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Rat brain docosahexaenoic acid metabolism is not altered by a 6 day intracerebral ventricular infusion of bacterial lipopolysaccharide

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Abstract

In a rat model of neuroinflammation, produced by a 6-day intracerebral ventricular infusion of bacterial lipopolysaccharide (LPS), we reported that the brain concentrations of non-esterified brain arachidonic acid (AA, 20:4 *n*-6) and its eicosanoid products PGE_2 and PGD_2 were increased, as were AA turnover rates in certain brain phospholipids and the activity of AA-selective cytosolic phospholipase A_2 ($cPLA_2$). The activity of Ca^{2+}-independent $iPLA_2$, which is thought to be selective for the release of docosahexaenoic acid (DHA, 22:6 *n*-3) from membrane phospholipid, was unchanged. In the present study, we measured parameters of brain DHA metabolism in comparable artificial cerebrospinal fluid (control) and LPS-infused rats. In contrast to the reported changes in markers of AA metabolism, the brain non-esterified DHA concentration and DHA turnover rates in individual phospholipids were not significantly altered by LPS infusion. The formation rates of AA-CoA and DHA-CoA in a microsomal brain fraction were also unaltered by the LPS infusion. These observations indicate that LPS-treatment upregulates markers of brain AA but not DHA metabolism. All of which are consistent with other evidence that suggest different sets of enzymes regulate AA and DHA recycling within brain phospholipids and that only selective increases in brain AA metabolism occur following a 6 day LPS infusion.

Keywords

docosahexaenoic acid; brain; lipopolysaccharide; neuroinflammation; metabolism

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Introduction

The brain is enriched in polyunsaturated fatty acids (PUFA) that fall into two categories based on a double bond at the *n*-6 or *n*-3 position. The most abundant brain PUFA are arachidonic (AA, 20:4 *n*-6) and docosahexaenoic (DHA, 22:6 *n*-3) acids, which are mainly esterified at the stereospecific numbered (*sn*)-2 position of the phospholipid moiety (Ansell 1973). Both AA and DHA, including their oxygenated derivatives, can modulate brain function, alter signal transduction, and disrupt other brain processes (Sergeeva *et al.* 2002, Crawford *et al.* 2003, Barcelo-Coblijn *et al.* 2003, Arvindakshan *et al.* 2003). The eicosanoids (AA derivatives) and docosanoids (DHA derivatives) may have antagonistic effects during inflammation. Eicosanoids, cyclooxygenase and lipoxygenase products, are considered pro-inflammatory (Shimizu & Wolfe 1990, Flower *et al.* 1972) while docosanoids, lipoxygenase products, are considered anti-inflammatory (Marcheselli *et al.* 2003, Hong *et al.* 2003, Serhan *et al.* 2002). Therefore, it is important to understand the contribution that different PUFA and their products have in the progression of neurodegeneration associated with inflammation.

Neuroinflammation can be produced experimentally in rat brain by infusing bacterial lipopolysaccharide (LPS) into the fourth cerebral ventricle (Hauss-Wegrzyniak et al. 1998a). This causes microglial activation, cytokine accumulation, and death of cholinergic neurons that result eventually in brain atrophy (Willard *et al.* 1999, Wenk *et al.* 2000, Rosi *et al.* 2004). In an earlier study, we reported that 6 days of LPS infusion at a rate of 1 ng/h increased brain activities of the AA-selective Ca^{2+}-dependent cytosolic phospholipase A_2 ($cPLA_2$) and secretory PLA_2 ($sPLA_2$) by 71% and 47%, respectively, without changing the activity of Ca^{2+}-independent PLA_2 ($iPLA_2$) (Rosenberger et al. 2004). The $iPLA_2$ isoform has been suggested to be selective for DHA release from membrane phospholipid (Strokin et al. 2004). A subsequent study from our laboratory using a more selective assay for $sPLA_2$ activity, found that the activity of $cPLA_2$ but not $sPLA_2$ or $iPLA_2$ was increased significantly by the infusion (Basselin et al. 2007). Brain concentrations of non-esterified AA, PGE_2, and PGD_2, also were elevated, as were turnover rates of AA in ethanolamine and choline glycerophospholipids (Rosenberger et al. 2004), indicating an increase in brain AA metabolism. Consistent with these observations (Rapoport 2001), a 6-day infusion of LPS increased the regional brain incorporation coefficients (k^*) of AA from plasma into brain by 31–71%, as imaged by quantitative autoradiography (Lee *et al.* 2004, Basselin et al. 2007).

