Delivery and turnover of plasma-derived essential PUFAs in mammalian brain

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Abstract **Polyunsaturated fatty acids (PUFAs) are critical to nervous system function and structure, but their rates of incorporation from plasma into brain have not been evaluated. In the adult rat, calculations based on our model show that at least 3–5% of esterified brain arachidonic acid (AA) and 2–8% of esterified brain docosahexaenoic acid (DHA) are replaced daily by unesterified PUFAs in plasma. These rates, when related to unlabeled brain PUFA composition, give half-lives of 1–2 weeks for plasma-brain exchange of AA and DHA. In the human brain, the arachidonate replacement rate is 0.3% per day. Although unesterified plasma PUFA concentrations are low, their rates of incorporation into brain are sufficient to compensate for metabolic and efflux losses, so that PUFA transport from plasma into brain as a component of a lipoprotein is unnecessary. Dietary supplementation, by altering plasma unesterified PUFA concentrations, can regulate brain PUFA content and may help to treat brain diseases involving PUFA imbalance.**—Rapoport, S. I., M. C. J. Chang, and A. A. Spector. **Delivery and turnover of plasma-derived essential PUFAs in mammalian brain.** ***J. Lipid Res.*** **2001.** 42: **678–685.**

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The nutritionally essential polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA; 22:6 n-3) and arachidonic acid (AA; 20:4 n-6) are critical to brain and retinal function and structure during early development (1–3) and in later life (4). DHA and AA are major components of brain and retinal cell membranes, and influence membrane fluidity, recovery from injury, signal transduction, and gene transcription (5–9). AA can be released from brain phospholipids through receptor-mediated activation of phospholipase A_2. AA and its metabolites (prostaglandins, hydroxyeicosatetraenoic acids, leukotrienes) are important second messengers with a plethora of biological effects (10, 11).

An imbalance between n-3 and n-6 PUFAs may play a role in infantile visual and cognitive dysfunction, Alzheimer disease, chronic alcoholism and fetal alcohol syndrome, genetic peroxisomal disorders, major depression, bipolar disorder, and human aging (1–4, 12–16). In bipolar disorder, chronic treatment with lithium or valproate may reduce brain AA turnover and availability (17, 18), whereas dietary supplementation of n-3 PUFAs is reported to be therapeutically effective (16).

If dietary replacement therapy is to be envisaged in human brain diseases, rates and mechanisms of incorporation of PUFAs from plasma into brain must be better understood and quantified (6, 19–21). In this article, we extend a published in vivo fatty acid (FA) model (22, 23) and apply it to experimental data to estimate the extent of regulation by plasma of brain PUFA content and turnover.

In this regard, it has been argued that plasma concentrations of unesterified PUFAs are not high enough to provide sufficient quantities to the brain and, thus, that esterified PUFAs within circulating lipoproteins must be directly transferred to brain (6, 24). It is true that lipoprotein receptors have been identified in brain capillaries (25) and shown to modulate phospholipid transfer in isolated capillaries (26). However, feeding experiments in adult rats suggest that FAs esterified within circulating lipoproteins do not measurably enter brain, and that only the unesterified form is incorporated (27). In this article, we extend and apply a previously published model (22, 23) to quantify the extents to which unesterified AA and DHA in plasma are incorporated into brain phospholipids to replace the amounts lost by metabolism or efflux. We thus show that diet, by regulating plasma PUFA concentrations, can indirectly influence brain PUFA metabolism. Unesterified circulating PUFAs may be derived from adipose tissue or by hydrolysis of lipids within circulating lipoproteins (28, 29).

MODEL

Our experimental method involves the intravenous injection of an albumin-bound radiolabeled PUFA, measur-

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; FA, fatty acid; GC, gas chromatography; PET, positron emission tomography; PUFA, polyunsaturated fatty acid; TLC, thin-layer chromatography.

