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SHORT CHAIN CERAMIDES AS SUBSTRATES FOR GLUCOCEREBROSIDE SYNTHETASE

DIFFERENCES BETWEEN LIVER AND BRAIN ENZYMES

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Summary

In order to increase the sensitivity of the assay for ceramide: UDPGlc glucosyltransferase, the enzyme that makes glucocerebroside, we synthesized a variety of ceramide homologues that might be better substrates than the naturally occurring ceramides. *N*-Octanoyl sphingosine proved to be the best lipid tested in liver and brain. It could be added to the tissue homogenate in the dry form, as a thin layer coated on Celite, or in liposomes, prepared from lecithin and cerebroside sulfate. The liposomal form produced better replication of assay values. It is suggested that the addition of cerebroside sulfate to liposomal preparations might be a good, and more physiological, replacement for the commonly used dicetyl phosphate.

A new homologue of DL-sphinganine, decasphinganine, was synthesized by an efficient series of steps and acylated with different fatty acids to form ceramide homologues. The best substrate in this series was the lauroyl amide and it is suggested that this lipid be used in cerebroside synthetase assays because of the convenience of preparing it, even though it is not as good as octanoyl sphingosine. Both compounds are distinctly better than natural ceramide or DL-sphinganine amides.

From comparisons of enzyme activity under various conditions, the tentative conclusion is drawn that the enzymes in liver and brain have different properties, and that liver has two different synthetases.

Introduction

The assay for the enzyme that forms glucocerebroside from fatty acyl amides of long chain bases ('ceramide') is usually run with a mixture of ceram-

ides prepared from galactocerebroside by degradation with periodate, borohydride, and weak acid [1,2]. The nonhydroxy acid ceramides are separated, with some difficulty, from the hydroxy ceramides and the former, a mixture of related acyl derivatives, are used as the lipoidal substrate [3]. It is also possible to prepare ceramide from sphingomyelin with a phospholipase. One can isolate naturally occurring nonhydroxy ceramide from some tissues, but the yield is low and it is difficult to obtain pure material. All of these methods yield a mixture of compounds, differing in base and acid composition. For some purposes it is desirable to use pure ceramides, which are readily made from individual fatty acids and D-sphingosine by a variety of methods.

All of the above approaches have their tedious and expensive aspects, and we thought that it might be feasible to synthesize a closely related compound, albeit not naturally occurring, that might act as a useful substrate. A shorter chain lipid would have the advantage, perhaps, of being more easily dispersed in water. Benjamins and Agranoff had previously found that a short chain diglyceride (didecanoin) was a superior substrate for the brain enzyme that makes phosphatidyl inositol [4]. Because of the recent availability of 2-decenoic acid, we investigated the possibility of preparing decasphinganine (DL-erythro-2-amino-decane-1,3-diol) and converting it to various amides ('short ceramides'). Sphingosine amides made from short chain fatty acids were also compared.

In our previous assay procedures [1,2,5], we used brain tissue that had been homogenized and lyophilized, then suspended in benzene with a ceramide solution. The solvent was removed and the powder was suspended in the incubation medium. This approach gave somewhat better enzyme activity than the use of wet tissue homogenates with Celite-coated ceramide, but it involved more steps and the replication was not as good as one gets with soluble enzymes and substrates. We thought that short chain ceramides might penetrate the wet tissue suspensions better than naturally occurring ceramide, making the use of benzene unnecessary. We also examined the possibility of using the ceramide in liposomes, as suggested by Costantino-Ceccarini and Suzuki [6].

Materials and Methods

Synthesis of decasphinganine. Decasphinganine was prepared through a series of six steps based on previously described procedures, some of which were used to make DL-sphinganine [7]. *trans*-2-Decenoic acid, 28.4 g (Research Organic/Inorganic Chemical Corp., Belleville, NJ) was converted to the methyl ester by a modification of our previously described method [8]. The acid was dissolved in 95 ml CH_3OH, 237 ml 2,2-dimethoxypropane, and 9.5 ml conc. HCl. After leaving overnight, the ester was isolated by vacuum evaporation and distillation (yield, 27.0 g (88%)).

