The Incorporation of Orally Administered Radiolabeled Dihomo γ-Linolenic Acid (20:3ω6) into Rat Tissue Lipids and Its Conversion to Arachidonic Acid

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ABSTRACT

Radioactivity from orally administered radiolabeled dihomo-γ-linolenic acid (20:3ω6) was recovered from the liver, plasma and brain lipid fractions. After administration the fatty acid was metabolized to arachidonic acid, the 22 carbon chain length fatty acid, and was also β-oxidized. However, 22 hr after administration of [1-^{14}C] 20:3 between one-third and one-half of the recovered radioactivity was still associated with dihomo-γ-linolenic acid in the liver and plasma lipid fractions. Orally administered dihomo-γ-linolenic acid is incorporated into lipid fractions and is, therefore, available in the metabolic pool for PGE_1 synthesis.

INTRODUCTION

The metabolism of linoleic acid (18:2ω6) – an essential fatty acid that must be supplied in the diets of vertebrates – to its longer chain polyunsaturated metabolites involves a Δ6 desaturation to γ-linolenic acid (18:3ω6) and followed by a chain elongation to dihomo-γ-linolenic (20:3ω6), the latter is then desaturated (Δ5 desaturase) to arachidonic acid (20:4ω6) (1). Previous studies in vitro (2) and in vivo (3,4) have demonstrated that the Δ6 desaturase is the key enzyme limiting the metabolism of linoleic acid to its metabolites.

Dihomo-γ-linolenic acid (20:3ω6) and arachidonic acid (20:4ω6), besides being principal components of cell structural lipids, are also the direct precursors of the physiologically active prostaglandins. Dihomo-γ-linolenic acid gives rise to PGE_1 – one of the most potent known inhibitors of platelet aggregation – and has other antithrombotic properties, while arachidonic acid gives rise to PGE_2, which potentiates platelet aggregation (5,6). The balance between these two series of prostaglandins and/or their precursors are thought to regulate platelet functions, and it has been suggested that dietary dihomo-γ-linolenic acid supplementation may be used as an antithrombotic agent (7).

It is thought that these prostaglandin precursor fatty acids are derived from the acyl phosphoglycerides. It has been shown that oral administration of dihomo-γ-linolenic acid results in the incorporation of this fatty acid into the tissue lipids (8,9). However, no detailed information is available on the distribution of the orally administered dihomo-γ-linolenic acid into the lipid classes or the extent to which it is desaturated to arachidonic acid.

TABLE I

The Incorporation of Radioactivity into the Liver, Brain and Plasma Lipids 22 hr after Oral Administration of [-1^{14}C]20:3[a]

	% administered dose
Liver	6.65 ± 0.95
Brain	1.02 ± 0.29
Plasma	0.27 ± 0.06

[a]Figures are mean of 4 animals ± SEM.

TABLE II

Percentage Distribution of Radioactivity after 22 hr. from Orally Administered [1^{14}C]20:3 in the Lipid Fractions of the Liver Brain and Plasma[a]

Lipid fractions	Liver	Brain	Plasma
Phospholipids	78.2 ± 1.64	88.7 ± 0.25	54.4 ± 1.84
Cholesterol	1.0 ± 0.07	8.0 ± 0.42	1.6 ± 0.91
Free fatty acids	0.4 ± 0.09	1.6 ± 0.10	1.3 ± 0.24
Triglycerides	19.4 ± 1.61	1.6 ± 0.28	18.7 ± 1.6
Cholesteryl esters	1.0 ± 0.19	0.2 ±0.10	24.1 ± 0.78

[a]Figures are mean of 4 animals ± SEM.

TABLE III

Percentage Distribution of Radioactivity in the Fatty Acids of the Liver, Brain and Plasma Lipid Fractions

Fatty acid fractions	Liver[a]		Plasma[b]		Brain[a]
	Phospholipids	Triglycerides	Phospholipids	Triglycerides	Phospholipids
16:0+16:1ω7+ 18:0+18:1ω9	1.5 ± 0.19	1.9 ± 0.36	3.1	3.8	22.1 ± 1.6
18:2ω6+ 18:3ω6+ 20:2ω6	0.5 ± 0.08	1.8 ± 0.20	1.4	3.5	2.1 ± 0.29
20:3ω6	26.4 ± 2.0	47.0 ± 1.6	46.8	51.1	15.2 ± 1.7
20:4ω6	67.1 ± 2.6	38.2 ± 3.4	42.5	28.8	51.5 ± 4.2
22:4ω6+ 22:5ω6	3.3 ± 0.74	10.0 ± 1.9	5.6	11.8	8.2 ± 0.66

[a]Mean of 4 animals ± SEM.
[b]Mean of 2 determinations from a pooled sample of 4 animals.

