Arachidonic Acid and the Brain

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Abstract

Kinetic methods in unanesthetized rodents have shown that turnover rates of arachidonic acid (AA) and docosahexaenoic acid (DHA) in brain membrane phospholipids are rapid and energy consuming and that phospholipase A$_2$ (PLA$_2$) and acyl-CoA synthetase enzymes that regulate turnover are specific for one or the other PUFA. Thus, AA turnover in brain phospholipids was reduced, and AA-selective cytosolic cPLA$_2$ or acyl-CoA synthetase, as well as cyclooxygenase (COX)-2, were downregulated in brains of rats given drugs effective against bipolar disorder, whereas DHA turnover and expression of DHA-selective calcium-independent iPLA$_2$ were unchanged. Additionally, the brain AA and DHA cascades can be altered reciprocally by dietary or genetic conditions. Thus, following 15 wk of dietary (n-3) PUFA deprivation, DHA loss from rat brain was slowed because of reduced iPLA$_2$ and COX-1 expression, whereas AA-selective cPLA$_2$, sPLA$_2$, and COX-2 were upregulated, as were AA and docosapentaenoic acid concentrations. Measured rates of AA and DHA incorporation into brain represent their respective rates of metabolic consumption, because these PUFA are not synthesized de novo or converted significantly from their precursors in brain. In healthy human volunteers, positron emission tomography (PET) was used to show that the brain consumes AA and DHA at respective rates of 17.8 and 4.6 mg/d, whereas in patients with Alzheimer disease, AA consumption is elevated. In the future, PET could be used to relate human brain rates of AA and DHA consumption to liver PUFA metabolism and dietary PUFA intake. J. Nutr. 138: 2515–2520, 2008.

Introduction

The conditionally essential PUFA arachidonic acid [AA, 20:4(n-6)] and docosahexaenoic acid [DHA, 22:6(n-3)] make up ~20% of fatty acids in the mammalian brain (1). They, as well as their respective shorter-chain precursors, linoleic acid [LA, 18:2(n-6)] and α-linolenic acid [α-LNA, 18:3(n-3)], cannot be synthesized de novo from 2-carbon fragments by vertebrate tissue, and biochemical pathways do not exist for their interconversion. Thus, they must be obtained from dietary sources. However, if sufficient LA or α-LNA is in a diet free of AA or DHA, respectively, normal brain DHA and AA concentrations can be maintained by elongation and desaturation of the precursors within the liver (2).

Multiple aspects of brain metabolism, function, and structure are thought to depend on having adequate brain concentrations of AA and DHA as well as on interactions among these PUFA and their metabolites (3). Furthermore, a number of human brain diseases, such as Alzheimer disease (4) and bipolar disorder (5), appear to involve disturbed PUFA metabolism. Thus, understanding the dynamics of brain PUFA metabolism could help to interpret brain function in health and disease.

Methods are available to quantitatively assess the kinetics and enzymatic regulation of brain PUFA metabolism in unanesthetized rodents and to image regional brain PUFA consumption in rodents and humans. This article briefly summarizes some of these methods and their applications, with a focus on AA metabolism.

Kinetic fatty acid methods

To measure brain PUFA kinetics and the enzymatic regulation of PUFA metabolism in rodents, a loosely restrained unanesthetized animal is injected intravenously with a radiolabeled PUFA bound to serum albumin. Labeled and unlabeled unesterified PUFA concentrations are measured in plasma at fixed times thereafter until the animal is killed. Its brain is removed after being subjected to high energy microwaving to instantaneously stop metabolism, or is removed and frozen. In the former case, lipids are extracted, and concentrations of labeled and unlabeled PUFA are measured by analytical procedures in brain unesterified fatty acid, acyl-CoA, and phospholipid pools. In the latter case, the frozen brain is cut into serial coronal slices on a freezing microtome, the slices are placed against X-ray film, and regional brain radioactivities are determined by quantitative autoradiography. The frozen brain also can be subjected to molecular or enzyme activity analysis. Another method involves injecting a radiolabeled PUFA intracerebrally, then measuring its specific activity in individual phospholipids of microwaved brain at...
different times thereafter, to determine loss half-lives and turnover rates of the unlabeled PUFA.

