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SHORT COMMUNICATION

Metabolism of Sphingosine Bases, I

Degradation and Incorporation of [3-^{14}C]*erythro*-DL-Dihydrosphingosine and [7-$^{3}H_2$]*erythro*-DL-Sphingosine into Sphingolipids of Rat Liver

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Zusammenfassung: Intravenös injiziertes [3-^{14}C]*erythro*-DL-Dihydrosphingosin wird bei Ratten rasch abgebaut. Als Abbauprodukte wurden isoliert: Abgeatmetes $^{14}CO_2$ (nach 6 Std. 20% der Gesamtaktivität), [1-^{14}C]-Palmitinsäure und in geringem Maße Stearinsäure, das Produkt der Verlängerung von Palmitinsäure. Die beiden Säuren wurden in den Ester- und Sphingolipoiden, die Sphingosinbasen in Ceramid und Sphingomyelin gefunden. [7-$^{3}H_2$]*erythro*-DL-Sphingosin, ebenso verabreicht, wird in ähnlicher Weise umgesetzt. Ebenso wie beim Dihydrosphingosin führt die Abspaltung eines C_2-Stückes zu Palmitinsäure. Es werden quantitative Angaben über die Verteilung der Radioaktivität gemacht.

The sphingosine bases of sphingolipids in animal tissue represent a group of closely related aliphatic 2-amino-1,3-diols. The substituents on C-2 and C-3 have D-*erythro*-configuration[1]. In general these bases have 18 and to a smaller extent 20 C-atoms. Sphingosine has an additional *trans*-double bond between C-4 and C-5. This and its saturated form, the dihydrosphingosine, are the predominant bases in all sphingolipids of animal origin. So far nothing is known about the degradation of the 2-amino-1,3-diol system present in dihydrosphingosine or the unsaturated system with the additional allylic *trans*-double bond.

Synthetic studies in this laboratory have made available a number of specifically labeled dihydrosphingosines, sphingosines and of intermediates which were required for the elucidation of the enzymatic degradation pathway[2,3,4].

In this communication we wish to report data of in vivo studies with [3-^{14}C]*erythro*-DL-dihydrosphingosine and [7-$^{3}H_2$]*erythro*-DL-sphingosine administered to rats. The results are concerned with the rate of oxidation of dihydrosphingosine, the identification of palmitic acid as the main degradation product of dihydrosphingosine and sphingosine, the incorporation of the two bases into ceramide (*N*-acylsphingosine) and sphingomyelin and of palmitic acid and its elongation product stearic acid into ester- and sphingolipids.

1. [3-^{14}C]*erythro*-DL-*dihydrosphingosine:* 8.0 μmoles (1.80 μC) were dissolved in 2 ml of a 5% serum albumin solution and injected into the tail vein of a rat. The animal was kept in a desiccator which was flushed continuously with CO_2-free air. Respiratory $^{14}CO_2$ was trapped by passing the exit air stream through three absorption vessels filled with 20 ml of absorption solution[5]. The absorption flasks were changed at intervals as indicated in figure 1. This experiment led to the surprising observation, that the dihydrosphingosine base is rapidly degraded at a rate comparable to palmitic acid. 23% of the injected radioactivity ($0.9 \cdot 10^6$ dpm) were trapped as $^{14}CO_2$ within the first 10 hours.

In another experiment two rats were killed 6 hours after the intravenous injection of 33 μmoles of [3-^{14}C]*erythro*-DL-dihydrosphingosine each and the liver lipids extracted with chloroform-methanol (2:1). 17% ($5.61 \cdot 10^6$ dpm) of the totally injected activity were recovered from liver. An aliquot of the lipid mixture ($2.20 \cdot 10^6$ dpm) was trans-esterified (5% HCl in methanol). The fatty acid methyl esters were extracted from the methanolic solution with hexane. They were analyzed by radio-gas-chromatography.

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Another aliquot of the lipid mixture ($2.20 \cdot 10^6$ dpm) was chromatographed on silicic acid. The elution pattern of the radioactive fractions is given in figure 2. The radioactive fractions proved to be homogenous in thin-layer chromatographic analysis. They were identified by cochromatography of authentic samples. Each ester- and sphingolipid fraction was hydrolyzed (5% HCl in methanol, 2 hours at reflux temp.). The methyl esters were extracted with hexane and the bases, after adjustment to pH 12, with ether. The distribution of the radioactivity in fatty acids of the esterlipids and long chain bases of ceramide and sphingomyelin is given in table 1. Quantitative analyses of the labeled fatty acids were achieved by radio-gas-chromatography. It is evident from figure 2 that no free base was present in liver 6 hours after the injection of [3-^{14}C]*erythro*-DL-dihydrosphingosine.