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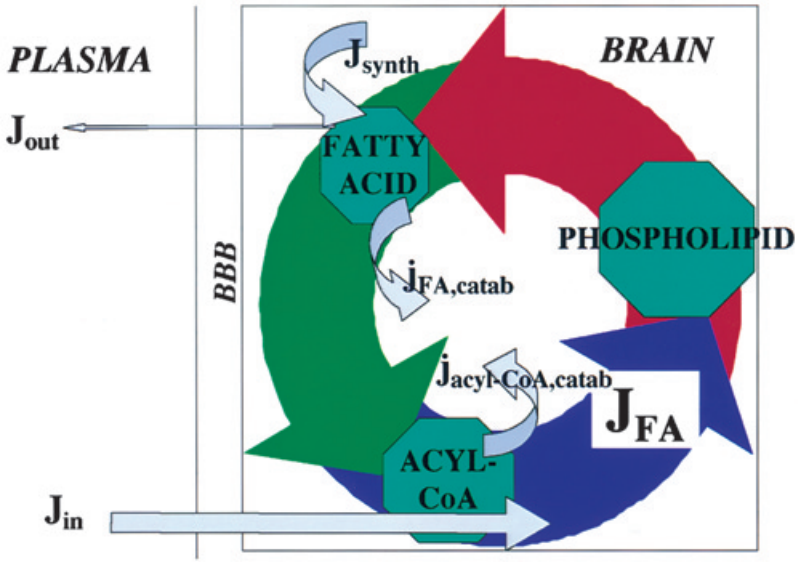

Fig. 1. Interacting "cycles" of intracerebral de-esterification and re-esterification and of plasma-brain exchange involving AA or DHA. For the intracerebral cycle, the PUFA in the *sn*-2 position of brain phospholipids is rapidly recycled through three brain compartments (large arrows). When released by phospholipase A_2, it enters the unesterified FA compartment. From there, a fraction is converted into bioactive eicosanoids or other metabolites $j_{FA,catab}$, whereas the remainder is transferred to the acyl-CoA compartment by an acyl-CoA synthetase. A small percentage of the acyl-CoA is lost through β-oxidation or other processes $j_{acyl\text{-}CoA,catab}$, whereas the remainder is reesterified into the phospholipids by an acyltransferase. The overall rate of brain PUFA loss due to metabolism equals J_{catab} ($j_{FA,catab}$ + $j_{acyl\text{-}CoA,catab}$). Synthesis within brain from linoleic or α-linolenic acid, at a rate J_{synth} also can contribute to brain acyl-CoA for the elongated PUFAs. In the second cycle, the unesterified PUFA in plasma diffuses across the blood-brain barrier (BBB), passes through the brain unesterified PUFA and acyl-CoA pools, and then is incorporated into phospholipids at a rate J_{in}. J_{out} represents the probably inconsequential net efflux rate of the unesterified PUFA from brain to plasma (see text).

ing labeled and unlabeled unesterified PUFA in plasma at fixed times thereafter, and then estimating tracer incorporation into brain lipids (22, 23). Incorporation into the lipids is very rapid. Within 1 min after the intravenous injection of tracer AA or DHA, about 90% of net brain nonvolatile radioactivity is found in these lipids, particularly at the stereospecifically numbered (*sn*)-2 position of phospholipids (30–33).

Figure 1 illustrates how AA and DHA metabolism and turnover in brain may be considered in terms of two interacting cycles. The first cycle (large arrows) involves the de-esterification and re-esterification of AA or DHA within brain lipids, mainly phospholipids. This cycle has been described extensively (11, 23, 34) and corresponds to the important energy-demanding roles of PUFAs in brain signal transduction and neuroplasticity. The second cycle involves plasma-brain exchange plus synthesis from a shorter chain PUFA precursor. In this cycle, incorporation of unesterified plasma AA or DHA into brain phospholipids, plus synthesis from linoleic acid (18:2 n-6) or α-linolenic acid (18:3 n-3), respectively, compensate for loss from brain owing to catabolism or back diffusion to plasma (small gray arrows). The relations between the two interacting cycles can be formulated as follows.