PUFA incorporation into stable brain lipids is unaffected by changes in cerebral blood flow, allowing us to image PUFA metabolism and signaling at rest and during functional activation in vivo, without correcting for concurrent variations in delivery by flow. Brain incorporation of AA and DHA also can be quantitatively imaged in human subjects using positron emission tomography (PET) and intravenously injected positron-emitting [1-11C]AA or [1-11C]DHA (6).

Compartmental representation of brain AA cascade

Pathways of plasma-brain exchange of AA and of its intravenously injected radiolabel (designated as AA*), as well as pathways and compartments of brain AA metabolism in what is termed the “arachidonic acid cascade” (7,8), are illustrated in Figure 1. DHA participates in a comparable cascade.

Circulating radiolabeled AA* will rapidly exchange with unesterified AA in the brain endoplasmic reticulum. Measuring the concentrations of AA and AA* in brain acyl-CoA and stable lipids in relation to integrated plasma specific activity can be used to calculate unesterified AA incorporation coefficients $k^*$,

$$k^* = \frac{c_{\text{brain}}(T)}{\int_0^T c_{\text{plasma}} dt}$$  \hspace{1cm} (1)

$c_{\text{brain}}(T)$ is incorporated brain radioactivity at time of death $T$ after tracer injection, and the denominator is the integral of plasma radioactivity $c_{\text{plasma}}$ (plasma input function) between the start of injection at time $t = 0$ and $T = T$.

The rate of incorporation of the plasma unesterified unlabeled PUFA into brain, $J_{\text{in}}$, equals the product of its unesterified, unlabeled plasma concentration $c_{\text{plasma}}$ and the incorporation coefficient $k^*$,

$$J_{\text{in}} = k^* c_{\text{plasma}}$$  \hspace{1cm} (2)

The turnover rate of the fatty acid in phospholipids is $J_{\text{in}}$ divided by the fatty acid concentration $c_{\text{brain}}$ in stable brain lipid, multiplied by an arachidonoyl-CoA dilution factor $k$,

$$\text{Turnover} = J_{\text{in}} / c_{\text{brain}}$$  \hspace{1cm} (3)

Because AA cannot be synthesized de novo or be converted in the brain from its shorter-chain LA precursor (9,10), $J_{\text{in}}$ equals the quantity of AA that has been lost by metabolism, that is, the rate of AA consumption within brain (11). A similar consideration applies to DHA.

The brain AA cascade involves 2 major cycles (Fig. 1). One cycle is a continuous process of AA deaclyation followed by reacylation in individual phospholipids, with short half-lives (hours) and high turnover rates (e.g., 15–30%/h) in rodent brain (12,13). The other is a cycle involving metabolic AA loss within brain, compensated for by AA replenishment from plasma, with half-lives of weeks (11,14).

In the first cycle, AA is released from the stereospecifically numbered (sn)-2 position of a synaptic membrane phospholipid by a phospholipase $A_2$ (PLA$_2$), whose activation is coupled to activation of any of a number of postsynaptic neuroreceptors by a G-protein or by calcium (15). A small fraction of the released AA (normally ~4%) is lost by metabolism to eicosanoids or other metabolites or by $\beta$-oxidation after being transferred to mitochondria from the acyl-CoA pool. This transfer requires carnitine palmitoyltransferase, whose affinity for arachidonoyl-CoA and other long-chain acyl-CoAs, compared with the affinity of acyltransferase, determines whether the fatty acid will be largely $\beta$-oxidized in mitochondria or esterified into lysophospholipid (16). AA and DHA are largely reacylated in brain, whereas LA, $\alpha$-LNA, and palmitic acid (16:0) are largely $\beta$-oxidized.

The larger fraction (~96%) of released AA in brain will be reincorporated into phospholipid after diffusing to the unesterified AA pool in the endoplasmic reticulum with the help of a fatty acid binding protein, then being converted to arachidonoyl-CoA by an acyl-CoA synthetase with consumption of 2 ATP (8). For AA to be a substrate for cyclooxygenase (COX)-2, it first must be reincorporated in phospholipid and then released by PLA$_2$ (17,18).