In the present study, we examined the effects of a 6 day LPS infusion on brain DHA metabolism. We proposed that the infusion would not change markers of brain DHA metabolism in view of the data showing that the $iPLA_2$ enzyme activity, thought to be selective for DHA, was not altered in the LPS-treated brain and that cytokine formation has been reported to be coupled to $cPLA_2$ and $sPLA_2$ activation (Strokin *et al.* 2003, Strokin et al. 2004, Dinarello 2002, Luschen *et al.* 2000). If this is the case, a pathological consequence of neuroinflammation induced by a 6 day LPS infusion may be considered a result of an imbalance between brain AA and DHA metabolism. Therefore, it is not unreasonable that the positive effect of LPS infusion on brain AA, but not DHA metabolism, reflects selective activation of AA-selective $cPLA_2$-mediated signaling.

Materials and Methods

Chemicals

Radiolabeled [1-^{14}C]arachidonic ([1-^{14}C]AA) and docosahexaenoic ([1-^{14}C]DHA) acids (52–60 mCi/mmol) were purchased from Moravek Biochemicals (Brea, CA). The specific radioactivity of the tracers was confirmed by scintillation counting and HPLC analysis. Phospholipid and neutral lipid standards and non-esterified AA and DHA were purchased from Nu-Chek-Prep (Elysian, MN). Bovine serum albumin “fatty acid free”, LPS (*E. coli*, serotype

055:B5, TCA extraction), reagent grade palmitic acid, potassium phosphate, ATP, and other chemical reagents were from Sigma Chemicals (St. Louis, MO). HPLC grade *n*-hexane, 2-propanol, and *n*-heptane were from EM Science (Gibbstown, NJ). Reagent-grade chloroform, methanol, and other chemicals were from Mallinckrodt (Paris, KY). A scintillation cocktail (Ready-Safe, Beckman, Fullerton, CA) containing 1.0 % glacial acetic acid was used to determine radioactivity. Sulfuric acid was from Aldrich Chemicals (Milwaukee, WI).

Rationale, cannula placement, and animal surgery

The rationale for choosing the 6 day infusion period is based on a pilot study performed measuring AA incorporation at 2, 3, 4, 6, 8, and 10 days of LPS infusion (data not shown). In this study we found no increase in total AA incorporation over control values until day 4 (10–15% increase) of infusion. The incorporation of AA reached a maximum at day 6 and remained elevated out to 10 days. Therefore to determine in the metabolism of DHA was altered similar to that found with AA these experiments were repeated using the 6 day infusion period to measure markers of brain DHA as outlined below. Surgery was performed following the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 80-23). Alzet osmotic mini-pumps (Model 2002; 0.5 µl/h, Plastics One, Roanoke VA) were used to continuously deliver either artificial cerebrospinal fluid (aCSF, 140 mM NaCl, 3.0 mM KCl, 2.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.2 mM Na_2PO_4, pH 7.4) or LPS dissolved in aCSF (1.0 µg/ml) to the fourth ventricle of the rat, as described (Hauss-Wegrzyniak et al. 1998b). After 6 days of infusion, a 12 h fasted rat was anesthetized with 3% halothane (Halocarbon, River Edge, NJ) and polyethylene catheters (PE 50, Becton Dickinson, Sparks, MD) filled with sodium heparin (100 IU) were implanted into the right femoral artery and vein. The skin was closed and 1% Lidocaine was applied to the wound. The hindquarters of the rat were wrapped loosely in a fast-setting plaster body cast then the rat was taped to a wooden block. The rat was allowed to recover from anesthesia for 3–4 h, while body temperature was maintained at 36.5°C using a feedback-heating device (Yellow Springs Laboratories, Yellow Springs, OH) equipped with a rectal thermometer.

Intravenous infusion of [1-^{14}C]DHA

The method, basis of tracer infusion, and a complete description of the kinetic analysis detailing the theory of steady-state analysis have been reported elsewhere (Rapoport 2001, Robinson *et al.* 1992, Washizaki *et al.* 1994, Rapoport 2008). With an infusion pump (Harvard Apparatus, South Natick, MA), an awake rat was infused intravenously for 6 min at a rate of 0.4 ml/min, with 2.0 ml isotonic saline containing 175 µCi/kg body wt [1-^{14}C]DHA suspended in 0.06 mg bovine serum albumin. Arterial blood samples (200 µl) were collected at 0, 30, 60, 120, 180 240, and 360 sec during infusion to determine radioactivity and concentrations of non-esterified fatty acids in plasma. Six min after starting infusion, the rat was killed with sodium pentobarbital (i.v., 100 mg/kg body wt) and immediately subjected to head-focused high-energy microwave irradiation to stop brain metabolism (5.5 kW, 3.4 sec; Cober Electronics, Stamford, CT). Animals used for enzyme assays, which were not infused with tracer, were killed with sodium pentobarbital and their brains were removed then frozen on dry ice. All samples were stored at −80° C until analyzed. To avoid artifacts from the cannula implant (Ghirnikar et al. 1996), analyses were performed only on cortical and basal forebrain regions.