The ester was converted to the epoxy compound by reaction with peroxytrifluoroacetic acid [9]. A fine suspension of 3.2 ml 90% H_2O_2 (FMC Corp., Philadelphia, PA) in 26 ml methylene chloride was stirred while 30.2 ml trifluoroacetic anhydride was added at 0°C. Stirring was continued for 30 min following disappearance of cloudiness, then the cold liquid was added dropwise to a stirred mixture of 14.85 g (80.6 mmol) methyl decenoate, 94 ml methylene

chloride, and 33.6 g anhydrous Na_2HPO_4, which was stirred at −3—0°C. Stirring was continued at the same temperature for 3 h, then for a further 3 h at room temperature. 220 ml water was added, the organic phase was separated, and the aqueous phase was extracted with 35 ml methylene chloride. The combined organic phases were dried with Na_2SO_4, the solvent evaporated, and 250 ml 10% methanolic KOH added to the residue. The mixture was refluxed 1 h, diluted with 600 ml water, and brought to pH 2 with 6 N HCl. The resultant oil was extracted with 2 X 100 ml ethyl acetate, which was backwashed with salt solution and dried with Na_2SO_4. Removal of the solvent yielded the epoxy acid (yield 15.1 g (101%) m.p. 56—57°C from hexane). The acid showed an R_F of 0.46 when chromatographed on a TLC plate with $CHCl_3/CH_3OH/CH_3COOH$ (90 : 2 : 8, v/v). Analysis for $C_{10}H_{18}O_3$ (M_r = 186.2); Calculated: C, 64.49; H, 9.74%; Found: C, 64.56; H, 9.65.

The next step was based on the observation that epoxides undergo ring opening with inversion of configuration at the carbon atom attacked [10], and the observation that benzylamine selectively attacks the 2-carbon of *trans*-2,3-epoxy acids [11]. 22.5 g benzylamine (210 mmol) was added to a suspension of 13.0 g epoxy acid (69.8 mmol) in 50 ml water and the mixture was stirred 1 h at 18—20°C and 3 h at 100—105°C. The mixture was cooled, diluted with 100 ml water, acidified with 22 ml 6 N HCl to pH 5, and the precipitate of DL-erythro-2-benzylamino-3-hydroxydecanoic acid was filtered, washed with water, and air-dried. The crude product was stirred in 200 ml hot CH_3OH, cooled to room temperature, filtered, and washed with 25 ml cold CH_3OH (yield, 10.3 g (50%), m.p. 225—227°C). The R_F was 0.53 after TLC with $CHCl_3/CH_3OH/CH_3COOH$ (75 : 10 : 20, v/v). Analysis: $C_{17}H_{27}NO_3$ (M_w 293.4); Calculated: C, 69.59; H, 9.28; N, 4.77%; Found: C, 69.57; H, 8.51; N, 4.95.

The amino acid was esterified by suspending 20 g acid in 240 ml CH_3OH and saturating the suspension with dry HCl, with cooling. The mixture was then refluxed for 6 h without stoppping the flow of gas and left overnight at room temperature. The precipitated hydrochloride was filtered and the filtrate evaporated to dryness (yield, 21.75 g (93%); m.p. 153—154°C from isopropanol); R_F, 0.61 with $CHCl_3/CH_3OH$ (97 : 3, v/v). Analysis: $C_{18}H_{29}NO_3 \cdot HCl$ (343.9); Calculated: C, 62.86; H, 8.79; N, 4.07%; Found: C, 62.72; H, 8.60; N, 4.00.