The rate of unesterified tracer incorporation from plasma into stable brain lipids (22, 23) equals

$$dc^*_{br}/dt = k^* c^*_{pl} \qquad Eq.\ 1)$$

where c^*_{br} is brain radioactivity, t is time after tracer injection has begun, c^*_{pl} is plasma radioactivity of the unesterified PUFA, and k^* is an incorporation coefficient. Integrating to time T (5–15 min after tracer infusion starts) gives an expression for k^*:

$$k^* = c^*_{br}(T) / \int_0^T c^*_{pl} dt \qquad Eq.\ 2)$$

k^* is the incorporation coefficient of both the labeled and unlabeled PUFA because they have identical physicochemical properties. Thus, the incorporation rate J_{in} (Fig. 1) of the *unlabeled* unesterified plasma PUFA equals

$$J_{in} = k^* c_{pl} \qquad Eq.\ 3)$$

As illustrated in Fig. 1, following its hydrolysis from phospholipids, AA or DHA can be lost metabolically at a rate J_{catab} due to *i*) oxidation to eicosanoids/docosanoids, *ii*) β-oxidation (35–37), *iii*) peroxidation to reactive oxygen species (38), or *iv*) conversion to products such as anandamide (39). Together with any PUFA that diffuses back to plasma at a rate J_{out}, the loss must be compensated for at the steady state by incorporation J_{in} from plasma plus synthesis J_{synth} from a PUFA precursor. Thus, at the steady state, we have the following balance equation:

$$J_{in} + J_{synth} = J_{catab} + J_{out} \qquad Eq.\ 4)$$

It is unlikely that unesterified brain PUFA diffuses to a significant extent into plasma, except if its concentration is raised in pathological conditions such as ischemia, trauma, or epilepsy (6, 40, 41). When they have entered

the brain, unesterified PUFAs are rapidly incorporated into the acyl-coenzyme (Co)A pool by the action of acyl-CoA synthetases, whose values for K_m exceed the unesterified brain PUFA concentrations (see below) (31). Thus, we can assume (as a first approximation) that $J_{out} \cong 0$. As we do not currently have an independent estimate for J_{synth}, however, equation 4 can be used as an inequality:

$$J_{in} \leqslant J_{catab} \qquad Eq.\ 5)$$

J_{in} thus represents a lower bound for the rate of loss of a PUFA from brain owing largely to metabolism.

The turnover (replacement) rate of AA or DHA within brain lipids, due to entry from plasma and intracerebral synthesis from a shorter chain precursor, is defined as Φ_{FA}:

$$\Phi_{FA} = (J_{in} + J_{synth}) / (c_{br} \geqslant J_{in}) / c_{br} \qquad Eq.\ 6)$$

where c_{br} is the unlabeled esterified brain concentration. With first-order kinetics (42, 43), the half-life of plasma-brain exchange is

$$t_{1/2} = 0.693 / \Phi_{FA} \qquad Eq.\ 7)$$

AA or DHA within the brain acyl-CoA pool may be derived from the following sources (Fig. 1): *i*) unesterified PUFA in plasma, *ii*) esterified PUFA within plasma (this contribution is likely insignificant in the adult brain) (27), *iii*) hydrolysis from phospholipids, or *iv*) synthesis from linoleic or α-linolenic acid, respectively. The contribution of the unesterified plasma PUFA to brain acyl-CoA compared with the contribution from all other sources is represented by the dilution factor λ of the acyl-CoA pool (22, 23, 33). λ approximates 0.02–0.04 in awake rats. It is determined by establishing a constant plasma specific activity of the PUFA with a programmed intravenous infusion of its radiolabel, and then measuring the ratio of brain acyl-CoA specific activity to plasma unesterified PUFA specific activity (22, 23, 31, 44):

$$\lambda = \frac{c^*_{br,acyl-CoA} / c_{br,acyl-CoA}}{c^*_{pl} / c_{pl}} \qquad Eq.\ 8)$$