Brain and plasma lipid extraction and chromatography

Total lipids from microwaved brains were extracted using *n*-hexane/2-propanol (3:2, by vol.) in a glass Tenbroeck homogenizer (Radin 1981). Plasma lipids were extracted in chloroform/methanol (2:1, by vol.) and partitioned with 0.9 % KCl. The total plasma lipids in the chloroform extract were washed once with 0.2 volumes of 0.9 % KCl to remove non-lipid contaminates prior to analysis (Folch et al. 1957). Standards and lipid extracts in chloroform

were applied to Whatman silica gel 60A LK6 TLC plates and separated using chloroform/methanol/acetic acid/H_2O (50:37.5:3:2, by vol.) (Jolly et al. 1997). Bands corresponding to ethanolamine and choline glycerophospholipid, phosphatidylinositol, or phosphatidylserine were scraped from the TLC plates. The plasmenylethanolamine and plasmenylcholine fractions were isolated from the ethanolamine and choline glycerophospholipid fractions as described (Murphy *et al.* 1993, Rosenberger et al. 2004). Neutral lipids were separated on silica gel 60 plates using the solvent system of heptane/diethyl ether/acetic acid (60:40:4, by vol.) (Breckenridge & Kuksis 1968). Gas chromatography was used to quantify esterified and non-esterified fatty acid levels. Liquid scintillation counting was used to measure radioactivity. Extracts were stored in n-hexane/2-propanol (3:2, by vol.) under N_2 at −80° C.

Quantification of labeled and unlabeled brain acyl-CoA

Long-chain acyl-CoA species were isolated from microwaved rat brain using oligonucleotide purification cartridges (Applied Biosystems, Foster City, CA) (Deutsch et al. 1994). Acyl-CoA concentrations and DHA-CoA radioactivity were measured using peak area analysis of chromatograms and liquid scintillation counting.

Methylation of esterified and non-esterified acids

Esterified fatty acids in the different phospholipid classes were methylated with 0.5 M methanolic potassium hydroxide at 37° C for 30 min. The reaction was stopped with methyl formate and the fatty acid methyl esters were extracted with *n*-hexane. The non-esterified brain fatty acids were methylated using 2 % sulfuric acid in toluene/methanol (1:1, by vol.) at 65° C for 4 h. The reaction was terminated with H_2O and the fatty acid methyl esters were extracted with petroleum ether (Akesson et al. 1970).

Gas chromatography

Fatty acid methyl esters were quantified with a gas chromatograph (Trace 2000, ThermoFinnigan, Houston, TX) equipped with a flame ionization detector using a capillary column (SP 2330; 30 m × 0.32 mm i.d., Supelco, Bellefonte, PA). Sample runs were initiated at 90° C with a temperature gradient to 230° C over 20 min. Fatty acid methyl ester standards were used to establish relative retention times and response factors. The internal standard, methyl heptadecanoate, and the individual fatty acids were quantified by peak area analysis (ChromQuest, Ver. 4.0, ThermoFinnigan, Houston, TX). The detector response was linear, with correlation coefficients of 0.998 or greater within the sample concentration range for all standards.

Microsomal long-chain acyl-CoA synthetase preparation

Total and individual brain microsomal long-chain fatty acyl-CoA synthetase activity was measured in non-microwaved rat brains as described (Wilson *et al.* 1982, Saunders *et al.* 1996). A frozen whole brain was homogenized in 10 mM HEPES buffer (pH 7.4) containing 0.32 M sucrose, 20 μg/ml phenylmethylsulfonyl fluoride, 1 mM benzamidine, 10 mM 2-mercaptoethanol, and 0.01 % soybean trypsin inhibitor. The homogenate was centrifuged at 1000 × g for 10 min at 4° C to remove cell debris and the microsomal membrane was isolated from the supernatant by centrifugation at 35,000 × g at 4° C for 1 hr. The microsomal pellet was re-suspended in 20 mM potassium phosphate buffer (pH 7.4) containing 1 % IGEPAL CA-630 detergent, 10 mM EDTA, and 10 mM 2-mercaptoethanol by vortexing at 4° C for 2 hr. Debris was removed by a second centrifugation at 35,000 × g at 4° C for 1 hr. A portion of the isolated microsomal fraction was removed to determine total microsomal long chain acyl-CoA synthetase activity. The remainder was partially purified on a 10-ml bed volume of Spectra/Gel HA hydroxyapatite (Spectrum, Los Angeles, CA) equilibrated with 20 mM potassium phosphate buffer, using a step gradient of 20 mM potassium phosphate, 80 mM