Enzyme selectivity allows independent brain AA and DHA cascades

Three major PLA$_2$ enzymes have been described in mammalian brain: 1) an AA-selective cytosolic cPLA$_2$ (85 kDa, Type IV),
while downregulating iPLA2 and COX-1 expression (25). Increased rat brain expression of cPLA2, sPLA2, and COX-2 (17, 22–24). Additionally, reduced dietary (n-3) PUFA for 15 wk and DHA-selective iPLA2 were unchanged in these conditions neuroinflammation and excitotoxicity, while DHA recycling in rats given mood stabilizers and upregulated in rat models of (19, 20). cPLA2 colocalizes with and is coupled to COX-2 at PLA2 (iPLA2) (101 kDa, Type VI) that is selective for DHA (21).

Thus, AA recycling and AA-selective cPLA2 were downregulated which requires <1 μmol/L Ca2+ for translocation to the membrane plus phosphorylation for activation; 2) an AA-selective secretory PLA2 (sPLA2) (14 kDa, Type II A), which is also Ca2+ (20 mmol/L) dependent; and 3) a Ca2+-independent PLA2 (iPLA2) (101 kDa, Type VI) that is selective for DHA (19, 20). cPLA2 colocalizes with and is coupled to COX-2 at postsynaptic sites, whereas iPLA2 is found at these sites and in astrocytes. Acyl-CoA synthetases also can be selective for AA or DHA (21).

As discussed in the following sections, the presence of AA- and DHA-selective enzymes within brain allows these PUFAs to recycle within phospholipids independently of each other and to be modified separately by drugs, genetics, disease, or diet (3). Thus, AA recycling and AA-selective cPLA2 were downregulated in rats given mood stabilizers and upregulated in rat models of neuroinflammation and excitotoxicity, while DHA recycling and DHA-selective iPLA2 were unchanged in these conditions (17, 22–24). Additionally, reduced dietary (n-3) PUFA for 15 wk increased rat brain expression of cPLA2, sPLA2, and COX-2 while downregulating iPLA2 and COX-1 expression (25).

### Table 2: Effective antipsychotic agents downregulate parts of rat brain AA cascade

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lithium</th>
<th>Carbamazepine</th>
<th>Valproate</th>
<th>Lamotrigine</th>
<th>Topiramate</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA turnover</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>DHA turnover</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>—</td>
<td>NC</td>
</tr>
<tr>
<td>mRNA, protein, activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cPLA2</td>
<td>↓5</td>
<td>↓5</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
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<td>sPLA2</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
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<td>NC</td>
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<tr>
<td>iPLA2</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>AA-selective acyl-CoA synthetase activity</td>
<td>NC</td>
<td>NC</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>COX-2 protein and/or activity</td>
<td>↓</td>
<td>↓</td>
<td>↓6</td>
<td>↑7</td>
<td>↓</td>
</tr>
<tr>
<td>Prostaglandin E2</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>NC</td>
<td>NC</td>
</tr>
</tbody>
</table>

1 Data adapted from Rao et al. (24) and Rapoport et al. (26). NC, no significant change; —, not tested.

2 Preferred for bipolar mania.

3 Preferred for bipolar depression.

4 Ineffective in Phase 3 trials.

5 Accompanied by reduced AP-2 expression.

6 COX-2 and COX-1 mRNA, NF-κB, and thromboxane B2 also decreased.

7 COX-2 mRNA also reduced.

Selected targeting of the AA cascade by antimanic mood stabilizers

Bipolar disorder is a neuropsychiatric disease that consists of repeated cycles of manic and depressive episodes (bipolar I) or of hypomanic and depressive episodes (bipolar II). It affects 1–2% of the U.S. population, appears initially in young adults, and has a 10–20% lifetime incidence of suicide. Agents called “mood stabilizers” are used to treat the disease. Of these, lithium, carbamazepine (5-carbamoyl-SH-dibenzo[b,f]azepine), and valproic acid (2-propylpentanoic acid) are FDA approved for treating bipolar mania, whereas lamotrigine [6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine] is approved for bipolar depression and rapid cycling. The mechanisms of action of these agents are not agreed on, but experiments in unanesthetized rodents suggest that they commonly target the brain AA cascade (12, 13, 24, 26).