The next step was reduction of the ester group. A suspension of 4.43 g hydrochloride (12.88 mmol) in 100 ml diethyl ether was shaken with 5 ml water and 5 ml concentrated NH_4OH. The organic phase was washed with water, filtered to remove insoluble material, dried with sodium sulfate, and evaporated to dryness. The free base was dissolved in 100 ml ether and added dropwise to a stirred suspension of 2 g $LiAlH_4$ in 100 ml dry ether kept at 0°C. After stirring overnight, the mixture was refluxed for 10 min, cooled, and treated dropwise with 5 ml ethyl acetate, followed by dropwise addition of water until a heavy precipitate settled down. The clear ether solution was decanted, dried, and evaporated to form an oil which solidified after a few hours (yield 3.7 g, m.p. 42—43°C (from hexane)); R_F ($CHCl_3/CH_3OH/NH_4OH/H_2O$, 140 : 35 : 2 : 2, v/v) was 0.65. Analysis: $C_{17}H_{29}NO_2$ (279.4); Calculated: C, 73.07; H, 10.46; N, 5.01%; Found: C, 73.14; H, 10.40; N, 5.04.

The benzyl group was removed by hydrogenolysis. A solution of 3.4 g amino

diol (12.2 mmol) in 35 ml acetic acid and 5 ml water was hydrogenated over 360 mg 5% Pd on charcoal at room temperature and atmospheric pressure for 24 h. The catalyst was removed by filtration and washed with 20 ml acetic acid. The decasphinganine acetate was obtained by evaporation of solvent, sonication with 50 ml ether, and filtration (yield, 2.62 g (86.3%); m.p. 114—115°C from isopropanol); R_F, 0.16 ($CHCl_3/CH_3OH/NH_4OH/H_2O$, 140 : 35 : 2 : 2, v/v). The overall yield, starting with decenoic acid, was calculated to be 35%.

The free base was obtained by shaking 500 mg of the acetate in 50 ml $CHCl_3$, 5 ml NH_4OH, 10 ml H_2O. The organic phase was washed with H_2O (yield, 0.35 g free base (92%), m.p. 58—61°C (from benzene-hexane)). Its infrared spectrum in a KBr pellet showed the expected bands and closely resembled that of dihydrosphingosine. The R_F value was 0.16 with the solvent used for the acetate salt. Analysis: $C_{10}H_{23}NO_2$ (M_w 189.3); Calculated: C. 63.44; H, 12.24; N, 7.40%; Found: C, 63.93; H. 12.28; N, 7.42.

Thin layer chromatography was run on silica gel plates and spots were located with an iodine spray (in CH_3OH) and a charring spray [12]. The aromatic intermediates were also located with ultraviolet light. Each compound yielded only a single spot after purification.

Synthesis of ceramides. Acylation was carried out by adding 1 mmol acyl chloride dropwise to a stirred mixture of 0.8 mmol decasphinganine acetate in 8 ml tetrahydrofuran and 8 ml 50% sodium acetate (w/v) at about 5°C [13]. The mixture was stirred for 30 min in ice and 30 min at room temperature. The amide was extracted with ethyl acetate and the organic phase was washed with water, 2 N HCl, water, bicarbonate, and water. Purification of the amides was accomplished with a silica gel column using hexane-isopropanol (90 : 10, v/v) or $CHCl_3/CH_3OH$ (98.5 : 1.5, v/v). Table I lists the ceramides prepared.

Amides of D-sphingosine were prepared similarly, using sphingosine isolated from brain galactocerebroside [14].

Acylation with DL-2-hydroxy fatty acids was carried out as described above. Fatty acids were brominated, then hydrolyzed (procedure to be published separately), acetylated, converted to the acyl chlorides, and reacted with decasphinganine as above. Alkaline methanolysis (2 mM NaOH in CH_3OH) removed the acetyl group and the amides were purified with a silica gel column

TABLE I

AMIDES OF DL-DECASPHINGANINE AND DL-SPHINGANINE

Ceramide	Yield (%)	Melting point	R_F *	Molecular weight
Octanoyl decasphinganine	56	96—98	0.21	315.5
Decanoyl decasphinganine	95	97—99	0.23	343.5
Lauroyl decasphinganine	51	97—99	0.24	371.6
Myristoyl decasphinganine	78	99—100	0.26	399.6
Palmitoyl decasphinganine	88	101—103	0.26	427.7
Stearoyl decasphinganine	73	105—106	0.29	455.7
Octanoyl sphinganine	—	99—100	0.26	427.7
Decanoyl sphinganine	71	97—98	0.28	455.7