potassium phosphate, and 300 mM potassium phosphate (Laposata et al. 1985). All chromatography buffers had a pH of 7.4 and contained 1 % IGEPAL CA-630 detergent and 10 mM 2-mercaptoethanol. The column eluate was collected in 1.75 ml fractions and each was assayed for enzyme activity. The protein content in total microsomal and column fractions, corresponding to the non-specific and AA-specific long-chain acyl-CoA synthetases, was measured by the method of Bradford (Bradford 1976).

Assay of microsomal long chain acyl-CoA synthetase activity

Total fatty acyl-CoA synthetase activity was measured in isolated microsomal fractions as described (Wilson et al. 1982, Saunders et al. 1996). A 50 µl aliquot of microsomal preparation or enzyme blank (re-suspension buffer) was added to 100 µl of an assay cocktail having a final concentration of 100 mM Tris/HCl (pH 8.0), 2 mM Triton X-100, 0.55 mM CoA, 6.6 mM ATP, 2.5 mM $MgCl_2$, and 12 µM of [1-^{14}C]AA or [1-^{14}C]DHA. The reactions were allowed to proceed for 10 min at 37° C, then were terminated by the addition of 2.25 ml of 2-propanol/*n*-heptane/2 M H_2SO_4 (40:10:1, by vol.). Non-esterified substrate was removed from the reaction mixture by extracting with 1.5 ml *n*-heptane and 1 ml water. The aqueous layer was re-extracted twice with 2 ml *n*-heptane containing 4 mg/ml palmitic acid. Radioactivity in a 1-ml portion of the aqueous layer was determined by liquid scintillation counting. Initial rates of reaction normalized to the enzyme blanks were calculated based on the specific radioactivity of the substrate and reported in the units of nmol/(min × g protein).

Calculations

Radioactivity of a brain phospholipid *i* of interest, $c^*_{br,i}$ (*T*) nCi/g, was calculated by correcting its net brain radioactivity for its intravascular radioactivity (Grange et al. 1995). Blood samples taken at the time of death, $T = 6$ min after starting tracer infusion, were extracted and analyzed to make this correction. Unidirectional incorporation coefficients, k^*_i ml/(g × sec), of [1-^{14}C] DHA from plasma into phospholipids *i* were calculated as follows,

$$k^*_i = \frac{c^*_{br,i}(T)}{\int_0^T c^*_{pl} dt} \quad \text{(Eq. 1)}$$

where *t* is time after beginning of infusion, and c^*_{pl} (nCi/ml) is the plasma concentration of radiolabeled DHA during infusion. Rates of incorporation of non-esterified DHA from plasma into brain phospholipid *i*, $J_{in,i}$, and from the brain docosahexaenoyl-CoA pool into brain phospholipid *i*, $J_{FA,i}$, were calculated as follows,

$$J_{in,i} = k^*_i c_{pl} \quad \text{(Eq. 2)}$$

$$J_{FA,i} = \frac{J_{in,i}}{\lambda_{acyl-CoA}} \quad \text{(Eq. 3)}$$

c_{pl} (nmol/ml) is the concentration of unlabeled non-esterified DHA in plasma. $\lambda_{acyl\text{-}CoA}$ represents the steady-state specific activity of docosahexaenoyl-CoA relative to that of plasma during [1-^{14}C]DHA infusion,

$$\lambda_{acyl-CoA} = \frac{c^*_{brain,\ docosahexaenoyl-CoA} / c_{brain,\ docosahexaenoyl-CoA}}{c^*_{pl,DHA} / c_{pl,DHA}} \quad \text{(Eq. 4)}$$

where the numerator is the specific activity of brain docosahexaenoyl-CoA and the denominator is the specific activity of plasma non-esterified DHA. The fractional turnover rate of DHA within phospholipid *i*, $F_{FA,\,i}$ (%/h), is defined as,

$$F_{FA,i} = \frac{J_{FA,i}}{c_{br,i}} \quad \text{(Eq. 5)}$$

Data and statistics

Integrals of plasma radioactivity were determined by trapezoidal integration (SigmaPlot, SPSS Science, Chicago, IL). Unpaired t-tests with a two-tail p value (Instat® Ver. 3.05, GraphPad, San Diego, CA) were used to compare means between LPS-infused and control aCSF-infused rats, where statistical significance was taken as $p \leq 0.05$. Data are presented as means ± SD.