A PUFA within the acyl-CoA pool enters brain phospholipids at a rate J_{FA}:

$$J_{FA} = J_{in} / \lambda \qquad Eq.\ 9)$$

Thus, turnover F_{FA} of the PUFA within phospholipids due to de-esterification and re-esterification (large arrows in Fig. 1) equals

$$F_{FA} = J_{FA} / c_{br} = J_{in} / c_{br}\lambda \qquad Eq.\ 10)$$

with half-life equaling

$$t_{1/2} = 0.693 / F_{FA} \qquad Eq.\ 11)$$

Comparison of turnover due to PUFA replacement by intracerebral synthesis plus entry from plasma (equation 6), with turnover due to de-esterification and re-esterification (equation 10), gives the following relation:

$$F_{FA} = \frac{\Phi_{FA}}{\lambda} \qquad Eq.\ 12)$$

As λ = 0.02–0.04 (see above), turnover due to de-esterification and re-esterification can be about 50 times faster than turnover due to plasma-brain exchange, with the half-life correspondingly 50 times shorter.

EXPERIMENTAL METHODS

In vivo experiments in awake rats

To obtain data for applying equations 1–12, a radiolabeled albumin-bound PUFA was injected or infused intravenously in an awake rat, and plasma radioactivity was measured until the animal was killed and the brain studied by quantitative autoradiography or biochemical analysis. Tracers infused were of high specific activity to minimize changes in unlabeled plasma PUFA concentrations, and had a thin-layer chromatography (TLC)-determined purity exceeding 95%. The tracers included [4,5-^{3}H]DHA, [1-^{14}C]DHA, [5,6,8,9,11,12,14,15-^{3}H(N)]AA, or [1-^{14}C]AA. Sources usually are Moravek Biochem (Brea, CA) or NEN Life Sciences (Boston, MA).

In a protocol approved by the Animal Care and Use Committee at the National Institutes of Health, adult male Fischer-344 rats were acclimatized for 1 week in an animal facility in which temperature, humidity, and light cycle were controlled, with free access to food and water. On the day of the study, a rat was anesthetized with 1–3% halothane, and polyethylene catheters were inserted into a femoral artery and vein. The animal was allowed to recover from anesthesia in a quiet dimly lit room for 3–4 h, with its lower body loosely wrapped in a plaster cast. Body temperature was maintained at 36.5°C by a feedback heating device and a rectal probe. This procedure is minimally stressful and is well accepted for in vivo studies of brain metabolism and blood flow in awake rodents (45). Although stress and ischemia can increase brain unesterified PUFA concentrations, the concentrations measured during this procedure were only 2–3 times higher than values in anesthetized rats (44, 46).

To determine regional incorporation coefficients, a radiolabeled PUFA, bound to FA-free bovine serum albumin in normal saline (Sigma Chemical, St. Louis, MO), was infused intravenously for 2–3 min. Labeled and cold PUFA concentrations in arterial plasma were measured at fixed times thereafter until the animal was killed after 10–15 min by an overdose of pentobarbital. The brain was removed, frozen, and sectioned for quantitative autoradiography. Values for k^* were calculated by dividing regional brain radioactivity obtained from the optical density by the integrated plasma unesterified PUFA radioactivity (equation 2).

To calculate appropriate "whole brain" fluxes, turnover rates, and half-lives given by equations 1–12, 5 min after a steady-state plasma radioactivity was established by regulated intravenous infusion of labeled AA or DHA, the animal was killed with an overdose of pentobarbital and its head was subjected to focused-beam microwave irradiation (5.5 kW) for 3 s (Cober Electronics, Stanford, CT) to stop brain metabolism. The rat was decapitated, the brain was removed and stored at −70°C. Frozen brains were used for lipid extraction and acyl-CoA quantification. The lipids in one half of the brain were extracted by the method of Folch, Lees, and Sloane Stanley (47). Incorporation of radiotracer in this fraction was determined using liquid scintillation counting. The other half of the brain was used to quantify acyl-CoA levels and specific activities (44).