* Thin-layer plates run with $CHCl_3/CH_3OH$ (95 : 5, v/v). A reference compound, *N*-decanoyl norephedrine, yielded an R_F of 0.52.

using $CHCl_3/CH_3OH$ (98 : 2, v/v) to elute the mixture of DL and LD forms; 95 : 5 eluted the DD, LL forms. This separation had been observed before with amides of DL-hydroxy acids and natural (D) sphingosine or galactosyl sphingosine [15,16]. The DD form is the naturally occurring one. Table II lists the DD, LL preparations used in this study.

Tables I and II also list ceramides made from DL-sphinganine (from Miles Laboratories, Elkhart, IN).

Other materials. Egg phosphatidylcholine was prepared from crude phosphatidylcholine by a new method [17]. Sulfatide was isolated from beef spinal cord lipids by chromatography through silica gel. Mixed nonhydroxy ceramides were prepared from galactocerebroside [3]. Labeled uridine diphosphoglucose was obtained from New England Nuclear Corp. and diluted to a specific activity of about 1000 cpm/nmol. Trasylol (aprotinin, a general protease inhibitor) was from FBA Pharmaceuticals (New York, NY).

Liposomes were made by evaporating to dryness a solution of 11.3 mg phosphatidylcholine, 2 mg cerebroside sulfate, and 4 μmol ceramide, using nitrogen and a warm bath. More complete drying was accomplished by adding 1 ml cyclohexane and lyophilizing the mixture for about 2 h. 1 ml water was added and the suspension was sonicated with a dipping probe (Branson Instruments, 2.1 amp) under N_2 for 5 min without a cooling bath. The liposomes were generally used promptly but could be stored at 4°C for a week provided they were sonicated again for 5 min.

Enzyme assay. Fresh or frozen brains and livers (stored at −70°C, not −20°C) from 16-days old male mice (Spartan Research, Haslett, MI) were homogenized with a Polytron (Brinkmann Instruments) with 4 vols. H_2O at 0°C for 30 s, then sonicated in an ice-containing ultrasonic bath for 1.5 min to remove trapped air bubbles. The homogenate was diluted further with water to contain 60 mg tissue/ml. Some livers were from mice 50—60 days old.

Incubation was carried out in triplicate with 0.1 ml homogenate, 0.05 ml liposomes, and 0.05 ml UDPGlc (32 nmol), Tris-HCl (pH 7.4), EDTA, dithioerythritol, Mg^{2+}, and ATP [1,5]. The tubes were shaken in an ultrasonic bath for 1 min, then incubated for 1 h and processed as described before [1].

Incubations carried out with coated Celite instead of liposomes were similar except that 20 mg Celite Analytical Filter Aid, coated by evaporation from solution with 50 or 100 μg ceramide, were weighed into each tube and the volume of incubation medium was raised to 0.1 ml. Incubation was for 40 min.

TABLE II

AMIDES OF DL-2-HYDROXY ACIDS AND DL-SPHINGOID BASES

The TLC solvent was $CHCl_3/CH_3OH/H_2O$ (144 : 25 : 2.8, v/v) and the R_F of a reference compound, stearoyl sphingosine, was 0.68. The R_F values shown are for the DD,LL-ceramides. The DL,LD form of OH-decanoyl decasphinganine had an R_F of 0.49.

Ceramide	R_F	Molecular weight
Hydroxy-hexanoyl decasphinganine	0.34	303.4
Hydroxy-octanoyl decasphinganine	0.36	331.5
Hydroxy-decanoyl decasphinganine	0.38	359.5
Hydroxy-stearoyl decasphinganine	—	471.7
Hydroxy-decanoyl sphinganine	0.57	471.7

Results and Discussion

The initial experiments were done with ceramides coated on Celite. A comparison of octanoyl and decanoyl ceramides with liver showed that octanoyl sphingosine was by far the best glucose acceptor (Table III). However decanoyl decasphinganine was rather effective, especially when allowance was made for its ability to saturate the transferase at a lower concentration (by weight). Dihydrosphingosine amides were quite poor acceptors, compared to the unsaturated amides. This finding suggests that it might be unwise to use tritium-labeled sphingolipids made by reducing the double bond in sphingosine-containing lipids.