Results

Plasma and brain lipid concentrations

To begin to determine the effect that LPS infusion has on brain DHA metabolism we measured the plasma and brain non-esterified fatty acid, brain acyl-CoA, and esterified brain fatty acid levels in control and LPS treated rats. We found that there were no statistically significant differences in the mean plasma concentration of any non-esterified fatty acid between control LPS-treated rats (Table 1). Additionally, the net mean brain long-chain acyl-CoA concentrations did not differ significantly between groups. As previously reported (Rosenberger et al. 2004), the 6-day LPS infusion significantly increased the concentration of non-esterified brain AA, whereas concentrations of the other non-esterified brain fatty acids, including DHA, were unchanged (Table 1). Further, there was no difference in the concentration of esterified DHA in any phospholipid class between groups (Figure 1). This data suggests that LPS infusion did not change basic parameters of DHA metabolism in that treatment did not result in a net change in brain or plasma DHA. The concentrations of non-esterified and esterified DHA found in these studies are comparable to those previously published (Contreras *et al.* 2001,Rosenberger et al. 2004).

DHA incorporation and turnover rates in individual brain phospholipids

Because a lack of changes in the concentration of DHA do not necessarily reflect changes in brain DHA metabolism we measured the incorporation and turnover rate using steady-state radiotracer kinetic analysis. This is not without precedence because in both the LPS-treated rat and the α-synuclein knockout mouse where brain esterified AA concentrations are not changed there is a profound and significant alteration in the incorporation and turnover rates of AA (Golovko *et al.* 2006). To begin this analysis we found that the mean arterial plasma radioactivity profiles during intravenous infusion of [1-^{14}C]DHA did not differ between control and LPS-treated rats (Figure 2). Further, a steady-state plasma radioactivity was achieved after 120 s following the start of intravenous [1-^{14}C]DHA infusion in both groups and the mean steady-state plasma integrals equal between the two groups being 338,650 ± 29,570 and 303,682 ± 39,624 (nCi x s)/ml, respectively. Because the dilution factor $\lambda_{acyl\text{-}CoA}$ (Eq. 4) for docosahexaenoyl-CoA in the two groups did not differ significantly and

equaled 0.027 ± 0.005 and 0.031 ± 0.006, respectively suggests that LPS-treatment did not alter the delivery or activation of DHA into the brain.

To expand on this analysis we calculated the rates of incorporation and turnover of DHA in control and LPS-treated rats by applying incorporation kinetic analysis. Table 2 shows that LPS infusion did not significantly alter the calculated incorporation coefficients k_i^* (Eq. 1) of DHA from plasma into any brain stable phospholipid *i*. This is in contrast to our report that the total incorporation coefficient (*k**) of AA was increased by 40% following a 6-day LPS infusion (Rosenberger et al. 2004). Because the derived parameters for each phospholipid were calculated by multiplying *k** by common constant factors with no significant changes in the cold concentrations of esterified DHA (Eqs. 1-6), the same pattern was found for the rates of incorporation of unlabeled DHA from the precursor DHA-CoA pool into phospholipids, given as $J_{FA,i}$ (Eq. 3), and for the fractional turnover $F_{FA,i}$ of DHA in the phospholipids (Eq. 5). Therefore, despite an increase in the phospholipase activity (Rosenberger et al. 2004;Basselin et al. 2007) and the subsequent increase in the turnover rates of brain AA (Rosenberger et al. 2004) found in this model the kinetic of DHA metabolism remain unaltered.

