ON THE OXIDATION OF LONG-CHAIN POLYUNSATURATED ALCOHOLS BY MYELINATING RAT BRAIN

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SUMMARY

[1-^{14}C]Octadecenol, [1-^{14}C]octadecadienol, and [1-^{14}C]octadecatrienol were administered intracerebrally to 18-day-old rats. After 6 hrs, the incorporation of radioactivity into the total fatty acids and into individual constituent fatty acids of the brain lipids was determined. The data presented allow the conclusion that myelinating rat brain is capable of oxidizing long-chain mono-, di- and triunsaturated alcohols to the corresponding fatty acids and that the rates of oxidation are not significantly different, regardless of whether the long-chain alcohols are natural constituents of the tissue or not.

It has long been known that saturated long-chain alcohols occur in the mammalian intestinal system (1) and that they can be oxidized biologically to the corresponding fatty acids (2). More recently, small amounts of saturated and monounsaturated alcohols have also been demonstrated in mammalian heart and brain (3,4). When di- and triunsaturated alcohols were fed to rats, the constituent polyunsaturated fatty acids of the lipids of heart and testis were found to be increased (5) which suggested a conversion of polyunsaturated alcohols to acids. Diunsaturated alcohol fed to rats was also shown to lead to the formation of diunsaturated O-alkyl and O-alk-1-enyl glycerophosphatides in the small intestine and the liver (6,7). We have recently shown that mono-, di- and triunsaturated alcohols administered to myelinating rat brain through intracerebral injection are incorporated into both the O-alkyl and O-alk-1-enyl moieties of the ethanolamine phosphatides (8) and into the O-alkyl moieties

of the choline phosphatides (9).

In this communication we report the results of comparative studies on the rates of oxidation of mono-, di- and triunsaturated long-chain alcohols administered intracerebrally to myelinating rat brain.

MATERIALS AND METHODS

[1-^{14}C]cis-9-Octadecenoic acid (62 mCi/mmole), [1-^{14}C]cis,cis-9,12-octadecadienoic acid (56 mCi/mmole) and [1-^{14}C]cis,cis,cis-9,12,15-octadecatrienoic acid (57 mCi/mmole) were purchased from Amersham/Searle Corporation, Arlington Heights, Illinois, U.S.A. Unlabeled fatty acid methyl esters were obtained from The Hormel Institute Lipids Preparation Laboratory, Austin, Minnesota. Alcohols were prepared by reduction of the methyl esters of the corresponding fatty acids with $LiAlH_4$ and were purified to a radiopurity of better than 99% as described (8). The alcohols were emulsified and administered intracerebrally to a group of four 18-day-old rats. After 6 hrs. the rats were killed and the brains were pooled (8-10).

The lipids were extracted, purified according to Folch et al. (11) and subjected to methanolysis by reaction with methanol-HCl (5%) at 80° for 2 hrs. The long-chain methyl esters were isolated by adsorption chromatography on layers of Silica Gel H, 0.3 mm thick, using benzene-ether (95:5, v/v) as the developing solvent and were eluted from the adsorbent with diethyl ether saturated with water. Part of the methyl esters was hydrogenated for 2 hrs. in hexane using 2.5 atm of H_2 and PtO_2 as catalyst.

Gas-liquid chromatography was carried out with a Victoreen 4000 instrument equipped with columns packed with ethylene glycol succinate (10% EGSS-X) on Gas-Chrom P, 100-120 mesh (Applied Science Laboratories, Inc.), and operated at 195°. Fractions were collected by means of glass tubes attached to the heated outlet and rinsed into

TABLE 1

INCORPORATION OF RADIOACTIVITY FROM [1-^{14}C]LABELED LONG-CHAIN ALCOHOLS INTO THE TOTAL FATTY ACIDS OF MYELINATING RAT BRAIN

	Octadecenol	Octadecadienol	Octadecatrienol
Radioactivity [cpm x 10^{-5}] administered per brain	9.37	11.80	7.50
Radioactivity [% of administered cpm] recovered in lipids other than precursor	29.8	18.9	19.7
Radioactivity in fatty acids [% of recovered cpm]	40.9	28.2	27.6

counting vials with the liquid scintillator (8-10). Recovery of the radioactivity injected into the column was 70-80%. The radioactivity was measured in a Packard Tri-Carb scintillation spectrometer (counting efficiency, 80-81%). Identification of peaks was made by reference to authentic standards, by the equivalent chain length (ECL) interpolation (12), and was supplemented by gas-chromatographic analyses of the hydrogenated products.

RESULTS AND DISCUSSION

We observed previously that, when about 2 μg of long-chain alcohols were administered to myelinating rat brain through intracerebral injection, only small amounts of precursor were left after 6 hrs. Therefore, we determined the percentage of radioactivity present in total fatty acids 6 hrs. after administration of [1-^{14}C] octadecenol (2.28 μg per brain), [1-^{14}C]octadecadienol (3.14 μg), and [1-^{14}C]octadecatrienol (2.03 μg) as a measure of the rates of oxidation of the three alcohols. The relative amounts of unmetabolized precursors were determined (3,8) as 10.1%, 26.3% and 2.6%,

respectively, of the total radioactivities recovered.

Table 1 summarizes the amounts of precursor administered, the amounts of total radioactivity recovered in lipids other than the precursor alcohols and the percentage of this radioactivity recovered in long-chain methyl esters.

