The Incorporation of Orally Fed Radioactive γ-Linolenic Acid and Linoleic Acid into the Liver and Brain Lipids of Suckling Rats

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ABSTRACT

The incorporation of radioactivity from orally administered γ-linolenic acid-1-^{14}C and linoleic acid-^{3}H into the liver, plasma, and brain lipids of suckling rats was studied. Significantly more radioactivity from the former compound was incorporated into the liver and brain lipids 22 hr after dosing. The distribution of the radioactivity in the fatty acids of the liver and brain lipids was different for each isotope. Most of the ^{3}H was still associated with linoleic acid, whereas most of the ^{14}C was in the 20:3 and 20:4ω6 fractions. These results suggest that the desaturation of linoleic to γ-linolenic acid in vivo is a rate-limiting step in the conversion of linoleic to arachidonic acid.

INTRODUCTION

In a previous paper we compared the incorporation of radioactivity from orally administered 18:2-1-^{14}C with 20:4-$^{3}H_8$ into the liver and brain 20:4 of suckling rats (1) and found that the majority of the radioactivity in tissue 20:4 was derived from the exogenously administered 20:4-$^{3}H_8$. The pathway of synthesis of arachidonic acid from linoleic acid involves an initial desaturation of linoleic to γ-linolenic acid (18:3ω6). This reaction has been demonstrated to be rate-limiting in vitro (2).

To test whether the slow endogenous synthesis of arachidonic acid in the above experiment was due, in part, to this rate-limiting step, we have compared the incorporation of radioactivity from 18:2-^{3}H and γ-18:3-1-^{14}C into the liver, brain, and plasma lipids of suckling rats.

METHODS

Suckling rats, between 15 and 16 days old, were used in these experiments. They were bred from female rats maintained on a semisynthetic diet (3).

In the first experiment, four pups were dosed with a mixture of 18:2-9,10,12,13-^{3}H methyl ester (85 mCi/m mole, radiopurity 99%) and γ-18:3-1-^{14}C (20 mCi/m mole, radiopurity 99%, Daiichi Pure Chemical Co., Tokyo, Japan). Ca. 3 μCi of each isotope (^{14}C/^{3}H dose ratio = 1.18) was added to 0.25 ml olive oil, and the mixture was administered orally to 15 day old pups. The pups were killed 22 hr later. This time interval was chosen so that a comparison could be made between the present results and our previous work (1).

In the second experiment, three pups were dosed with ca. 1.5 μCi of each of 18:2-^{3}H and 18:2-1-^{14}C (61 mCi/m mole, The Radiochemical Center, Amersham, U.K.). The isotopes (^{14}C/^{3}H dose ratio = 1.01) were added to 0.25 ml olive oil, and the mixture was administered orally to 16 day old pups. The pups were killed 22 hr later.

Lipids were extracted from the tissue as previously described (1). Blood was collected into citrated tubes, and plasma was extracted in chloroform:methanol (1:1 v/v). Aliquots of the total lipids were assayed for radioactivity by liquid scintillation counting using an Intertechnique SL 30 scintillation spectrometer. The efficiency of counting was determined by the use of internal standards of n-hexadecane-1-^{14}C and n-hexadecane-1,2-^{3}H (The Radiochemical Center, Amersham, U.K.).

Tissue lipids were separated by thin layer chromatography (1); the cholesteryl esters (CE), triglycerides (TG), free fatty acids, cholesterol (Chol), and phospholipids (PL) were scraped quantitatively into scintillation vials and 1 ml water was added to each vial, followed by 10 ml Unisolve 1 (Koch Light Laboratories, Bucks, U.K.). In this system, the efficiency of counting, determined by the use of internal standards (above) was 47% for ^{14}C in the B channel and 22 and 38% for ^{14}C and ^{3}H, respectively, in the A channel. Using this method, the recovery of radioactivity from the plates was greater than 95% for each isotope.

