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Research Article

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Incorporation of Chylomicron Fatty Acids into the Developing Rat Brain

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Abstract

The developing brain obtains polyunsaturated fatty acids from the circulation, but the mechanism and route of delivery of these fatty acids are undetermined. ^{14}C-labeled chylomicrons were prepared by duodenal infusion of [1-^{14}C]16:0, [1-^{14}C]18:2(n-6), [1-^{14}C]18:3(n-3), or [1-^{14}C]22:6(n-3) into adult donor rats, and were individually injected into hepatectomized 2-wk-old suckling rats. After minor correction for trapped blood in the brain, the incorporation of chylomicron fatty acids after 30 min was nearly half that of a coinjected free fatty acid reference. [1-^{14}C]22:6(n-3)-labeled chylomicrons showed an average 65% greater incorporation than chylomicrons prepared from the other fatty acids. This apparent selectivity may have been partly due to lower oxidation of 22:6(n-3) in the brain compared to the other fatty acids tested, based on recovered water-soluble oxidation products. The bulk of the radioactivity in the brain was found in phospholipid and triacylglycerol, except that animals injected with [1-^{14}C]22:6(n-3) chylomicrons showed considerable incorporation also into the fatty acid fraction instead of triacylglycerol. These data show that chylomicrons may be an important source of fatty acids for the developing rat brain. (*J. Clin. Invest.* 1994. 93:2764–2767.)
Key words: brain • chylomicrons • docosahexaenoic acid • palmitic acid • linoleic acid • linolenic acid • rat

Introduction

Long-chain polyunsaturated fatty acids are processed into the developing rat brain principally during the first 3 wk of postnatal life (1, 2). Docosahexaenoic acid (22:6[n-3]) is the most prevalent n-3 polyunsaturated fatty acid found in the brain (for review see reference 3), and its uptake and incorporation into the brain has been examined (4–8). However, it is still not clear how 22:6(n-3) reaches the brain. One potential delivery route for fatty acids, via chylomicrons, has only been examined in adult rats (9). In that study it was found that very little radioactivity from [1-^{14}C]16:0-labeled chylomicrons was incorporated into the brain.

Lipoprotein lipase, the endothelial enzyme responsible for hydrolyzing triacylglycerol in chylomicrons and VLDL, is present in the brain (10–13), and in fact peaks during the period of postnatal development in the rat when polyunsaturated fatty acids are being actively laid down (10). We present data here on the incorporation of chylomicron-bound 22:6(n-3) and other fatty acids into the developing rat brain.

Methods

Isotopes were purchased from New England Nuclear Research Products (Boston, MA); [9,10-^{3}H]18:1(n-9) was purified by silica gel thin layer chromatography before use. Sprague-Dawley rat litters were purchased from Simonsen (Gilroy, CA) and were allowed at least one day to recover from any stress associated with shipment. Only 13–15-d-old male pups were used in the experiments.

Chylomicrons were prepared in lymph-fistula rats as described (14). Adult donor rats were fitted with cannulae in the duodenum and the mesenteric lymph duct. 50–100 μCi of a 1-^{14}C-fatty acid (16:0, 18:2[n-6], 18:3[n-3], or 22:6[n-3]; 40–60 mCi/mmol) was emulsified into 3 ml of a lipid emulsion containing phosphate-buffered saline, pH 6.4, 40 μmol triolein, 8.8 μmol egg lecithin, and 57 μmol sodium taurocholate. The labeled emulsion was infused into the duodenum over a 30-min period, followed by steady infusion of the nonradioactive lipid emulsion at 3 ml/h. Lymph was collected for 4 h, with most of the radioactivity recovered in the first 3 h. Recovery of radioactivity in the lymph ranged from ~ 30% (for [1-^{14}C]16:0) to 50–60% ([1-^{14}C]18:2[n-6], [1-^{14}C]18:3[n-3], and [1-^{14}C]22:6[n-3]). Chylomicrons were isolated by ultracentrifugation (15) with 85% recovery of lymph radioactivity. The purity of the preparation was estimated by recentrifugation of an aliquot of VLDL (from control rat lymph) under the above conditions designed for isolation of chylomicrons. Only 3–5% of the VLDL was recovered in the traditional chylomicron fraction. Since VLDL is a comparatively minor component of intestinal lymph, this indicates that VLDL contamination of the original chylomicron preparation was negligible. Chylomicron fractions were assayed for triacylglycerol content on an Abbott VP Chemistry Analyzer (Abbott Laboratories, Abbott Park, IL). The specific activity of the chylomicrons ranged between 0.3–0.4 μCi/mg triacylglycerol.

