MAMMALIAN HYDROCARBON METABOLISM: OXIDATION OF 1-HEPTADECENE BY DEVELOPING RAT BRAIN

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1. Introduction

Long-chain aliphatic hydrocarbons, as part of the normal mammalian diet, are absorbed in appreciable amounts [1–3]. They have been found as minor constituents of mammalian brain [4], human arterial tissue and plaques [5,6], meninges and meningiomas [7].

In the rat, dietary aliphatic hydrocarbons are absorbed mainly through the small intestine [3] and are oxidized to fatty acids [8–10]. Oxidation of saturated long-chain hydrocarbons proceeds by terminal hydroxylation [11] catalyzed by microsomal mono-oxygenases [12–14], which have been found in mammalian intestine, liver, lung and kidney.

We have previously shown [15] that myelinating rat brain can oxidize intracerebrally administered *n*-hexadecane to the corresponding fatty alcohol and fatty acid, and that both *n*-hexadecanol and palmitic acid are incorporated as such into brain phospholipids.

We now present evidence for the biological oxidation of intracerebrally administered 1-heptadecene at both the saturated and unsaturated ends of the molecule. Thus, 16-heptadecen-1-ol and 16-heptadecenoic acid, as well as 1,2-heptadecanediol, are produced and incorporated into brain phospholipids.

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2. Materials and methods

Lipid standards such as long-chain alcohols, aldehydes, 1,2-alkanediols, 1-*O*-2′-hydroxyalkyl glycerols and their derivatives were prepared as described previously [15–22]. 1-Heptadecene was purchased from Lachat Biochemical Co., Chicago, Ill. and 1-[1-^{14}C]heptadecene (sp. act. 18.5 mCi/mmole) from ICN Isotope and Nuclear Division, Irvine, Cal. The latter was purified by t.l.c. on Silica Gel H using hexane as developing solvent to a radiopurity (by t.l.c. and g.l.c.) of 99.5%. The substrate was emulsified with aqueous sodium choleate (10 mg/ml) and injected into the brains of 18-day-old male Sprague–Dawley rats (Dan Rolfsmeyer Co., Madison, Wis.). Animals, in groups of five, were killed after 12, 24 and 48 h, the brains of each group were pooled, and the lipids were extracted [23]. Methods for the fractionation of neutral and polar lipids by t.l.c., their isolation, degradation and analysis by g.l.c. were as described previously [15–21].

Periodate cleavage of 1,2-heptadecanediol was carried out in pyridine [22]. Water was added and the reaction products were extracted with diethyl ether. The formaldehyde present in the aqueous phase was precipitated with dimedone (in 50% aqueous ethanol) in the presence of sodium arsenite and the dimedone derivative analyzed by t.l.c. using hexane–diethyl ether, 3:2 (v/v).

Identifications of radioactive fractions in t.l.c. and g.l.c. were based on the use of synthetic internal standards. Radioactivities were determined with a Packard Tri-Carb liquid scintillation spectrometer in Permablend (Packard) counting solution except for

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phospholipids for which Aquasol (New England Nuclear) was used.

3. Results and discussion

Radioactivity from intracerebrally administered 1-[1-^{14}C]heptadecene (2.75 X 10^6 dpm per brain) was found to be incorporated into the neutral and polar lipids of the brain. After 12, 24 and 48 h, approx. 20% of the administered radioactivity was recovered in the total lipids (5.30, 5.06 and 5.19 X 10^5 dpm per brain, respectively). Analysis of small aliquots by t.l.c. showed most of the radioactivity to be associated with unmetabolized substrate and with polar lipids as demonstrated in fig.1 for the 48 h group.

Small amounts of radioactivity were also present (fig.1 A) in fractions corresponding to fatty alcohol (b) and 1,2-alkane-diol (c). They were isolated and identified as 16-heptadecene-1-ol and 1,2-heptadecanediol by the following methods:

Fatty alcohol: In g.l.c., using a homologous series of alkyl acetates as carrier, the acetylated compound cochromatographed with octadecyl acetate and, after catalytic hydrogenation, with heptadecyl acetate.

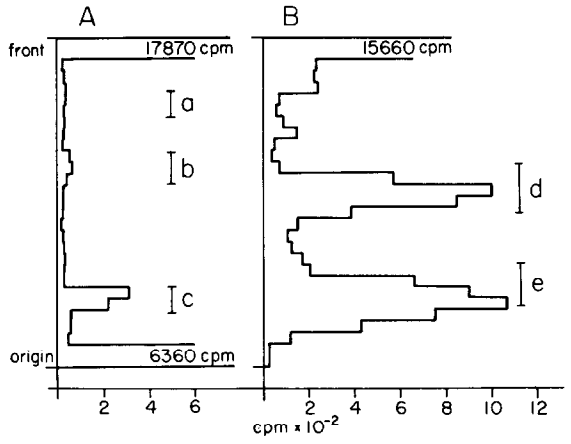

Fig.1. Radioactivity in t.l.c. fractions of total brain lipids (48 h group). Silica Gel H (Merck). (A) Hexane-ethyl acetate-acetic acid, 70:30:2 (v/v/v); Permablend scintillator. (B) Chloroform–methanol–water, 65:25:4 (v/v/v); Aquasol scintillator. Migration rates of standards: (a) fatty acid, (b) fatty alcohol, (c) 1,2-alkanediol, (d) ethanolamine phosphatides, (e) choline phosphatides.