Obviously, the percentage of radioactivity present in total fatty acids, i.e., mostly in acyl moieties of glycerophosphatides, is not the full measure of alcohol oxidation, since the data do not consider loss of radioactivity through fatty acid degradation. It is apparent from the data listed in Table 1 that both the recovery of radioactivity and the percentage of the recovered radioactivity present in fatty acids was greater when monounsaturated alcohol was used as precursor than when either di- or triunsaturated alcohol were used. This difference could have been due to the different rates of degradation of the fatty acids produced from either precursor and, thus, the actual rates of oxidation of the three alcohols could have been quite similar. Analyses of individual fatty acids showed that both the di- and triunsaturated fatty acids had been further metabolized extensively.

The relative amounts of radioactivity incorporated into individual long-chain fatty acids from each precursor are shown in Table 2.

In each case, the fatty acid corresponding to the precursor alcohol contained the highest amount of radioactivity, but major portions of radioactivity derived from the diunsaturated and, especially, from the triunsaturated alcohol were found in products of chain elongation and desaturation. From each precursor some radioactivity was also incorporated into palmitic acid, obviously through utilization of labeled acetate. These amounts were higher from the di- and triunsaturated alcohols than from the monounsatu-

TABLE 2

WEIGHT PERCENT AND PERCENT OF RADIOACTIVITY OF FATTY ACIDS DERIVED FROM TOTAL LIPIDS AFTER ADMINISTRATION OF [1-^{14}C]OCTADECENOL, [1-^{14}C]OCTADECADIENOL AND [1-^{14}C]OCTADECATRIENOL

	Fatty Acid	Weight [%]	Radioactivity [%] [1-^{14}C]18:1-OHω9	[1-^{14}C]18:2-OHω6	[1-^{14}C]18:3-OHω3
	14:0	0.8	-	-	-
	16:0	29.6	2.1	5.9	9.0
	16:1	1.1	-	-	-
	18:0	19.5	3.0	1.9	4.8
	18:1	15.4	92.1	2.4	-
a	18:2ω6	1.1	-	67.4	-
	20:0	tr.	-	-	-
b	18:3ω3	tr.	-	-	23.5
b	20:1	tr.	2.5	-	-
	20:2ω6	tr.	-	11.8	0.8
	20:3ω6	0.6	-	-	-
c	20:3ω3	tr.	-	-	8.8
c	20:4ω6	13.3	tr.	9.8	-
	20:4ω3	tr.	-	-	5.3
	20:5ω3	0.8	-	0.8	8.9
	22:4ω6	3.7	-	-	tr.
	22:5ω6	1.2	-	-	1.1
	22:5ω3	tr.	-	-	22.4
	22:6ω3	11.6	-	-	15.0

(a) ω:Position of methylene interrupted double bonds (counting from methyl end of fatty acid).

(b) Not separable, radioactivity determined after hydrogenation.

(c) Not separable, tentative assignment of radioactivity.

rated one. As expected, however, most of the radioactivity of the di- and triunsaturated precursors was associated with the linoleic acid (ω6) and linolenic acid (ω3) families (13,14). If one compares the rates of formation of fatty acids from alcohols as listed in Table 1, the significant differences in the fatty acid metabolism

through chain elongation-desaturation reactions must be taken into consideration. The more extensive metabolism of linoleic and linolenic acids, as reflected in the presence of chain elongation/desaturation products, suggests that these fatty acids were also more readily available for metabolic degradation than oleic acid.

Thus, the data presented here allow the conclusion that myelinating rat brain is capable of oxidizing long-chain mono-, di- and triunsaturated alcohols to the corresponding fatty acids and that the rates of oxidation are not significantly different, regardless of whether the long-chain alcohols are natural constituents or not. It appears that biological oxidations of alcohols to fatty acids proceed regardless of degree of unsaturation, whereas reductions of fatty acids to alcohols are more selective for saturated and monounsaturated substrates (15).

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REFERENCES

1. R. Schoenheimer and G. Hilgetag, J. Biol. Chem. 105 (1934) 73.
2. D. Stetten, Jr., and R. Schoenheimer, J. Biol. Chem. 133 (1940) 347.
3. T. Takahashi and H. H. O. Schmid, Chem. Phys. Lipids 4 (1970) 243.
4. M. L. Blank and F. Snyder, Lipids 5 (1970) 337.
5. E. Aaes-Jørgensen, O. S. Privett and R. T. Holman, J. Nutr. 67 (1959) 413.
6. Z. L. Bandi, H. K. Mangold, G. Hølmer and E. Aaes-Jørgensen, FEBS Lett. 12 (1971) 217.
7. Z. L. Bandi, E. Aaes-Jørgensen and H. K. Mangold. Biochim. Biophys. Acta 239 (1971) 357.
8. K. L. Su and H. H. O. Schmid, J. Lipid Res. 13 (1972) 452.
9. H. H. O. Schmid, T. Muramatsu and K. L. Su, Biochim. Biophys. Acta, in press.
10. H. H. O. Schmid and T. Takahashi, J. Lipid Res. 11 (1970) 412.
11. J. Folch, M. Lees and G. H. Sloane Stanley, J. Biol. Chem. 226 (1957) 497.

12. H. H. Hofstetter, N. Sen and R. T. Holman, J. Amer. Oil Chem. Soc. 42 (1965) 537.
13. K. Miyamoto, L. M. Stephanides and J. Bernsohn, J. Neurochem. 14 (1967) 227.
14. G. A. Dhopeshwarkar, C. Subramanian and J. F. Mead, Biochim. Biophys. Acta 231 (1971) 8.
15. D. M. Sand, J. L. Hehl and H. Schlenk, Biochemistry 8 (1969) 4851.