Comparison of the last four ceramides in Table III, derived from racemic long chain bases, shows the value of using a shorter base. In earlier tests, not shown in the table, we found octanoyl sphingosine to be somewhat more effective than mixed, natural ceramides.

It is easier to pipette liposomal suspensions than to weigh out Celite and, thus, we tried making liposomes from octanoyl sphingosine. Egg phosphatidylcholine was included, partly because it is frequently included in liposomes, and partly because it has been found to be a stimulant for cerebroside synthetases in brain [14,18] and kidney [19]. Sulfatide was added to the liposomes because an acidic lipid is commonly included in liposomes and because we had found it to stimulate ceramide galactosyltransferase [1]. Sulfatide seemed to enhance the stability of the liposomal suspensions and produced a modest increase in transferase activity. A comparison between such liposomes and coated Celite, using 20 mg liver, showed the former yielded about 10% more product. However, the liposomes had an additional advantage: equal amounts of cerebroside could be formed with only 6 mg liver while the yield was somewhat reduced when less liver was used with Celite-ceramide. Also useful was the finding that replication of assay data was distinctly improved by the use of liposomes. Using octanoyl sphingosine in liposomes with liver homogenate, we found that the amount of synthesized cerebroside was proportional to liver weight up to 8 or 10 mg.

A comparison in brain and liver homogenates of different ceramides in lipo-

TABLE III

SYNTHESIS OF GLUCOCEREBROSIDES FROM DIFFERENT CERAMIDES

Incubations were run with ceramides coated on 20 mg Celite with 20 mg liver homogenate for 40 min.

Ceramide	Amount of cerebroside formed (nmol)	
	50 μg ceramide	100 μg ceramide
Octanoyl sphingosine	0.96	1.43
Decanoyl sphingosine	0.85	1.06
Stearoyl sphingosine	0.14	0.20
Octanoyl dihydrosphingosine	0.28	0.31
Decanoyl dihydrosphingosine	0.20	0.31
Octanoyl decasphinganine	0.57	0.67
Decanoyl decasphinganine	0.74	0.79

somes again showed octanoyl sphingosine was the best substrate (Table IV). There was a distinct advantage to using shorter chain fatty acids.

The glucosyltransferase in brain seemed to be different from the liver enzyme, judging by the relative activities toward the different substrates (column 3). Ceramides having a total carbon number of 26 or less were favored by the brain.

Comparison of the two analogous ceramides, hydroxydecanoyl and decanoyl decasphinganine, showed that the glucosyltransferases of both organs did not discriminate against the hydroxyl group. This nondiscrimination had been observed before in brain when using the dry brain-benzene or coated-Celite method of assay [5,20,21]. However use of the completely unnatural mixture of diastereoisomers (DL,LD-hydroxydecanoyl decasphinganine) yielded only a slight amount of cerebroside.

Trials with ceramide-Celite and higher liver concentrations showed that transferase activity slowed down noticeably after a 30-min incubation, and the amount of cerebroside formed ultimately reached a peak and then decreased. This suggested the intervention of cerebroside glucosidase, which was apparently destroying some of the cerebroside being formed. While the pH optimum of this enzyme is around 5.4, it possesses appreciable activity at the pH used for synthetase assay [22]. The time course with octanoyl sphingosine liposomes and only 6 mg liver showed that good activity was maintained at least for 3 h, with only modest slowing after 60 min. The inclusion of *N*-hexyl glucosyl sphingosine, a strong glucosidase inhibitor [22], produced only a small increase in activity, possibly because the effect of glucosidase in this more dilute enzyme suspension was minor. The failure of the glucosidase inhibitor to inhibit the synthesis of cerebroside, incidentally, shows that the synthesis was not the result of transglucosylation by glucosidase [23].