Palmitic acid is the predominant degradation product, some additional activity is found in stearic acid, the elongation product of palmitic acid. The label is present

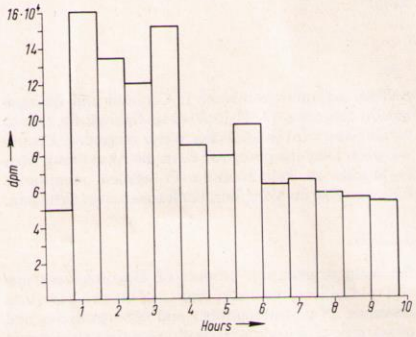

Fig. 1. Recovery of respiratory $^{14}CO_2$ after injection (intravenously) of [3-^{14}C]*erythro*-DL-dihydrosphingosine into the rat.

Table 1. Distribution of radioactivity in lipid fractions 6 hours after administration of [3-^{14}C]*erythro*-DL-dihydrosphingosine.

Radioactive lipid	Total radioactivity [dpm]	Total radioactivity in fatty acids [%]	
Triglycerides	422,800	16:0 18:0	80 20
Ceramide*	525,000	16:0 18:0	90 10
Phosphatidyl-ethanolamine	464,400	16:0 18:0	62 38
Phosphatidyl-choline	314,000	16:0 18:0	70 30
Sphingomyelin*	455,000	16:0 18:0	90 10

* The fatty acids of ceramide contain 44% and of sphingomyelin 45% of the total radioactivity.

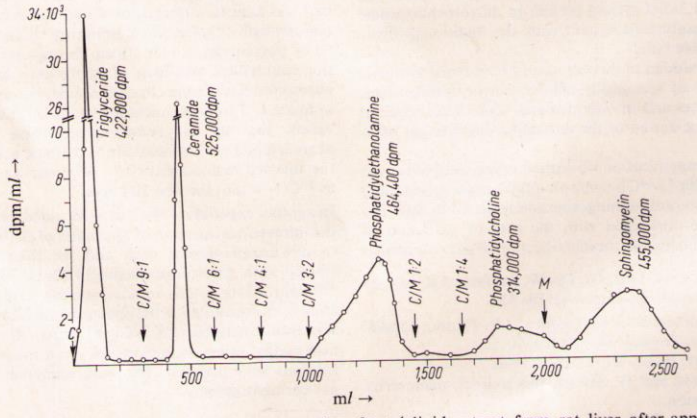

Fig. 2. Elution pattern of silicic acid chromatography of total lipid extract from rat liver after application of [3-^{14}C]*erythro*-DL-dihydrosphingosine. C = chloroform; M = methanol.

in the COOH-group (>90%) of palmitic acid, as revealed by DAUBEN-degradation[3].

2. [7-$^3H_2$]erythro-DL-sphingosine: 33 μmoles, dissolved in a 5% serum albumin solution, were administered intravenously to a rat and the liver lipids isolated after a 3-hour period. They were analyzed in the same manner as described in the experiment with radioactive dihydrosphingosine.

The recovery of the injected radioactivity from liver was 37% ($9.0 \cdot 10^6$ dpm), 30% of which was present in the fatty acids of ester- and sphingolipids, the rest as sphingosine, free or bound in ceramide and sphingomyelin. The distribution of radioactivity in the different lipid classes is given in table 2. When the fatty acid methyl esters, obtained from the total lipid mixture by acid hydrolysis, were analyzed by radio-gas-chromatography again *palmitic acid was the main radioactive fatty acid (80%)*, the rest of the radioactivity was in stearic acid. $^3H_2$ on C-7 of sphingosine (C-3 of the resulting palmitic acid) is lost during β-oxidation of palmitic acid and cannot be utilized via the acetate pool.

Our results prove that *the degradation of dihydrosphingosine and sphingosine is initiated by the loss of a two-carbon unit thus yielding palmitic acid*, which is either further degraded to CO_2, elongated to stearic acid and/or incorporated into ester- and sphingolipids. The enzymatic reaction sequence of the conversion of dihydrosphingosine and sphingosine to palmitic acid, the chemical structure of the intermediates and their chemical synthesis will be described in the following papers[3,4].

When this manuscript was submitted we received note of an investigation of BARENHOLZ and GATT[6] on the degradation of phytosphingosine. These authors found pentadecanoic acid as degradation product. They suggest that phytosphingosine is the common intermediate in dihydrosphingosine and sphingosine degradation. Our results disprove this hypothesis.

Table 2. Distribution of radioactivity in lipid fractions 3 hours after administration of [7-$^3H_2$]*erythro*-DL-sphingosine.

Radioactive lipid	Total radioactivity [dpm]	Total radioactivity in fatty acids [%]	
Triglycerides	1,075,000	16:0	92
		18:0	8
Ceramide*	624,000	16:0	73
		18:0	27
Phosphatidyl-ethanolamine	340,000	16:0	75
		18:0	25
Phosphatidyl-choline	670,000	16:0	72
		18:0	28
Sphingomyelin*	650,000	16:0	85
		18:0	15
Free sphingosine	1,141,000	—	—

* The fatty acids of ceramide contain 34% and those of sphingomyelin 25% of the total radioactivity.

[6] Y. BARENHOLZ and S. GATT, Biochem. biophysic. Res. Commun. **27**, 319 [1967].