Thus, lithium, valproic acid, or carbamazepine, when administered chronically to rats to produce plasma levels therapeutically relevant to bipolar disorder, reduced turnover of AA but not of DHA or palmitic acid in brain phospholipids (Tables 1 and 2). The reduced AA turnover produced by lithium and carbamazepine corresponded to reduced transcription of AA-selective cPLA2 and reduced expression of its transcription factor, activator protein-2. iPLA2 and sPLA2 were unaffected. On the other hand, the reduced AA turnover caused by valproic acid corresponded to its inhibiting AA conversion to arachidonoyl-CoA by an AA-selective microsomal acyl-CoA synthetase. Each of the 3 agents also reduced rat brain COX-2 activity and the concentration of prostaglandin E2 produced from AA via COX-2 (12, 13, 24, 26). When reduced COX-2 transcription was the cause of reduced activity, it was related to reduced expression of the COX-2 transcription factor, NF-κB (Table 2). Topiramate (2,3-4,5-di-O-isopropylidene-α-D-fructopyranose sulfamate), an anticonvulsant suggested by initial Phase II trials to work in bipolar disorder but later proven ineffective in phase III trials (27), had no effect on any of the above markers, whereas lamotrigine reduced transcription and expression of COX-2 without changing either AA or DHA turnover.

Downregulating the brain AA cascade may be one way in which the antimanic mood stabilizers work in bipolar disorder, in view of evidence that AA cascade enzymes—cPLA2, sPLA2,
and COX-2—are overexpressed in the postmortem bipolar disorder brain (5).

Other examples of alterations of the AA but not DHA cascade are experimental neuroinflammation and excitotoxicity, where AA turnover and cascade enzymes cPLA2 and COX-2 are upregulated, whereas DHA turnover and iPLA2 expression are unchanged (17,22,23,25).

Upregulation of brain AA cascade by dietary (n-3) PUFA deprivation

The rat brain AA cascade is upregulated and the DHA cascade downregulated by reducing the dietary content of (n-3) PUFA, indicating reciprocal regulation of the AA and DHA cascades. Thus, feeding rats an (n-3) PUFA-deficient or (n-3) PUFA-adequate diet (containing no DHA and 0.2% or 4.6% α-LNA, respectively) for 15 wk postweaning reduced the brain concentration of esterified DHA by 30% while reciprocally increasing concentrations of esterified AA and the AA elongation product docosapentaenoic acid [DPA, 22:5(n-6)]. Phospholipid and neutral lipid concentrations were unchanged (2).

The rats fed the deficient compared with the adequate diet demonstrated increased scores on tests of aggression and depression, which are like symptoms found in bipolar disorder (28).

The half-life of DHA loss from brain was prolonged in rats on the (n-3) PUFA-deficient diet (14) (Fig. 2). This prolongation reflected a transcriptional downregulation of the DHA-selective iPLA2 and of COX-1, whereas the AA-selective cPLA2, as well as sPLA2 and COX-2, were reciprocally upregulated (Fig. 3) (25). The latter changes were accompanied by elevated brain AA and DPA concentrations.

Reciprocal changes in the brain AA and DHA cascade also occur in mice in which the gene for α-synuclein, which can be mutated in families with autosomal dominant Parkinson disease, is deleted. These mice have increased DHA turnover but reduced AA turnover in their brain phospholipids. They also have a marked reduction in brain arachidonoyl-CoA concentration and in microsomal acyl-CoA synthetase activity toward AA (29).

Quantifying regional PUFA consumption by the human brain

The regional rate of brain PUFA consumption equals $J_{\text{in}}$, the product of the incorporation coefficient $k^*$ and the plasma concentration of unesterified PUFA (Eq. 2), because AA or DHA cannot be synthesized de novo to replace the PUFA that is lost by metabolism. Because of this, PET has been used to quantify $J_{\text{in}}$ in the human brain for both PUFA.