Microsomal long-chain acyl-CoA synthetase activity

To further examine if the increased metabolism of AA and not DHA in this model were due solely to increases in the activities of AA-selective PLA_2 we measured the total acyl-CoA synthetase activity using labeled AA and DHA. Total long chain acyl-CoA synthetase activities from isolated brain microsomes were generated by assaying for acyl-CoA synthetase in the presence of 12 μM [1-^{14}C]AA or [1-^{14}C]DHA, as described in the Methods Section. The rates of conversion of AA or DHA to their respective CoA derivatives did not differ significantly between LPS-infused and control samples (Figure 3). The averaged microsomal long chain acyl-CoA synthetase activities toward AA and DHA were 1.5 ± 0.6 and 0.6 ± 0.2 nmol/(min × g protein), respectively, and were similar to previously reported values (Saunders et al. 1996). The acyl-CoA synthetase activity from fractions corresponding to the non-specific and AA-specific microsomal long-chain acyl-CoA synthetases (Wilson et al. 1982,Saunders et al. 1996) also did not differ between control and LPS-treated rat brain (data not shown), suggesting that LPS infusion did not alter the activity or expression of microsomal long-chain acyl-CoA synthetases. These data suggests that the LPS-induced increases in brain AA but not DHA metabolism are due primarily to increases in the activity of AA-selective PLA_2, which confirms our previous studies.

Discussion

Despite marked increases in regional incorporation (Lee et al. 2004, Basselin et al. 2007) and fractional turnover rates (Rosenberger et al. 2004) of brain AA after 6 days of LPS infusion, no significant difference was found with regard to the brain metabolism of DHA. Further, the microsomal conversion rates of both AA and DHA to their respective acyl-CoA derivatives also were not changed, suggesting that LPS infusion did not alter the activity or expression patterns of those enzymes involved in the activation of these fatty acids. LPS infusion did increase the concentration of brain non-esterified AA (Table 1), confirming our prior results (Rosenberger et al. 2004). That no change was found in the incorporation and turnover rates of DHA or in the rates of acyl-CoA formation further supports the premise that enzymes regulating brain AA metabolism differ from those regulating brain DHA metabolism.

Selective alterations in the incorporation and turnover rates of brain AA and DHA have been reported in rats treated chronically with lithium (Calabrese & Woyshville 1995, Calabrese *et al.* 1995), and in rats subject to chronic nutritional *n*-3 fatty acid deprivation (Greiner et al. 2001). Chronic treatment of rats with lithium chloride, to produce therapeutically relevant

concentrations of plasma and brain lithium (Bosetti et al. 2002), reduced the incorporation and turnover rates of brain AA by 80% (Chang et al. 1996) with no effect on brain DHA (Chang et al. 1999). This selective decrease of brain AA metabolism by lithium has been attributed to a reduction in the activity and expression of the AA-selective type IVA $cPLA_2$ (Rintala et al. 1999). Lithium also reduces the activity and expression of COX-2 resulting in reduced brain prostaglandin levels (Bosetti et al. 2002). Similarly lithium treatment does not alter brain $iPLA_2$ or $sPLA_2$ activity or expression (Weerasinghe et al. 2004). In astrocytes DHA release is mediated in large part by the stimulation of type VI $iPLA_2$, and can be reduced by a selective $iPLA_2$ inhibitor, 4-bromoenol lactone (Strokin et al. 2003), with only a minimal effect on AA release (Ackermann et al. 1995). On the other hand, AA release can be selectively and completely blocked with methyl arachidonoyl fluorophosphonate, a general inhibitor of both $cPLA_2$ and $iPLA_2$ (Riendeau et al. 1994). Therefore the selective decrease in AA turnover found with lithium treatment is likely due to a selective decrease in brain $cPLA_2$ activity and expression. Therefore, it is not unreasonable that the positive effect of LPS infusion on brain AA, but not DHA metabolism, reflects selective activation of AA-selective $cPLA_2$-mediated signaling.

Further, chronic nutritional deprivation of *n*-3 fatty acids, which reduces the incorporation and turnover rates of DHA into brain phospholipids by 30–70% (Contreras et al. 2000) does not affect the incorporation or turnover of AA within brain phospholipids (Contreras et al. 2001). Our results showing that $cPLA_2$ activity and brain AA metabolism (Rosenberger et al. 2004, Basselin et al. 2007) are increased in LPS infused rats, with no significant effect on DHA metabolism, are consistent with this idea and support the premise that an increase in AA metabolism following LPS infusion is due to an increase in AA-selective brain $cPLA_2$. $cPLA_2$ is calcium dependent and hydrolyzes AA in preference to other fatty acids esterified at the *sn*-2 position of the phospholipid moiety (Dennis 1994, Clark *et al.* 1995) (Kramer & Sharp 1997). In contrast, the brain activity of calcium-independent $iPLA_2$ was unchanged by LPS infusion (Rosenberger et al. 2004). Collectively, these data support the idea that different brain PLA_2 isoforms mediate the release of AA and DHA independently, with Group VI $iPLA_2$ being responsible for DHA release and group IV $cPLA_2$ for AA release (Strokin et al. 2003, Strokin et al. 2004).