Frozen brains were homogenized in methanol (31, 46) containing 0.01% (wt/v) butylated hydroxytoluene, and then the homogenate was extracted by a modified Folch procedure (47). The upper phase and protein fractions were re-extracted once with chloroform. Lipids in the organic extract were separated by

TLC (Silica Gel 60 A plates, Whatman, Clifton, NJ) and identified using unlabeled standards. Neutral lipids were separated from phospholipids using heptane–diethylether–glacial acetic acid 60:40:2 (v/v/v) as the solvent system (48). Phospholipid classes were resolved by TLC using chloroform–methanol–glacial acetic acid–water 60:50:1:4 (v/v/v/v) as the solvent system. Each band, corresponding to a specific standard, was identified using primuline dye and scraped, and then analyzed for radioactivity by liquid scintillation counting or methylated for later gas chromatography (GC). FA analysis in the total phospholipid fraction or in each phospholipid class was achieved by a transesterification reaction of the extracted fraction using sodium methoxide to convert the FAs to their methyl esters. Known amounts of di-heptadecanoyl-phosphatidylcholine were added to each fraction as a standard.

The unesterified FA fraction obtained from the TLC plate was extracted with chloroform-methanol 2:1, with heptadecanoic acid as a standard, and then converted to methyl esters using diazomethane. The FA methyl esters were separated by GC and quantified using flame ionization detection, with a limit of sensitivity of 10 pmol. Identities of peaks were confirmed with FA methyl ester standards. Additionally, arterial plasma samples (50 μl) were extracted by the Folch method (47) following the addition of known concentration of heptadecanoic acid. Unesterified FAs were methylated with diazomethane and analyzed by GC.

In vivo positron emission tomography (PET) in human subjects

In human subjects, PET was used to noninvasively determine regional brain incorporation coefficients k^* of [1-^{11}C]AA, whose radioactive half-life is 20 min (49, 50). [1-^{11}C]AA was synthesized and formulated in 0.2% human serum albumin in 0.9% saline (51). The solution was sonicated and sterilized by filtration. Radio-high performance liquid chromatography analysis shows it to be more than 99.6% (n = 9) radiochemically pure, with a specific activity of ~1,000 Ci/mmol.

Under an Institutional Review Board-approved protocol, a subject was placed in a General Electric Advance positron emission tomograph (Waukesha, WI), with his head in a face mask attached to the scanner bed (50). [1-^{11}C]AA (20.9 mCi to 26.5 mCi) was infused intravenously during 3 min, and serial dynamic three-dimensional scans (30 s to 5 min) were acquired for 1 h. Arterial blood (0.5–1.5 ml) that is removed during this hour contains [1-^{11}C]AA with minimal labeled metabolites (49). Plasma radioactivity was counted and decay-corrected in a gamma counter, and then integrated. After correcting for attenuation, scatter, random coincidences, and dead time, regional brain radioactivities were determined on PET slices with the help of an anatomic template (52) to calculate values for k^* (equation 2).

RESULTS

Distribution of intravenously injected labeled PUFAs in rat brain lipids

About 1% of the administered intravenous dose of [5,6,8,9,11,12,14,15-^{3}H(N)]AA or [4,5-^{3}H]DHA was incorporated into brain lipids of awake rats at 15 min after injection. Of the net amount of nonvolatile tracer in brain, 93.9% and 89.6% of ^{3}H-labeled AA and DHA, respectively, was within tissue lipids, 79.6% and 71% being in phospholipids and about 1% in cholesterol and cholesterol esters (**Table 1**). Thus, there was minimal β-oxidation and de novo sterol synthesis (36, 37) in this time. Tracer AA was mainly in brain phosphatidylcholine and phosphatidylinositol; tracer DHA mainly in phosphatidylethanolamine and phosphatidylcholine. The high fraction of phospholipid labeling by the labeled PUFAs justifies relating their incorporation into brain, mainly to their esterification into brain phospholipids (Fig. 1).

