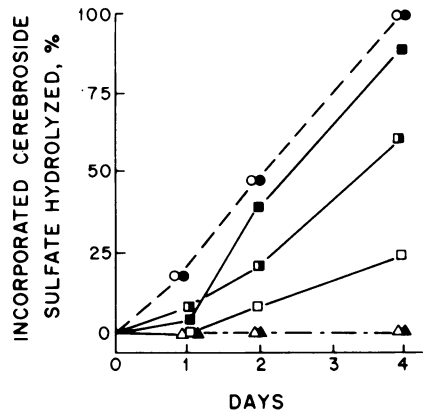

FIG. 3.—Effect of cerebroside sulfatase activator on response of various fibroblast strains in cerebroside sulfate loading test. Activator and sulfatide were provided simultaneously on day 0 and amounts of sulfatide hydrolyzed over course of test period were determined. Total sulfatide incorporated by fibroblasts was determined from the values obtained on day 4, as in figure 2. Atypical MLD, □ = without, ◨ = 4 U, and ■ = 24 U activator; normal, O = without and ● = 24 U activator; late infantile MLD, Δ = without and ▲ = 24 U activator.

substrate was affected. Hydrolysis of cerebroside sulfate in the bile salt-promoted system and in the cerebroside sulfatase-activator-promoted system was identical with the hydrolysis by the normal enzyme. A defective enzyme appeared to be precluded.

An indication of the cause of the enigma was provided by the observation that attentuated cerebroside sulfate hydrolysis by growing fibroblasts was normalized by supplementation with cerebroside sulfatase activator. The simplest explanation was that the cells were deficient in activator and that the exogenous material corrected the deficit. However, in the correction experiment, the activator supplement and sulfatide were provided simultaneously so it might be implied that an extracellular interaction between activator and sulfatide altered metabolic events, for example, by facilitated uptake of activator-sulfatide complex. In separate experiments, normalization of cerebroside sulfate hydrolysis was also achieved when fibroblasts were first provided activator, then challenged with sulfatide. Moreover, the amount of sulfatide taken up was the same in both experimental paradigms—sequential and simultaneous supplementations. In addition, neither the amounts of sulfatide taken up nor the hydrolysis patterns by normal and late infantile MLD fibroblasts were affected by activator in each presentation scheme.

Other explanations might be broached in lieu of the "activator deficiency" postulate because a demonstration of the absence of activator in tissues is lacking. Such experiments require relatively large samples of tissue, and since both patients are living, this approach is not feasible. Direct demonstration of activator deficiency must await further developments.

The concept that MLD is caused in the present patients by a deficiency of activator is not totally without precedence. The absence of a different activator as the cause of a related sphingolipidosis has been established by Conzelmann and

Sandhoff [18]. They showed that tissue obtained at autopsy from a patient with the AB variant of G_{M2} gangliosidosis was deficient in the activator required for the hydrolysis of G_{M2} ganglioside by β-N-acetylhexosaminidase A. A similar activator deficiency was found recently by Hechtman [19] in tissue of another case of the AB variant of G_{M2} gangliosidosis.

In addition to the cerebroside sulfatase and β-hexosaminidase A systems, other sphingolipid hydrolases have been noted to require auxiliary protein activators for their reactions with natural substrates. Ho and O'Brien [20] observed that such material facilitated the hydrolysis of glucocerebroside by β-glucosidase. An activator for the cleavage of G_{M1} ganglioside by β-galactosidase has been isolated and shown to be distinct from that required for G_{M2} ganglioside hydrolysis by Li et al. [21, 22]. An activator has also been implicated for the reaction of sphingomyelinase with its substrate [23, 24]. It should not be surprising if similar activators are involved in the enzymatic hydrolysis of other complex lipoidal substrates.

The implication that fibroblasts are deficient in cerebroside sulfatase activator suggests by extension that the patients from whom they were derived are activator deficient. Thus, the defective catabolism of cerebroside sulfate and the clinical manifestations mimic MLD, but the molecular basis is distinct from MLD in which there is a deficiency of ARS A. It is anticipated that other sphingolipidoses may be mimicked by deficiencies of activators, so an effort to develop experimental models for their demonstration appears warranted.

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