Table 1
Incorporation of radioactivity from 1-[1-^{14}C]heptadecene into rat brain lipids

	Percent of total ^{14}C recovered after		
	12 h	24 h	48 h
Substrate	89.8	84.2	67.8
16-heptadecene-1-ol	0.3	0.5	0.4
1,2-heptadecanediol	1.2	1.5	2.0
Ethanolamine phosphatides	2.8	5.4	12.2
Choline phosphatides	5.9	8.4	17.6

Alkanediol: In t.l.c. and, after preparation of the isopropylidene derivative, in g.l.c. the radioactive fraction cochromatographed with 1,2-heptadecanediol. Periodate cleavage of the vicinal hydroxy groups and precipitation of the formaldehyde with dimedone yielded virtually all radioactivity in the dimedone derivative.

The relative amounts of radioactivity present in various lipid fractions are listed in table 1. From the presence of a long-chain 1,2-alkanediol in the brain lipids, one would expect the formation of hydroxy- and oxo-substituted alkyl glycerophosphatides [17, 21] as well as phospholipids having a 1,2-alkanediol backbone [19]. A long-chain ω-unsaturated alcohol should yield ω-unsaturated alkyl, alk-1-enyl and acyl moieties in the glycerophosphatides [16,18]. Degradation of the total polar lipids (48 h) by converting the alk-1-enyl moieties to 1,3-dioxanes followed by hydrogenolysis with Vitride reagent and t.l.c. of the reaction products as described [20], confirmed the presence of these structures as demonstrated in fig.2.

Analysis of the alcohols (as acetates) by g.l.c. showed that about 75% of the radioactivity in the acyl moieties had been in 16-heptadecenoic acid, the rest in unidentified fractions. After catalytic hydrogenation, about 80% of the radioactivity was eluted with the C_{17} and 20% with the C_{19} derivative. Almost all radioactivity (>95%) in both alk-1-enyl glycerols (as dioxanes) and alkyl glycerols (as isopropylidene derivatives) was found to be in ω-unsaturated C_{17}-derivatives. Virtually all radioactivity in the alkanediol fraction was in 1,2-heptadecanediol (g.l.c. of isopropylidene derivative) and in the hydroxyalkyl

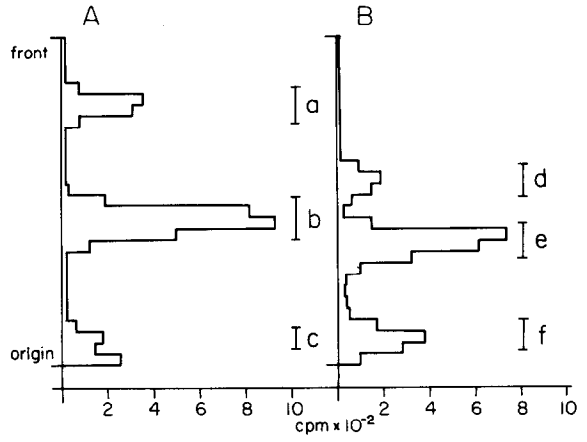

Fig.2. Radioactivity in t.l.c. fractions of products derived from total polar lipids (48 h group). (A) Hexane–diethyl ether, 1:1 (v/v); (B) Refractionation of polar products of (A) after acetylation. Hexane–diethyl ether, 9:1 (v/v), developed three times. Permablend scintillator. Migration rates of standards: (a) alkyl dioxane, (b) fatty alcohol, (c) alkyl glycerol, (d) 1,2-alkanediol diacetate, (e) alkyl-glycerol diacetate, (f) hydroxyalkyl glycerol triacetate.

glycerol fraction in 1-*O*-2′-hydroxyheptadecyl glycerol (GLC of the triacetate). Analysis of ethanolamine and choline phospholipids individually (table 2) confirmed these findings.

Our results show that myelinating rat brain is capable of metabolizing intracerebrally administered 1-heptadecene not only by oxidation of the methyl group to yield an ω-unsaturated alcohol, but also by oxidation of the double bond to yield a 1,2-alkanediol, presumably through the corresponding epoxide.

A microsomal epoxide hydratase of rat liver has been studied extensively because of its importance in the metabolic inactivation of the epoxides produced from polycyclic hydrocarbons and other potentially carcinogenic xenobiotics ([24,25] and references therein). To our knowledge, the formation of an alkanediol from a long-chain monounsaturated hydrocarbon in a mammalian system has not been reported. The resulting 1,2-alkanediol is not only incorporated into brain lipids but is efficiently degraded by oxidation and decarboxylation [17,21].

As previously demonstrated [18] for polyunsaturated alcohols, our present data show that an ω-unsaturated alcohol is readily incorporated into ether lipids and the resulting ω-unsaturated alkyl moiety of the alkylacyl EPG is desaturated to yield the corresponding plasmalogen.

Table 2
Distribution (%) of radioactivity in hydrogenolysis products of rat brain ethanolamine (EPG) and choline (CPG) phosphoglyceride fractions 48 h after administration of 1-[1-^{14}C]heptadecene

	EPG	CPG
Alcohols (from total acyl groups)	37.5	79.4
1-*O*-alk-1′-enyl glycerols	45.3	–
1-*O*-alkyl glycerols	10.0	11.8
1-*O*-2′-hydroxyalkyl glycerols	7.2	5.3
1,2-alkanediols	tr	3.5

Acknowledgements

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