In brain, fatty acid incorporation into lipids is initiated by acyl-CoA synthetases that catalyze the formation of acyl-CoA from fatty acid, ATP, and CoA (Watkins 1997, Waku 1992) making transport of fatty acid into the cell unidirectional (Lewin et al. 2001). To date, seven acyl-CoA synthetases having different cDNA have been cloned, each the product of a different gene (Coleman *et al.* 2002, Uberti *et al.* 2003, Tang *et al.* 2001). The different acyl-CoA synthetases, which differ in their tissue distribution and transcriptional regulation, can impart fatty acid selectivity (Marszalek et al. 2005) (Coleman et al. 2002). In this regard, acyl-CoA synthetase-2 and -3 preferentially convert long chain polyunsaturated fatty acids to their CoA derivates when compared to acyl-CoA synthetase-1 (Iijima *et al.* 1996, Fujino *et al.* 1996), whereas acyl-CoA synthetase-2 preferentially promotes DHA metabolism (Marszalek et al. 2005). Modulation in the activity of brain long chain acyl-CoA synthetases by α-synuclein have been found to disrupt the incorporation and turnover rates of brain fatty acids in the knockout mouse (Golovko et al. 2006). The lack of a significant change in the rates of AA-CoA or DHA-CoA formation in LPS infused rats found in this study further supports the principle that selective changes in the incorporation and turnover of AA found in this model reflect primarily increased $cPLA_2$.

Microglia and astrocytes respond to inflammatory stimuli by producing pro-inflammatory cytokines that initiate a cascade of biochemical and molecular events that propagate the inflammatory response. These changes stimulate PLA_2 (Farooqui et al. 1997) and increase the production of prostaglandins by the coordinated action of PLA_2 with COX-1 and COX-2 (Hernandez et al. 1999). Following injury and in response to intracellular Ca^{2+}, type IVA

$cPLA_2$ is functionally coupled to COX-2 and co-localized to the nucleus (Scott *et al.* 1999, Sandhya *et al.* 1998, Evans *et al.* 2001). The activation and co-localization of these enzymes can have a propagating influence on other brain PLA_2 enzymes, as non-esterified AA and the formation of prostaglandins can result in a feedback loop that stimulates the activation of types IIA and V secretory PLA_2, and increases the production of membrane-derived inflammatory mediators (Bazan et al. 2002). Therefore, LPS-mediated activation of $cPLA_2$ and the subsequent conversion of AA to prostaglandins can have both an immediate response as well as a propagating influence on inflammation-induced neurodegeneration. The presence of activated $cPLA_2$ and increased brain concentrations of PGE_2 and PGD_2 found in the LPS model (Rosenberger et al. 2004) are consistent with the propagating potential of $cPLA_2$ in the neuroinflammatory response.

Since excess PLA_2 activation is associated with membrane breakdown, mitochondrial dysfunction, and impaired ATP synthesis (Bonventre 1997), sustained PLA_2 activation may lead to failure of excitable membranes and result in loss of membrane stability and cell death. Collectively, these studies suggest that chronic inflammatory events may lead to the selective disruption in the turnover of brain AA resulting in a loss of membrane homeostasis. Thus, the increase in PLA_2 activity, increased conversion of AA due to prostaglandins, and disruption in AA recycling under sustained neuroinflammatory conditions may lead to the loss of membrane phospholipid and result in cell death. Therefore, targeting PLA_2-mediated signaling in response to a neuroinflammatory insult may be beneficial in attenuating the temporal progression of degenerative events associated with inflammation.

In conclusion, the absence of changes in markers of brain DHA metabolism in rats subjected to a 6-day intracerebral ventricular infusion of LPS is in marked contrast to studies showing upregulated markers of AA metabolism (Rosenberger et al. 2004, Lee et al. 2004, Basselin et al. 2007). The lack of a change of brain $iPLA_2$ with no significant change in the rate of microsomal acyl-CoA synthetase activity or in the rate DHA incorporation or turnover, suggests that these DHA-related pathways are not altered following LPS-treatment. Therefore, targeting the AA cascade, particularly $cPLA_2$-mediated events, during neuroinflammation may provide a useful therapeutic tool to attenuate the degenerative events associated with neuroinflammation.