Incorporation and turnover rates due to plasma-brain exchange

Table 2 presents calculated incorporation rates J_{in} of unesterified AA and DHA from plasma into the brain of awake rats and of unesterified AA into the human brain. J_{in} was calculated by multiplying experimental values for k^* in brain gray matter by the respective unesterified plasma concentration (equation 3). Incorporation rates of AA and DHA equal 0.35–1.2 μmol/g/day in the rat, whereas

TABLE 1. Distribution of radioactivity within brain lipids in awake rats 15 min after beginning a 3-min intravenous infusion of labeled arachidonate and docosahexaenoate

	Fatty Acid Tracer (% Total Nonvolatile Brain Radioactivity)	
Brain Fraction	[5,6,8,9,11,12,14,15-^{3}H(N)]Arachidonate	[4,5-^{3}H]Docosahexaenoate
Total lipids	93.9 ± 1.4	89.6 ± 0.4
Phospholipids	79.6 ± 1.7	71.0 ± 1.2
Sphingomyelin	−1.3 ± 0.6	−0.5 ± 0.1
Phosphatidylcholine	−38.5 ± 1.1	−17.5 ± 1.0
Phosphatidylserine	−4.7 ± 0.4	−2.2 ± 0.4
Phosphatidylinositol	−26.1 ± 1.6	−8.3 ± 0.6
Phosphatidylethanolamine	−9.1 ± 0.7	−42.5 ± 1.7
Unesterified fatty acid	5.4 ± 0.8	3.9 ± 0.1
Monoacylglycerol	0.7 ± 0.2	0.4 ± 0.1
Cholesterol	0.4 ± 0.1	0.5 ± 0.1
Diacylglycerol	3.4 ± 0.3	3.6 ± 0.4
Triacylglycerol	3.9 ± 0.6	9.8 ± 0.7
Cholesterol ester	0.5 ± 0.1	0.6 ± 0.1

Values given are means ± SD, n = 5 rats.

TABLE 2. Incorporation and turnover rates from plasma of polyunsaturated fatty acids into brain of adult awake rats and humans

Fatty Acid	Plasma Unesterified Concentration	k^*	J_{in}	Brain Esterified Concentration	Φ_{FA}
	nmol/ml	sec^{-1}	*μmol/g/day*	*μmol/g*	*% per day*
Awake rat					
20:4 n-6 (arachidonic)	9–16.1[a]	4.5×10^{-4}	0.35–0.62	13.0[d]	3–5
22:6 n-3 (docosahexaenoic)	7.7–24.7[a]	5.5×10^{-4}	0.37–1.16	15.3[d]	2–8
18:2 n-6 (linoleic)	94–238[a]	?	?	0.3–0.98[e]	
18:3 n-3 (α-linolenic)	7.6–28.8[a]	?	?	ND[e]	
Awake human					
20:4 n-6 (arachidonic)	4.0[f]	0.81×10^{-4},[g]	0.028	10.5[h]	0.3
22:6 n-3 (docosahexaenoic)	2.2[f]	?	?	10[h]	?
18:2 n-6 (linoleic)	61.2[f]	?	?		
18:3 n-3 (α-linolenic)	9.2[f]	?	?	?	

Daily rate of incorporation J_{in} of unesterified PUFA from plasma into brain equals product of plasma unesterified concentration and incorporation coefficient k^*. k^* for linoleic and α-linolenic acids not known. Dividing J_{in} by the esterified brain concentration c_{br} gives turnover ΦFA. ND, not detectable in adult rat brain, concentration is 0.02 μmol/g in 10-day-old rat. ?, not available.

[a] From refs. (33, 34).
[b] From refs. (77–80).
[c] From refs. (75, 79, 80).
[d] From ref. (33).
[e] From refs. (46, 81).
[f] From ref. (61).
[g] From ref. (50).
[h] From ref. (13).

the incorporation rate of AA equals 0.028 μmol/g/day for the human brain. Brain incorporation coefficients k^* of linoleic acid and α-linolenic acid have not been quantified in rats or humans, and are not shown or used for additional calculations in Table 2

Dividing J_{in} by the esterified brain PUFA concentration provided a lower bound for turnover Φ_{FA} of AA and DHA within brain phospholipids, reflecting replacement from plasma (equation 6). Φ_{FA} in the rat brain was 3–5% per day and 2–8% per day for AA and DHA, respectively (Table 2). Corresponding half-lives were 4–14 days (equation 7). In contrast, in human brain, Φ_{FA} for AA was 0.3% per day, one-tenth the rate in rat brain.