When 500 units Trasylol, a protease inhibitor, were included in the incubation medium, there was a slight slowing of cerebroside synthesis but the rate of

TABLE IV

CEREBROSIDE SYNTHESIS BY LIVER AND BRAIN USING CERAMIDE LIPOSOMES

Ceramides made from 0.2 μmol nonhydroxy or 2-hydroxy fatty acids were dispersed in lecithin-sulfatide liposomes and incubated with 6 mg tissue. The data below are from two experiments.

Ceramide used	Glucocerebroside synthesized (nmol)		
	Brain	Liver	Brain/liver ratio
Octanoyl sphingosine	1.06	0.83	1.28
Decanoyl sphingosine	0.44	0.60	0.73
Stearoyl sphingosine	0.05	0.17	0.29
Oleoyl sphingosine	0.12	0.29	0.41
Mixed ceramides (nonhydroxy)	0.07	0.26	0.27
Decanoyl dihydrosphingosine	0.12	0.32	0.38
Octanoyl dihydrosphingosine	0.28	0.38	0.74
Decanoyl decasphingenine	1.27	0.65	1.97
Hydroxy-hexanoyl decasphingenine	0.33	0.16	2.01
Hydroxy-octanoyl decasphingenine	0.79	0.39	2.02
Hydroxy-decanoyl decasphingenine	1.21	0.60	2.02
Hydroxy-stearoyl decasphingenine	0.18	0.45	0.40

synthesis was more constant with time, so that the total amount of cerebroside formed by 180 min was the same with or without Trasylol. It is possible that a small portion of the later decrease in activity of the transferase is due to proteolytic attack by endogenous proteases.

While octanoyl sphingosine was the best substrate found in this study, decasphinganine amides seemed worth more study because of the greater ease of preparing large quantities of the amine. Additional amides were prepared with this base and tested in liposomes with brain and liver (Fig. 1). Dodecanoyl decasphinganine proved to be the best in this series for both organs. However, there was an unexpected second peak in the case of liver with palmitoyl decasphinganine. This relationship, which was repeatedly observed, may indicate the presence in liver of a second transferase, not occurring in brain, that works better with ceramides having a longer fatty acid.

Using the same preparation of tissue, octanoyl sphingosine was only 8% more active than dodecanoyl decasphinganine in brain, and 44% more active in liver. This is additional evidence for a difference between the liver and brain enzymes.

The predominant chain length of mammalian amino diols is 18 carbons and we wanted to be sure that the product of reaction with the 'short ceramides' was indeed a cerebroside. This point was checked by a radioautograph of a TLC plate run with $CHCl_3/CH_3OH/H_2O$ (24 : 7 : 1, v/v) on silica gel, using the product of reaction with lauroyl decasphinganine. Only a single radioactive band could be seen, with R_F 0.81 relative to nonhydroxy galactocerebroside. This placed the new 'short cerebroside', as expected, close the hydroxy galactocerebrosides. When the TLC adsorbent was scraped into sections and counted, 94% of the recovered activity was in the band made visible by radioautography.

Additional experiments were performed in an effort to obtain additional evidence for a multiplicity of glucosyltransferases. Liver homogenate was incubated at 37°C, then incubated further with labeled UDPGlc and three dif-

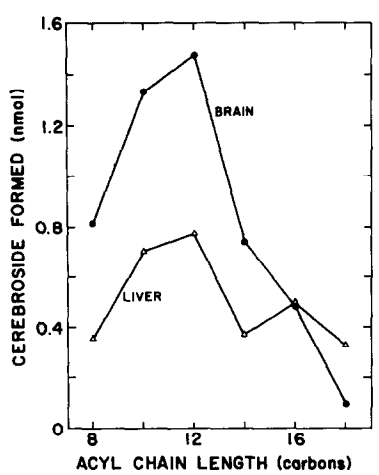

Fig. 1. Amount of glucocerebroside formed by 6 mg liver and 6 mg brain homogenates. Liposomes were prepared from 'short ceramides' prepared from different fatty acids and from DL-decasphinganine.