The distribution of radioactivity in the fatty acids of TG and PL was determined by separation and fraction collection of the methyl esters using a preparative gas liquid chromatograph (4). The methyl esters were prepared as previously described (1), and the preparative gas liquid chromatography was carried out in a glass column 2.1 m in length x 7 mm inside diameter packed with 10% polyethylene glycol adipate (PEGA) on Diatomite C-AW 60-70 mesh at 177 C. Methyl-γ-linolenate (nonradioactive) was added to all samples prior to

TABLE I

Incorporation of Radioactivity from γ-18:3-1-^{14}C and 18:2-^{3}H into Liver and Brain Lipids of Suckling Rats

Tissue	Percent of dose		
	18:2-^{3}H	γ-18:3-1-^{14}C	p[a]
Liver lipids	3.94 ± 0.43[a]	6.05 ± 0.48	<0.0025
Brain lipids	0.27 ± 0.003	0.41 ± 0.003	<0.0005

[a]P>0.05 = no significant difference.

[b]Mean ± standard error of the mean from four pups which received a mixture of the two labeled fatty acids. The ^{14}C/^{3}H dose ratio was 1.18:1.

TABLE II

Percentage Distribution of Isotopes in Liver, Plasma, and Brain Lipid Fractions

Tissue fraction[a]		Percent distribution	
		γ-18:3-1-^{14}C	18:2-^{3}H
Liver	PL	54 ± 1[c]	28 ± 1
	TG	43 ± 1	68 ± 0.2
Plasma	PL	35[d]	45
	TG	21	17
	CE	41	34
Brain	PL	83 ± 0.01	95 ± 0.02
	Chol[e]	12 ± 0.02	1 ± 0.04

[a]PL = phospholipids, TG = triglycerides, CE = cholesteryl esters, and Chol = cholesterol.

[b]Percentage distribution of isotope in lipid fractions of each tissue (see "Methods").

[c]Mean ± standard error of the mean from four animals.

[d]Pooled analysis from four animals.

[e]Cholesterol counts were not contaminated by radioactive diglycerides.

chromatography to determine the exact position of this compound. The retention times of the methyl esters of 18:2ω6 and γ-18:3ω6 relative to 18:0 were 1.44 and 1.72, respectively.

Fatty acid fractions were decarboxylated by the Schmidt procedure as described by Goldfine and Bloch (5).

Analyses for changes were performed on the data using the paired Student t-test (6).

RESULTS

Radioactivity from both γ-18:3-1-^{14}C and 18:2-^{3}H was incorporated into the liver, plasma, and brain of the suckling rats (Table I). In the liver, the radioactivity from γ-18:3-1-^{14}C was distributed evenly between the TG and PL fractions, whereas the radioactivity from 18:2-^{3}H was incorporated preferentially into the TG fraction (Table II). In the plasma, the major lipid fractions labeled with both isotopes

TABLE III

Percentage Distribution[a] of Radioactivity in the Fatty Acids of Liver Triglycerides (TG), Phospholipids (PL), and Brain PL

Fatty acid fraction[b]	Liver TG		Liver PL		Brain PL	
	18:2-^{3}H	γ-18:3-1-^{14}C	18:2-^{3}H	γ-18:3-1-^{14}C	18:2-^{3}H	γ-18:3-1-^{14}C
16:0 + 16:1	---[c]	---	---	---	1.9 ± 0.4	18 ± 0.3
18:0 + 18:1	---	---	---	---	2.3 ± 0.3	10 ± 0.2
18:2ω6	67 ± 3.2[d]	---	67 ± 2.4	---	39 ± 1.7	---
18:3ω6	14 ± 1.0	31 ± 0.4	13 ± 1.1	4.3 ± 0.6	8.3 ± 0.5	5.6 ± 0.2
20:0-20:2	8.1 ± 1.9	4.8 ± 0.7	7.4 ± 0.5	---	5.0 ± 0.6	---
20:3ω6	3.5 ± 0.3	24 ± 0.9	2.8 ± 0.6	12 ± 1.0	6.8 ± 0.2	9.9 ± 0.1
20:4ω6	3.4 ± 0.5	20 ± 0.5	5.8 ± 0.6	56 ± 0.3	25 ± 0.8	43 ± 1.1
20:5	3.0 ± 0.4	11.0 ± 0.7	2.4 ± 0.1	13.0 ± 0.7	4.0 ± 0.8	5.0 ± 0.8
22:4 + 22:5ω6	---	6.3 ± 0.2	1.3 ± 0.3	9.7 ± 0.3	5.0 ± 0.2	5.4 ± 0.3