DISCUSSION

About 3–5% of AA and 2–8% of DHA within brain phospholipids is replaced daily in the awake adult rat by the respective unesterified PUFA in plasma. A replacement rate of 0.3% per day is estimated for AA in the human brain. Actual replacement rates may be higher, to the extent that the circulating precursors linoleic and α-linolenic acids contribute to brain AA and DHA, respectively. In the future, the method and model outlined in this article could be used to quantify the contributions of the precursors as well.

In this regard, it is evident that smaller amounts of the precursors than of their elongated AA and DHA products are esterified within brain lipids (Table 2). Thus, precursors that enter and remain in brain would, for the most part, be elongated and desaturated to AA or DHA, probably within astroglia and blood vessels (53–55). Feeding ^{13}C-labeled linoleic or α-linolenic acids to 3-month-old rats indicates substantial β-oxidation by the liver at 24–48 h, at which time sterols and saturated and monounsaturated FAs are heavily labeled (35–37). However, what happens in brain soon after the intravenous infusion of the precursors is unknown.

Comparable octanol/water partition coefficients and molecular weights of the PUFA precursors and of their elongated AA and DHA products suggest that the unesterified precursors in plasma can diffuse into brain as fast as the products (22, 43, 56). Indeed, they are taken up by the immature rat brain to the same extent as are the elongated products (57) and are converted at high rates into their PUFA products in isolated microsomes from the immature brain (58). If they also are converted to AA or DHA within adult mammalian brain to a significant extent, net rates of incorporation of unesterified n-3 and n-6 PUFA classes from the brain acyl-CoA pool into phospholipids, as well as turnover rates, will exceed the values calculated for AA and DHA alone in Table 2 (equations 5 and 6).

In this regard, it is not known whether the adult mammalian brain has sufficient enzymatic capacity, particularly of Δ^6-desaturase, to convert the shorter chain PUFA precursors to AA and DHA at acceptable rates, or to what extent the liver must make this conversion for the brain (6, 53). It has been suggested that the liver can convert sufficient α-linolenic acid to DHA to maintain an adequate plasma DHA level in breast-fed human infants (59). However, liver conversion may be unnecessary if the infant brain itself can perform significant conversion.

It is not immediately apparent why AA turnover due to plasma-brain exchange is less in the human brain than in the rat brain. The difference is not solely related to a species

difference in net brain energy metabolism, as adenosine 5′-triphosphate (ATP) consumption is only about 2.5 times higher in the rat brain than in human brain (45). Perhaps, phospholipase A_2 signaling in rat brain consumes a greater proportion of net ATP consumed than in the human brain owing to differences in neurotransmitter patterns (34, 60).

The linear dependence of J_{in} on the plasma unesterified PUFA concentration (equation 3) suggests that diet, by modifying the plasma unesterified PUFA concentration (61), can indirectly influence brain PUFA content and turnover. This is relevant because brain PUFA concentrations are abnormal in a number of human disorders (see Introduction), and because dietary supplementation has been suggested to be effective in some of them (16, 62). Additionally, lowering the plasma n-3 PUFA concentration by nutritional deprivation can alter their brain and retina concentrations and turnovers, as well as the functions of these organs (1, 46, 63, 64).

PUFA incorporation from plasma into brain phospholipids is not limited by diffusion at the blood-brain barrier, but is regulated by the ATP-dependent conversion of the unesterified PUFA within brain to acyl-CoA by an acyl-CoA synthetase (Fig. 1) (22, 23, 31). One reported acyl-CoA synthetase is selective for AA and eicosapentaenoic acid; another is less discriminating (65, 66). The former has a K_m for AA of 30 μM, whereas K_ms of the latter are 13, 29, and 15 μM for palmitate, oleate, and AA, respectively. For both enzymes, K_m equals or exceeds the respective brain unesterified PUFA concentration, which in the awake rat is about 8 nmol/g for AA and 2 nmol/g for DHA (44, 46, 67). This relation, and because unesterified PUFAs in plasma are largely bound to albumin and contribute to only 2–4% of the brain unesterified PUFA pool (31, 32), explain why incorporation J_{in} is proportional to the unesterified plasma FA concentration over a wide concentration range (42).