ferent ceramides in liposomes: octanoyl sphingosine, dodecanoyl decasphinganine, and palmitoyl decasphinganine. Increasing duration of preincubation led to increasing loss of activity, to an extent that was quite similar for all three ceramides. The losses were about 26% after 10 min, 67% after 30 min, and 87% after 50 min. (Inactivation was complete in 10 min at 50°C with all three substrates.) A similar nondiscrimination was observed when an inhibitor of ceramide:UDPGlc glucosyltransferase, 2-decanoylamido-3-morpholinopropiophenone (to be described in a separate paper), was included with both the lauroyl and palmitoyl amides. In both cases, 45% inhibition was found with 0.3 mM inhibitor. These experiments do not support the idea that glucosylation of the two decasphinganine amides is performed by two different liver enzymes.

Further comparisons of the brain and liver transferases furnished additional evidence that they are somewhat different. Brain homogenate incubated various times before addition of substrate, as above, lost activity somewhat more slowly than liver: about 50% of the activity remained after 50 min of preincubation. There seemed to be slightly greater instability when the brain activity was assayed with the C_{12} amide but the difference was not examined further. When liver and brain were compared with the enzyme inhibitor and C_{12} decasphinganine, brain was found to be more sensitive: the inhibition was 71% as opposed to 56%. With octanoyl sphingosine as substrate, brain was again more sensitive than liver: 56% inhibition vs. 43%. A study of the effect of incubation pH also revealed a difference. The optimum pH for brain seemed to be around 8.2 while it was around 7.8 for liver.

Some tests were made with ceramide:UDPGal galactosyltransferase, the enzyme that makes galactocerebroside. This enzyme in brain, assayed with the lyophilized tissue and benzene solutions of ceramides, had been found to react far faster with short hydroxy acid ceramides than with ceramides of typical chain lengths [5]. The reaction rate went up with chain lengths shorter or longer than 18, hydroxyheptanoyl sphingosine being best. Similar comparisons with 100 μg of ceramide (Table V) showed that the nonhydroxy ceramides made from decasphinganine were similar to other nonhydroxy ceramides in being very poor galactose acceptors. In addition we found that substituting dihydrosphingosine for sphingosine greatly reduced the effectiveness of

TABLE V

SYNTHESIS OF GALACTOCEREBROSIDE FROM DIFFERENT CERAMIDES

Assays were run with 1 mg of rat brain powder and 0.1 mg of ceramide by the benzene procedure [4], with a 90 min incubation.

Substrate	Amount of cerebroside (nmol)
Octanoyl decasphingenine	0.04
Decanoyl decasphingenine	0.10
Hydroxy-decanoyl decasphingenine	2.10
Hydroxy-stearoyl decasphingenine	0.97
Octanoyl sphingenine	0.10
Decanoyl sphingenine	0.12
Hydroxy-decanoyl sphingenine	0.14
Hydroxy-decanoyl sphingenine	6.60

hydroxy ceramide. Comparison of the two best hydroxydecanoyl amides showed that decasphinganine was not a good substitute for sphingosine in the case of galactosyltransferase.

Comparison should be made of the merits of synthesizing decasphinganine and sphingosine. The latter compound has been synthesized by Shapiro [24] and Shoyama et al. [25]. The overall yields cannot be readily determined from the published data, but eight steps are involved (opposed to six in our procedure) and the sphingosine method involves a nonspecific reduction that produces a mixture of threo and erythro isomers. Thus there is a 50% loss in this step alone. Our procedure yields the erythro form stereospecifically. The fact that synthetic sphingosine is not commercially available, while dihydrosphingosine is, suggests that the synthesis of the more common base is difficult.

It is now possible to purchase methyl 2-decenoate (K&K Labs, ICN Pharmaceuticals, Plainview, NY), so our synthesis of decasphinganine can be shortened to five steps, with a corresponding increase in yield.

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