Horizontal brain sections showing AA and DHA incorporation coefficients $k^*$ (Eq. 1) in healthy human volunteers are presented in Figure 4. These scans were obtained by injecting [1-11C]AA or [1-11C]DHA intravenously and then measuring brain radioactivity with PET (30, J. C. Umhau, W. Zhou, R. E. Carson, S. I. Rapoport, A. Polozova, J. Demar, N. Hussein, A. K. Bhattacharjee, K. Ma, G. Esposito, S. Majchrzak, P. Herscovich, W. C. Eckelman, K. A. Kurdzil, and N. Salem, Jr, unpublished material). When $k^*$ was summed over the entire brain and multiplied by the respective unesterified plasma concentration, $J_{\text{in}}$ for the whole brain equaled 17.8 mg/d for AA and 4.6 mg/d for DHA. The calculated rate of DHA consumption is 2.5–5% of the average daily dietary intake of EPA + DHA in the United States, 100–200 mg/d (31).

Alzheimer disease is associated with neuroinflammation and excitotoxicity, and the postmortem Alzheimer brain demonstrates elevated expression of cPLA2, sPLA2, and COX-2 (32).
Consistent with these findings and evidence that both excitotoxicity and neuroinflammation upregulated the rat brain AA cascade, PET demonstrated increased brain AA incorporation in patients with Alzheimer disease compared with healthy age-matched controls, particularly in neocortical regions reported to have activated microglia and high levels of inflammatory cytokines (4).

**Discussion**

During neurotransmission, the brain AA cascade is initiated when AA is released from synaptic membrane phospholipid by neuroreceptor-initiated activation of cPLA2 (15). In conditions such as neuroinflammation and excitotoxicity, additional AA is released at cytokine and glutamatergic N-methyl-D-aspartate receptors, and AA cascade enzymes are overexpressed. Kinetic methods and models in unanesthetized rodents have been used to show that PUFA turnover in brain membrane phospholipids is rapid and energy consuming. AA and DHA recycling (deesterification-reesterification) in brain are independent processes that are catalyzed, respectively, by AA- and DHA-selective enzymes. The AA cascade can be targeted independently of the DHA cascade or altered in a reciprocal fashion to downregulate AA cascade enzymes and their transcription factors in post-mortem frontal cortex from bipolar disorder patients. Soc Neurosci Abstr. 2007; 7: S4Z.

For example, AA but not DHA turnover in brain membrane phospholipids is downregulated in unanesthetized rats that have been chronically administered lithium, carbamazepine, or valproic acid, mood stabilizers that are used to treat the mania of bipolar disorder. This downregulation correlates with reduced expression of AA-selective cPLA2 or acyl-CoA synthetase and of CYP-2. Such effects are relevant to the therapeutic action of the agents, as the downregulated enzymes are overexpressed in postmortem brain from bipolar disease patients.

In the face of dietary (n-3) PUFA deprivation, homeostatic mechanisms slow brain DHA loss while increasing AA metabolism. Thus, in rats subjected to (n-3) PUFA deprivation for 15 wk, the half-life of DHA in brain phospholipids is prolonged, and the DHA-selective iPLA2 and COX-1 are transcriptionally downregulated. In contrast, expression of AA-selective enzymes cPLA2, sPLA2, and COX-2 is upregulated, as are concentrations of AA and its elongation product, DPA. These changes may exacerbate neuroinflammation and excitotoxicity.

PET has been used with positron-emitting tracers, [1-11C]AA or [1-11C]DHA, to quantitatively image regional rates of AA and DHA consumption by the normal human brain. AA consumption is increased in patients with Alzheimer disease compared with aged-matched healthy controls, likely because of neuroinflammation and excitotoxicity. In the future, PET could be used to determine brain rates of AA and DHA consumption in relation to human aging, brain or liver disease, or dietary PUFA intake.

Other articles in this symposium include references (33) and (34).

**Literature Cited**

14. DeMar JC Jr, Ma K, Bell JM, Rapoport SI. Half-lives of docosahexaenoic acid in rat brain phospholipids are prolonged by 15 weeks of...