To further characterize the chylomicrons, aliquots were extracted by the method of Folch et al. (16) and subjected to silica gel TLC with hexane:chloroform:ether:acetic acid (80:10:10:1.5). Triacylglycerol represented 86–96% of the lipid radioactivity, with small amounts of phospholipid (2–8%), cholesterol (1–4%), fatty acid (1–5%), and cholesteryl ester (0–0.5%). ^{14}C-labeled water-soluble oxidation products constituted 0.03–1.32% of total chylomicron radioactivity. Before injection, the chylomicron preparations were complexed with [9,10-^{3}H]18:1(n-9) to compare incorporation of chylomicron fatty acids with that of a free fatty acid. Since results from ^{14}C- and ^{3}H-labeled compounds are not necessarily strictly comparable, the incorporation of [^{3}H]18:1 cannot be compared directly with individual ^{14}C-labeled fatty acids. Thus, incorporation data for [^{3}H]18:1 in the form of free fatty acid are included only to put the incorporation of chylomicron fatty acids into perspective, since free fatty acids are the "presumed" vehicle for fatty acid delivery to the brain (17). The [9,10-^{3}H]18:1(n-9) was dried under nitrogen, a sample of the chylomicron preparation added, and the solution mixed gently with magnetic stirring for 15 min at room temperature. TLC of a lipid extract of the [^{3}H]18:1 complexed chylomicrons showed that > 98% of the tritium was recovered as fatty acid. An aliquot of [^{3}H]18:1 complexed chylomicrons was subjected to ultracentrifugation as above. The majority of the tritium label, 62%,

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Table I. Incorporation of Fatty Acids into the 2-wk-old Developing Rat Brain After Intravenous Injection of ^{14}C-Chylomicrons and ^{3}H-Free Fatty Acid

	^{14}C-chylomicrons*				^{3}H-FFA
	16:0	18:2	18:3	22:6	18:1
Brain weight (*g*)	1.02±0.06	1.08±0.05	1.03±0.02	0.99±0.04	1.03±0.05
Radioactivity (*DPM*)‡	4790±860	4550±810	5120±1230	7590±1640	12630±2720
Incorporation§ (10^2 × [% *dose/g*])	10.6±2.1[a]	9.4±1.5[a]	11.2±2.6[a]	17.2±3.4[b]	27.8±6.4
n	7	4	4	5	19

* The indicated ^{14}C-labeled fatty acids were infused with triolein into adult donor rats and the mesenteric lymph collected (see Methods). For free fatty acids (FFA), [^{3}H]18:1(n-9) was complexed with each chylomicron preparation. Data are means±SD. ‡ Normalized to a dose of 2 μCi. § Values with unlike superscripts are different at $P = 0.003$.

was present in the infranatant fraction (presumably associated with fatty acid binding protein[s], such as albumin) while the rest, 38%, was present in the chylomicron fraction.

Rat pups were anesthetized with ketamine/xylazine/acepromazine and a Biemer clip (Aesculap Instruments Corp., Burlingame, CA) placed on the porta hepatis to interrupt circulation to the liver. The jugular vein was then exposed and 0.55-ml chylomicrons (containing 5–22 μmol triacylglycerol, 2–5 μCi 1-^{14}C-fatty acid and 2–5 μCi [^{3}H]18:1, suspended in 0.9% NaCl) was injected as described (4). After 30 min a blood sample was taken from the heart, the heart removed, and normal saline infused into the aorta for 2 min. The saline-perfused brain (which was noticeably white compared with unperfused brain) and a serum aliquot were extracted by the method of Folch et al. (16). Aliquots of the organic and aqueous phases, containing an average of 500 dpm, were counted in a liquid scintillation spectrometer capable of simultaneously determining ^{3}H and ^{14}C, with quench and background correction. The distribution of label within the lipid fractions of the serum and brain was determined by silica gel TLC (4).