TABLE IV

Decarboxylation Studies on the Liver TG, PL and Brain PL Fatty Acids Collected by Preparative Gas Liquid Chromatography

Fatty acid fraction collected by preparative GLC		Relative Carboxyl activity (RCA) $\left(\frac{^{14}C \text{ in the -COOH group}}{^{14}C \text{ in total fatty acids}}\right)$	Activity in -COOH groups as a % of RCA in the original [1-^{14}C]20:3[a]
Liver	Liver TG		
	20:3ω6	0.81[b]	100
	20:4ω6	0.79	98
	22:4 + 22:5ω6	0.57	70
	Liver PL		
	20:3ω6	0.81	100
	20:4ω6	0.82	101
	22:4 + 22:5ω6	0.47	58
	Brain PL		
	16:0 + 16:1ω7	0.14	17
	18:0 + 18:1ω9	0.07	9
	20:3ω6	0.76	94
	20:4ω6	0.78	96
	22:4 + 22:5ω6	0.51	63

[a]The original [1-^{14}C]20:3 had a relative carboxyl activity of 0.81 ± 0.66 (mean ± standard error of six determinations).
[b]Mean of two determinations of pooled samples.

MATERIALS AND METHODS

Suckling rat pups bred from females (Wistar strain) raised in this laboratory on a semisynthetic diet were used. Four pups aged between 15 and 16 days were dosed orally with [1-^{14}C]8,11,14-20:3 (57 mCi/mmole, radiopurity 99%, New England Nuclear Chemicals, Boston MA). About 6 μCi of isotope were administered to each pup. The pups were killed 22 hr later, and blood, liver and brain were collected for lipid analysis.

Lipids were extracted, and the distribution of isotope into the lipid fractions, after separation on thin layer chromatography, was determined by means of techniques described previously (3,10). The distribution of the radioactivity in the fatty acid fractions of lipids was determined by the separation and collection of fatty acid methyl esters on a preparative gas liquid chromatograph and followed by measurement of the radioactivity in a liquid scintillation spectrometer (3,10). Fatty acid fractions were also decarboxylated by the Schmidt procedure, as described by Goldfine and Bloch (11), after separation and collection on a preparative gas liquid chromatograph.

RESULTS AND DISCUSSION

After an oral dose of [1-^{14}C]20:3, about

8% of the administered dose was recovered from the liver, plasma and brain lipids (Table I), which is less than recoveries found for arachidonic acid, but is much greater than those found for linoleic acid (10). In the liver lipids, the radioactivity was mainly in the phosphoglyceride (PL) and triglyceride (TG) fractions (Table II). The brain lipids contain very little triglycerides, and the radioactivity was mainly recovered from the PL fraction. In the plasma lipids, besides PL and TG fractions, radioactivity was also recovered from the cholesteryl ester (CE) fraction. The phosphoglyceride fractions accounted for more than 50% of the recovered activity.

The distribution of the radioactivity in the fatty acids of these lipid fractions is presented in Table III. In the liver PL fraction, two-thirds of the radioacitivity was in the arachidonic acid (20:4ω6), while 26% of the activity was still as dihomo-γ-linolenic acid (20:3ω6). In the liver TG, the radioactivity was evenly distributed between dihomo-γ-linolenic acid (47%) and its metabolites, namely arachidonic (20:4ω6), docosatetraenoic (22:4ω6) and docosapentaenoic (22:5ω6) acids, which in total accounted for 48% of the recovered activity. In the plasma PL and TG fractions, the recovered radioactivity was again more or less evenly distributed between dihomo-γ-linolenic acid and its longer chain metabolites. However, in the brain PL fraction, half of the recovered radioactivity was arachidonic acid, 8% in the 22 carbon chain length metabolites and 15% as 20:3ω6. Significant amounts of radioactivity (22%) were also present as 16 and 18 carbon chain length saturated and monounsaturated fatty acids in the brain PL fraction.

The presence of radioactivity in the brain phosphoglyceride 16:0, 18:0, 16:1ω7 and 18:1ω9 fatty acids and the brain cholesterol fraction suggests β-oxidation of the orally-administered [1-^{14}C] 20:3, and this was confirmed by decarboxylation of these 16 and 18 carbon fatty acids (Table IV). The β-oxidation and the reincorporation of the isotope into structural lipids by *de novo* synthesis of fatty acids has also been shown to take place for both linoleic (18:2ω6) and γ-linolenic (18:3ω6) acids, both of which are precursors of dihomo-γ-linolenic acid (20:3ω6) (3,12). Comparison of these earlier studies with the present study indicates that dietary dihomo-γ-linolenic acid undergoes β-oxidation to an extent less than that shown by linoleic or γ-linolenic acids.

In conclusion, up to one-third or even one-half of the radioactivity recovered from the liver and plasma lipid fraction, 22 hr after oral administration of [1-^{14}C] 20:3, is still present as dihomo-γ-linolenic acid. Therefore, orally administered 20:3ω6, besides being metabolized to arachidonic acid and the 22 carbon chain length derivatives, is also available for incorporation as 20:3ω6 into the tissue lipids, the Δ5 desaturase regulating the conversion of dihomo-γ-linolenic acid to arachidonic acid. Such a regulation would result in the availability of dihomo-γ-linolenic acid for PGE_1 synthesis. This is important for the normal homeostatic balance in which the prostaglandins of the E_1 series inhibit platelet aggregation, while the prostaglandins of the E_2 series have the opposite effect.

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[Received May 15, 1978]