Published results (30, 68) indicate that 89.4% and 87.1% of nonvolatile net brain radioactivity 15 min following the intravenously injection of [1-^{14}C]AA and [1-^{14}C]DHA, respectively, is within brain lipids, with 72.9% and 64.7% in phospholipids. The lesser percentage of lipid labeling by the [^{14}C] compared with the [^{3}H] PUFAs (Table 1) arises because β-oxidation of the latter releases 3 of 4 [^{3}H] atoms as volatile [^{3}H]H_20, whereas β-oxidation of the former produces mostly nonvolatile [^{14}C]glutamate and [^{14}C]aspartate (68–70). Phospholipid labeling is specific not only with regard to the PUFA and type of phospholipid (Table 1), but also to the *sn* position. In the awake rat, all ^{14}C-labeled AA is found in the *sn*-2 position of phosphatidylinositol, 92% in the *sn*-2 position of phosphatidylcholine, with the remainder in the *sn*-1 position. All ^{14}C-labeled DHA within phosphatidylethanolamine is at the *sn*-2 position, whereas 77% and 23% DHA is at the *sn*-2 and *sn*-1 position, respectively, of phosphatidylcholine (30).

Our estimated AA and DHA turnover rates in adult rat brain owing to brain-plasma exchange (Table 2) correspond to half-lives of 4–14 days (equation 7). Such long half-lives explain slow rates of recovery of brain DHA content noted in n-3 nutritional deprivation studies and long half-lives calculated following intracerebral injection of a tracer. Thus, after rats subjected to an n-3-deficient diet for several generations are placed on a DHA-adequate diet, 8 weeks are required for 90% recovery of normal brain DHA, equivalent to a recovery half-life of 12–14 days. In contrast, serum and liver DHA levels in these rats become normal within 2 weeks on the adequate diet (71). Comparable nutritional deprivation-replacement studies in the macaque give DHA recovery half-lives of 17–21 days in different brain phospholipids (72). Finally, half-lives calculated by intratissue injection of tracer, then following tracer disappearance from a phospholipid, are 11–14 days in mouse brain for AA (73) and 19 days in rat retina for DHA (63).

In contrast to these long plasma-brain exchange half-lives, half-lives of de-esterification and re-esterification of AA and DHA within brain phospholipids (large arrows in Fig. 1) can be much shorter (minutes to hours), and turnover rates can be correspondingly much faster (23, 33, 74). Intracerebral recycling due to these processes is largely related to phospholipase A_2-mediated release of AA and DHA from phospholipids, induced by receptor-mediated signal transduction involving GTP proteins (7, 11, 75, 76). The 50-fold or more differences in half-lives and turnover rates due to brain-plasma exchange on the one hand, and intracerebral de-esterification and re-esterification on the other (equation 9), reflect the fact that the dilution factor λ of the acyl-CoA pool that relates these two processes approximates 0.02–0.04 in the awake rat.

In summary, a modified model and operational equations describing brain FA metabolism provide estimates of incorporation rates of unesterified n-3 and n-6 PUFAs from plasma into brain phospholipids, as well as turnover rates and half-lives for each of two cycles: plasma-brain exchange and de-esterification and re-esterification. Rates of incorporation and turnover from plasma may be higher than calculated rates in Table 2, to the extent that linoleic and α-linolenic acid enter brain from plasma and are elongated and desaturated to AA and DHA, respectively. These latter rates need to be measured in the future if we wish to better understand the complete relation between brain PUFAs and diet. Nevertheless, by identifying the kinetic relations between cycles of plasma-brain exchange and of intracerebral de-esterification and re-esterification (Fig. 1), we now have a reasonable explanation for the differences in turnover rates and half-lives reported following intravenous injection of a labeled PUFA on the one hand, and nutritional manipulation or intracerebral injection on the other.

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