Since the saline perfusion may not have removed all of the blood from the brain (18), it was necessary to estimate the amount of trapped blood in the current experiments. ^{51}Cr-labeled red cells were prepared by standard methods (19) and injected into control 2-wk-old rats in the same manner as described above. After 30 min a blood sample was taken and the radioactivity remaining in the brain was measured with and without saline perfusion. From these data it was calculated that an average of 1.1 μl of blood remained trapped in the brain after rinsing. Since blood radioactivity was measured at termination for each rat injected with ^{14}C-chylomicrons, it was thus possible to estimate the amount of brain radioactivity in each experiment which was actually due to trapped blood. This number was subtracted from total brain radioactivity to give a corrected value for brain incorporation. The correction averaged only 10% of total brain radioactivity. Likewise, the distribution of label in the various lipid fractions of the brain was corrected based on the volume of trapped blood and the distribution of label in the serum lipids.

Differences between fatty acids were detected by one-way ANOVA, followed by post-hoc testing with the appropriate t-statistic (20). The Bonferroni inequality (21) was used to control the overall alpha level. Where needed, the data were first log transformed to pass Bartlett's test for equal variances.

Results

Intravenous injection of chylomicrons labeled in the fatty acid moiety of triacylglycerol resulted in a significant accumulation of radioactivity in the brains of hepatectomized 2-wk-old rats after 30 min (Table I). There was selectivity for chylomicrons labeled with [1-^{14}C]22:6(n-3), which were incorporated to a greater extent than chylomicrons labeled with [1-^{14}C]16:0, [1-^{14}C]18:2(n-6), or [1-^{14}C]18:3(n-3). The average incorporation of 1-^{14}C-labeled chylomicron fatty acids, expressed as a percentage of injected dose, was almost half that of unesterified ^{3}H]18:1(n-9), which was coinjected with the chylomicrons. The incorporation of unesterified [^{3}H]18:1(n-9) complexed initially with the chylomicron preparation was nearly the same as the incorporation of [^{3}H]18:1(n-9) complexed initially with rat plasma (4).

The increased incorporation of 22:6(n-3) compared with the other fatty acids might be explained at least in part by differences in fatty acid oxidation. There were fewer ^{14}C-labeled water-soluble oxidation products recovered from the brains of animals injected with [1-^{14}C]22:6(n-3) chylomicrons (Table II). The lower apparent susceptibility of 22:6 to oxidation in the brain did not apply to the whole animal, however, as the amount of recovered oxidation products in the serum was generally higher for 22:6 than for the other fatty acids.

The distribution of ^{14}C-label within the lipid fractions of the brain is shown in Table III. For most of the fatty acids tested, phospholipid contained the majority of the label, followed by triacylglycerol. The channeling of significant amounts of labeled fatty acid into brain triacylglycerol, while not expected from the bulk lipid composition, has been noted before (8, 12). For [1-^{14}C]22:6(n-3), however, the next most prominent radiolabeled fraction after phospholipid was fatty acid. The lower proportion of [^{14}C]cholesterol (derived from ^{14}C-acetate after ^{14}C-fatty acid catabolism) in the animals injected with [1-^{14}C]22:6(n-3) is consistent with the data in Table II showing

Table II. Water-soluble ^{14}C-labeled Oxidation Products Recovered After Intravenous-Injection of ^{14}C-Chylomicrons into 2-wk-old Rats

	^{14}C-chylomicrons*			
	16:0	18:2	18:3	22:6
	10^2 × (% *dose/g*)			
Brain	0.5±0.2[a]	0.4±0.4[a,c]	1.0±0.4[b]	0.1±0.1[c]
Serum	35.1±19.1[a]	86.2±59.3[a,b]	34.3±16.1[a]	160.3±116.2[b]
n	7	4	4	5

* See footnote to Table I. Means±SD; values with unlike superscripts are different at $P = 0.029$ and $P = 0.005$ for brain and serum, respectively.

Table III. Distribution of Radioactivity in Brain Lipids After Intravenous Injection of ^{14}C-Chylomicrons into 2-wk-old Rats

	^{14}C-chylomicrons*			
	16:0	18:2	18:3	22:6
	Percentage total dpm			
Phospholipid	52.9±7.9	59.6±11.7	51.4±8.3	57.0±4.4
Cholesterol	9.3±2.0[a]	6.1±1.3[b]	6.0±1.6[b]	3.4±0.2[c]
Fatty acid	5.7±2.0[a]	12.5±2.8[b]	13.7±1.2[b]	33.9±2.9[c]
Triacylglycerol	32.1±8.4[a]	22.1±14.5[a]	29.0±10.5[a]	5.7±2.9[b]
n	7	4	4	5

* See footnote to Table I. Means±SD; values with unlike superscripts are different at $P \leq 0.022$.

less oxidation of this fatty acid and subsequently less synthesis of cholesterol.