[a]Percentage of radioactivity in a fraction relative to total radioactivity collected. Samples were collected continuously from the solvent front to after the 22:6ω3 fraction. Values above 10% are underlined.

[b]The 18:3ω6 fraction includes 18:3ω3; the 20:0-20:2 fraction includes 20:1.

[c]Less than 1%.

[d]Mean ± standard error of the mean from four animals.

were PL, TG, and CE (Table II). In the brain lipids, most of the radioactivity was associated with the PL fraction.

The distribution of the radioactivity in the fatty acids of liver TG and PL in the 18:2-^{3}H experiment showed that most of the ^{3}H was still associated with the 18:2ω6 fraction and little with the 20:4ω6 fraction (Table III). In the brain PL, some 25% of the ^{3}H was in the 20:4ω6 fraction. On the other hand, in the γ-18:3-1-^{14}C experiment, most of the radioactivity in the polyunsaturated fatty acids of liver TG, PL, and brain PL was associated with 20:3ω6 and 20:4ω6—the longer chain metabolites of the ω6 series. The distributions of the radioactivity in the fatty acid fractions of the plasma TG, CE, and PL were similar to the respective distributions (^{14}C or ^{3}H) obtained in the liver lipids (results not shown).

Decarboxylation of the 16:0 + 16:1 and 18:0 + 18:1 fractions of the brain lipids showed that the carbon 14 in these acids was derived from a combination of de novo synthesis and chain elongation from acetate-^{14}C (Table IV). The presence of significant amounts of ^{14}C in these fatty acids and in brain cholesterol has been commented on previously (1). The very low carboxyl carbon values in the 20:3ω6 and 20:4ω6 fraction of the liver and brain showed that the radioactivity was derived directly from the fed γ-18:3-1-^{14}C.

The total incorporation of ^{14}C into the brain lipids exceeded that of the ^{3}H incorporation (Table I), but it was calculated, from Tables II and III, that, of the total radioactivity in the brain lipids, ca. 30 and 5% of the ^{14}C and ^{3}H, respectively, were associated with the brain cholesterol together with the saturated and monounsaturated fatty acids. In previous experiments in which pups were orally fed 18:2-1-^{14}C, α-18:3-1-^{14}C, and 20:4-1-^{14}C, it was shown that a substantial amount of the radioactivity in the brain lipids was associated with cholesterol and the saturated and monounsaturated fatty acids (1). It was suggested that this incorporation was a property of the type of label in the fed acid (^{14}C or ^{3}H) rather than a property of the fatty acid itself. To confirm this, 18:2-1-^{14}C and 18:2-^{3}H were fed simultaneously to 16 day old pups. The ^{14}C/^{3}H ratios in the liver and plasma lipids were similar to the dose ratio. However, in the brain lipids, the ^{14}C/^{3}H ratio was significantly greater than the dose ratio (Table V). The excess of ^{14}C over ^{3}H in the brain lipids was accounted for by the high ^{14}C/^{3}H ratio in the brain cholesterol and the saturated and monounsaturated fatty acids. As in the previous experiment (γ-18:3-1-^{14}C plus 18:2-^{3}H), it was calculated that there was ca. six times as much ^{14}C as ^{3}H in these compounds.

