METABOLISM OF *n*-HEXADECANE IN MYELINATING RAT BRAIN *

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1. Introduction

Long-chain aliphatic hydrocarbons can be absorbed by the mammalian intestinal system. They also were found to be oxidized to the corresponding fatty acids *in vivo* [1–4] and *in vitro* by mammalian liver homogenates [3] and by subcellular preparations of intestinal mucosa [5]; the formation of long-chain primary alcohols was proposed [5] as an intermediate step in this reaction. If long-chain alcohols are produced in the course of hydrocarbon oxidation by a mammalian tissue capable of synthesizing plasmalogens, it can be expected that such a tissue would utilize them for the formation of *O*-alkyl and *O*-alk-1-enyl moieties of glycerophosphatides.

Studies from this laboratory have shown that myelinating rat brain incorporates various long-chain alcohols into *O*-alkyl and *O*-alk-1-enyl moieties of ethanolamine phosphatides [6–8] and into *O*-alkyl moieties of choline phosphatides [8]. It was found that all *O*-alkyl and *O*-alk-1-enyl moieties of the glycerophosphatides had the same chain length and degree of unsaturation as the precursor alcohol, but that the acyl moieties contained substantial amounts of elongation-desaturation products of the fatty acids derived through alcohol oxidation [6–8].

Thus, comparative analysis of the *O*-alkyl, *O*-alk-1-enyl and acyl moieties of the ethanolamine phosphatides of myelinating rat brain after intracerebral injection of [1-^{14}C]*n*-hexadecane are expected to yield information not only on whether biological oxidations of hydrocarbons occur in mammalian tissues other than the gastrointestinal system and the liver, but also on the role of long-chain alcohols as intermediates in this reaction. Results of such an experiment are reported here.

2. Materials and methods

[1-^{14}C]*n*-Hexadecane (47.2 mCi/mmole) was purchased from Amersham/Searle Corporation, Arlington Heights, Illinois, USA. Analysis by TLC confirmed the purity of 99% stated by the manufacturer. The preparation was found to be virtually free of labeled long-chain alcohols or fatty acids. All experimental procedures have been described previously [6–8]. [1-^{14}C]*n*-Hexadecane was emulsified as described for alcohols and was administered intracerebrally to 18-day-old rats. After 3, 6 and 24 hr, the rats, in groups of five, were killed and the brains within each group were pooled. The lipids were extracted, purified, and analyzed [6–8]. The ethanolamine phosphatides were isolated and purified by thin-layer chromatography [6]; they were subjected to $LiAlH_4$-reduction followed by treatment with HCl. The reaction products were isolated by TLC and analyzed by GLC of their derivatives using synthetic standards as described [6–8]. Recoveries of total radioactivity in GLC were 70–80%, the counting efficiency of the scintillation spectrometer was 80–81%.

3. Results and discussion

When [1-^{14}C]*n*-hexadecane was administered intracerebrally to 18-day-old rats, significant amounts

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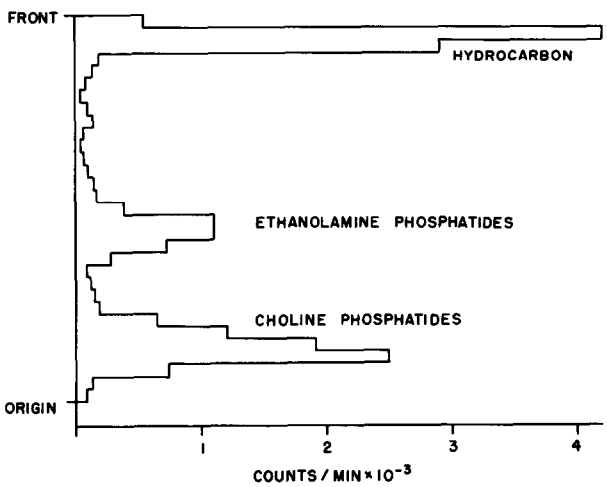

Fig. 1. Distribution of radioactivity in TLC fractions of total brain lipids 24 hr after administration of [1-^{14}C] *n*-hexadecane. Silica Gel H; chloroform–methanol–water (65:25:4, v/v/v). Migration rates of authentic standards are indicated.

of radioactivity were recovered in both ethanolamine and choline phosphatides of the brain. Fig. 1 demonstrates the distribution of radioactivity in the total brain lipids. The ethanolamine phosphatides derived from each group of brains were purified and reacted with $LiAlH_4$. As in previous experiments, the aldehydes (and small amounts of their condensation products) derived from *O*-alk-1-enyl moieties, alcohols derived from the total acyl moieties, and glycerol ethers derived from the *O*-alkyl moieties were isolated by TLC. The amounts of radioactivity administered per brain and those recovered in total lipids, ethanolamine phosphatides, and their aliphatic moieties are listed in table 1.

After 24 hr labeled hexadecane was still found to represent a major portion (41%) of the total radioactivity of the brain lipids, but during this period of time, significant proportions of radioactivity appeared in the polar lipids (5, 9 and 59% after 3, 6 and 24 hr). The distribution of radioactivity among the various aliphatic moieties of the ethanolamine phosphatides resembled that obtained after administration of labeled long-chain alcohols [6–8].

Proof for the conversion of *n*-hexadecane to *n*-hexadecanol was obtained by analysis of the individual aliphatic moieties of the ethanolamine phosphatides. Derivatives from both the *O*-alkyl and *O*-alk-1-enyl

Table 1

Incorporation of radioactivity from [1-^{14}C] *n*-hexadecane into ethanolamine phosphatides.

	3 hr	6 hr	24 hr
Injected [cpm × 10^{-6} per brain]	1.68	1.68	1.68
Recovered [cpm × 10^{-5} per brain]	9.32	9.64	5.28
Ethanolamine phosphatides [cpm × 10^{-4} per brain]	1.07	1.92	7.52
% 1-*O*-Alkyl moieties*	33.8	27.0	23.7
% 1-*O*-Alk-1′-enyl moieties*	26.8	31.8	39.2
% Total acyl moieties*	39.4	41.2	37.1

* Disregarding traces of radioactivity in other fractions.

moieties showed, even after 24 hr, all radioactivity associated with the 16:0 chain length. However, the acyl moieties of the ethanolamine phosphatides exhibited 21, 28 and 44% of their radioactivity after 3, 6 and 24 hr in the 18:0 and 18:1 derivatives. The data suggest that in myelinating rat brain *n*-hexadecanol is an obligatory intermediate in the oxidation of *n*-hexadecane to palmitic acid and that it becomes available for glycerol ether formation before being further oxidized. This finding disagrees with recent data by Paltauf [9] concerning the utilization of hydrocarbons for alkyl formation in the intestine. Apparently, in the intestine the octadecane administered was oxidized to stearic acid which was subsequently reduced to octadecanol before being incorporated into *O*-alkyl groups [9].

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