Discussion

The presence of lipoprotein lipase in the brain naturally suggests the possibility that chylomicrons could supply fatty acids for formation of neural membranes. In fact, lipoprotein lipase activity in the developing rat brain rises sharply after birth, peaks at day 4, and then declines to approximately adult levels by day 20 (10). This time course parallels the burst of polyunsaturated fatty acid accretion in the brain (1, 2). Only one study has reported the incorporation of chylomicron fatty acids by the brain, and this was in adult rats (9). In that study, very little radioactivity from [1-^{14}C]16:0-labeled chylomicrons reached the brain, on the order of 0.01–0.02% of dose. This is much lower than our reported incorporation of 0.4% of dose for [1-^{14}C]16:0 injected as free fatty acid into hepatectomized 2-wk-old rat pups (4). In the present study an incorporation of 0.1% of the injected dose was observed when [1-^{14}C]16:0-labeled chylomicrons were injected into hepatectomized 2-wk-old rat pups. That is, 10 times the incorporation found in adult rats. Since the plasma of suckling rats contains much more lipid in the form of chylomicrons than unesterified fatty acids, this raises the possibility that chylomicrons are quantitatively a more important source of fatty acids for the developing rat brain than are fatty acids bound to carrier proteins, such as albumin (17) or alpha-fetoprotein (7, 22, 23).

In the intact, i.e., non-hepatectomized, rat other possible uptake routes exist, such as incorporation of phospholipid (24, 25) from HDL (26) or other lipoproteins derived from the liver (5, 26, 27). The present experiments were designed to separate variables, so that incorporation from chylomicrons could be studied without complication from secondary metabolism by the liver, which occurs quickly (28, 29). For example, in a 2-wk-old non-hepatectomized rat pup injected with [^{14}C]16:0-labeled chylomicrons under the conditions described in this report, we measured half-again as much incorporation of radioactivity into the brain as in hepatectomized rats (data not shown). However, all of this additional radioactivity was present in the form of water-soluble oxidation products presumably derived from metabolism of [^{14}C]16:0 in the liver. It is apparent that additional quantitative work will be necessary to determine how the various pathways contribute to accretion of fatty acids in the brain. Further complicating the picture is a recent study which found no evidence for incorporation of orally fed deuterated 16:0 into the brain and concluded that the brain derives saturated fatty acids entirely from de novo synthesis (30).

The currently reported preferential incorporation of [1-^{14}C]22:6(n-3)-labeled chylomicrons compared to 16:0-, 18:2-, and 18:3-labeled chylomicrons is analogous to the preferential incorporation we (4) and others (31) observed when [1-^{14}C]22:6(n-3) and other fatty acids were injected into rats in the free fatty acid form. In that case, we found that the preferential incorporation was probably not due to sparing of 22:6 from oxidation. In the current situation, with [1-^{14}C]22:6(n-3) injected as triacylglycerol in a lipoprotein, we cannot be sure. Since there were fewer oxidation products recovered from the brains of rats injected with [1-^{14}C]22:6(n-3)-labeled chylomicrons, it is possible that some or even all of the differential incorporation was due to differential processing after uptake.

Differential action of lipoprotein lipase towards substrates with differing chain lengths and degrees of unsaturation has been reported (32–36), and offers a possible explanation for preferential incorporation of [1-^{14}C]22:6(n-3)-labeled chylomicrons by the brain. However, in these cases triacylglycerols containing longer and more polyunsaturated acyl chains were poorer substrates. Thus, it is unlikely that brain lipoprotein lipase was responsible for the differential incorporation of 22:6, although it is possible that a plasma membrane fraction could act selectively on 22:6 immediately following the action of lipoprotein lipase.

Another difference between 22:6 and the other fatty acids injected was evident in the fate of the label in the brain. Although most of the label was found in phospholipid in all cases, a significant amount was present in triacylglycerol in rats injected with labeled 16:0, 18:2, or 18:3. This was not the case with labeled 22:6, however, as unesterified fatty acid constituted the second most prominent lipid fraction. Very little of the label was found in triacylglycerol, suggesting that 22:6 entering the brain from chylomicrons is channeled into a different pathway. This different pathway apparently prevents 22:6 from being routed into depot fat.

The data reported here show that chylomicrons are a source of fatty acids for the developing rat brain. Rat milk contains all of the essential fatty acids (23, 37), including 20:4(n-6) and 22:6(n-3), so it is possible that the brain derives a significant fraction of its essential fatty acid requirements from chylomicrons.

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