TABLE IV

Decarboxylation Studies on Fatty Acids Collected by Preparative Gas Liquid Chromatography

Fatty acid fraction	Relative carboxyl activity[a]		
	Liver TG	Liver PL	Brain PL
16:0 + 16:1	ND[b]	ND	0.15
18:0 + 18:1	ND	ND	0.17
18:3ω6	0.89	0.90	0.85
20:3 + 20:4ω6	0.06	0.01	0.03
22:4 + 22:5ω6	0.31	0.04	0.08

[a] ^{14}C in the -COOH group/^{14}C in total fatty acid. The fatty acid fractions from four experiments were pooled prior to decarboxylation, and the analyses were done in duplicate. The original γ-18:3-1-^{14}C had a relative carboxyl activity of 0.87 ± 0.01 (mean ± standard error of the mean for six determinations).

[b] ND = not determined.

TABLE V

The Incorporation of Radioactivity from 18:2-1-^{14}C and 18:2-^{3}H into Tissue Lipids and Fatty Acids of Suckling Rats

Tissue fraction	^{14}C/^{3}H ratio[a]
Liver lipids	1.2 ± 0.02[b]
Plasma lipids	1.0 ± 0.01
Brain lipids	2.4 ± 0.002[c]
Brain cholesterol	13 ± 1.4[d]
Brain 16:0 + 16:1	27 ± 4.2[e]
Brain 18:0 + 18:1	16 ± 1.0[d]

[a] Ratio = ^{14}C/^{3}H in fraction ÷ ^{14}C/^{3}H in dose. The dose ratio was 1.01:1.

[b] Mean ± standard error of the mean for 3 animals.

[c] The ratio differed significantly from the dose ratio, (P) <0.0025.

[d] P<0.025.

[e] P<0.05.

DISCUSSION

The 18:2-1-^{14}C–18:2-^{3}H experiment (Table V) confirms our earlier suggestion that the labeling of the cholesterol, saturated and monounsaturated fatty acids in the brain is due to the position of the label on the fatty acid and its subsequent loss, depending upon the metabolism, rather than the nature of the fatty acid itself (1). The results show that care must be taken when comparing the incorporation of radioactivity from fatty acids labeled with carbon 14 at the carboxyl carbon with more uniformly ^{3}H labeled acids into brain lipids in suckling rats.

The liver is the major site for the desaturation and chain elongation of fatty acids (7). It

commonly is assumed that linoleic acid is converted to arachidonic acid without difficulty (8). However, in vitro studies (2) have shown that the initial desaturation of 18:2ω6 to 18:3ω6 is a rate-limiting step. The amount of radioactivity found in arachidonate may be dictated, not only by the rate of conversion of linoleate or γ-linolenate to arachidonate, but also by the size of the metabolic pool of linoleate and γ-linolenate. In the present experiment, the total liver content of linoleate is much greater than that of γ-linolenate, and this is consistent with the results obtained when rats are fed up to 6.4% of their calories as either linoleate or γ-linolenate (9). That is, when γ-linolenate is fed to essential fatty acid-deficient rats there is an increase in the amount of this compound in the liver. However, rather than a large expansion of the γ-linolenate pool, the γ-linolenate is metabolized to, and results in, an increase in the liver content of 20:3 and 20:4ω6. On the other hand, when linoleate is fed to the essential fatty acid-deficient rats, there is a considerable increase in the total liver content of linoleate and a more modest and slower increase in the content of 20:3 and 20:4ω6 in the liver (9). In the 18:2-^{3}H–γ-18:3-1-^{14}C experiment, most of the radioactivity in the liver PL from orally fed 18:2-^{3}H was in the 18:2ω6 fraction, whereas most of the ^{14}C (from γ-18:3-1-^{14}C) was in the 20:4ω6 fraction and less than 5% in the γ-18:3 fraction.

Clearly, care must be taken in extrapolating from results based upon a single time point. Nevertheless, the results of this experiment demonstrate that one day after dosing the desaturation of 18:2ω6 to γ-18:3 is rate-limiting in vivo as it is in vitro (2). Further work is planned to establish if this rate-limiting step remains of importance in the long term.

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