Acknowledgments

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Abbreviations

AA	arachidonic acid
DHA	docosahexaenoic acid
LPS	lipopolysaccharide
PLA_2	phospholipases A_2
$cPLA_2$	cytosolic phospholipase A_2
$sPLA_2$	secretory phospholipase A_2
iPLA2	calcium-independent PLA_2
PUFA	polyunsaturated fatty acids
AA-CoA	arachidonoyl-CoA
DHA-CoA	docosahexaenoyl-CoA

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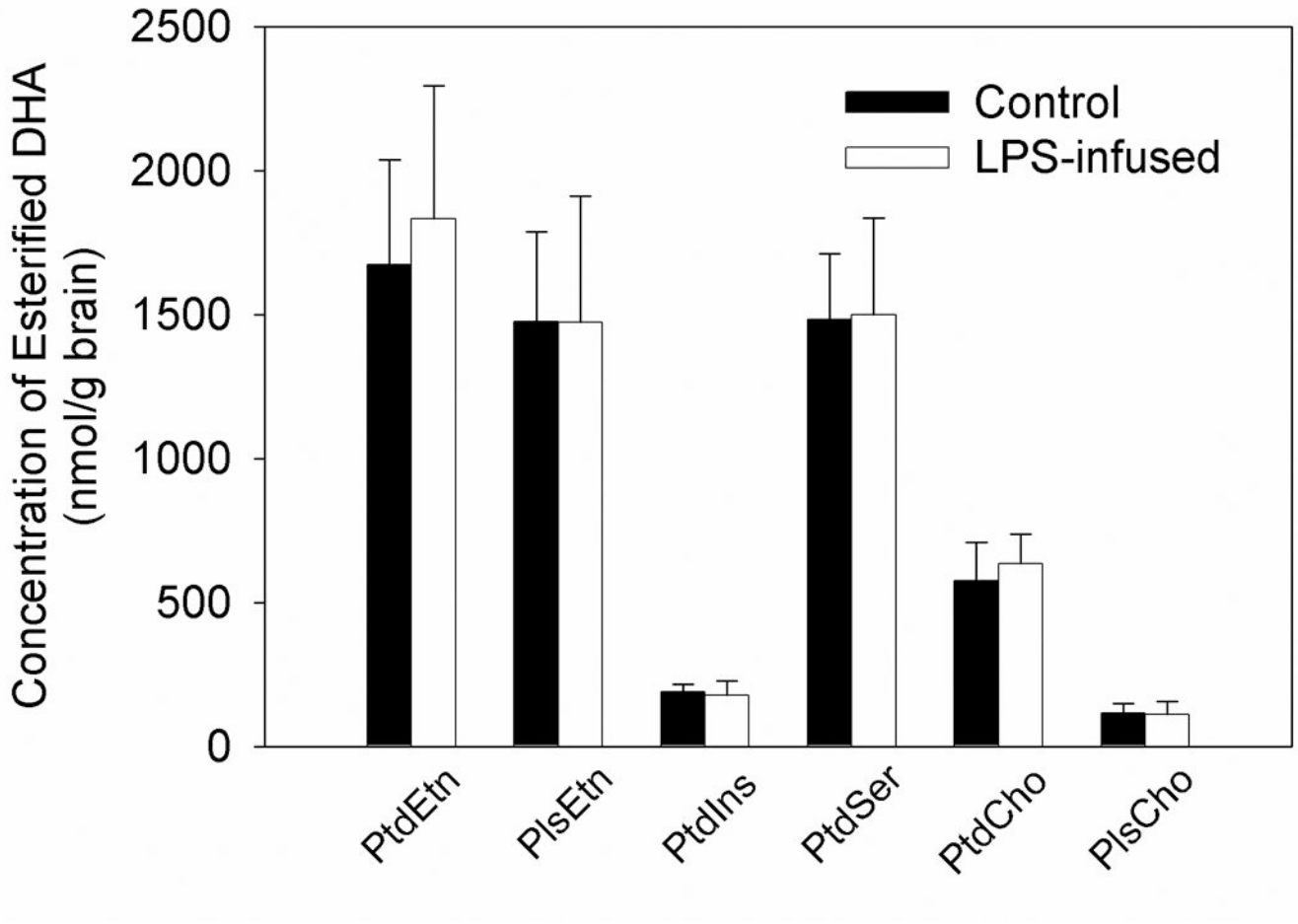

Figure 1.

Concentration of esterified DHA in the stable brain phospholipid pools from control and LPS-treated rats following an intravenous infusion of [1-^{14}C]DHA. Values are the means ± SD (n=7). Abbreviations are: DHA, docosahexaenoic acid; PtdEtn, phosphatidylethanolamine; PlsEtn, plasmenylethanolamine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; PtdCho, phosphatidylcholine; PlsCho, plasmenylcholine.

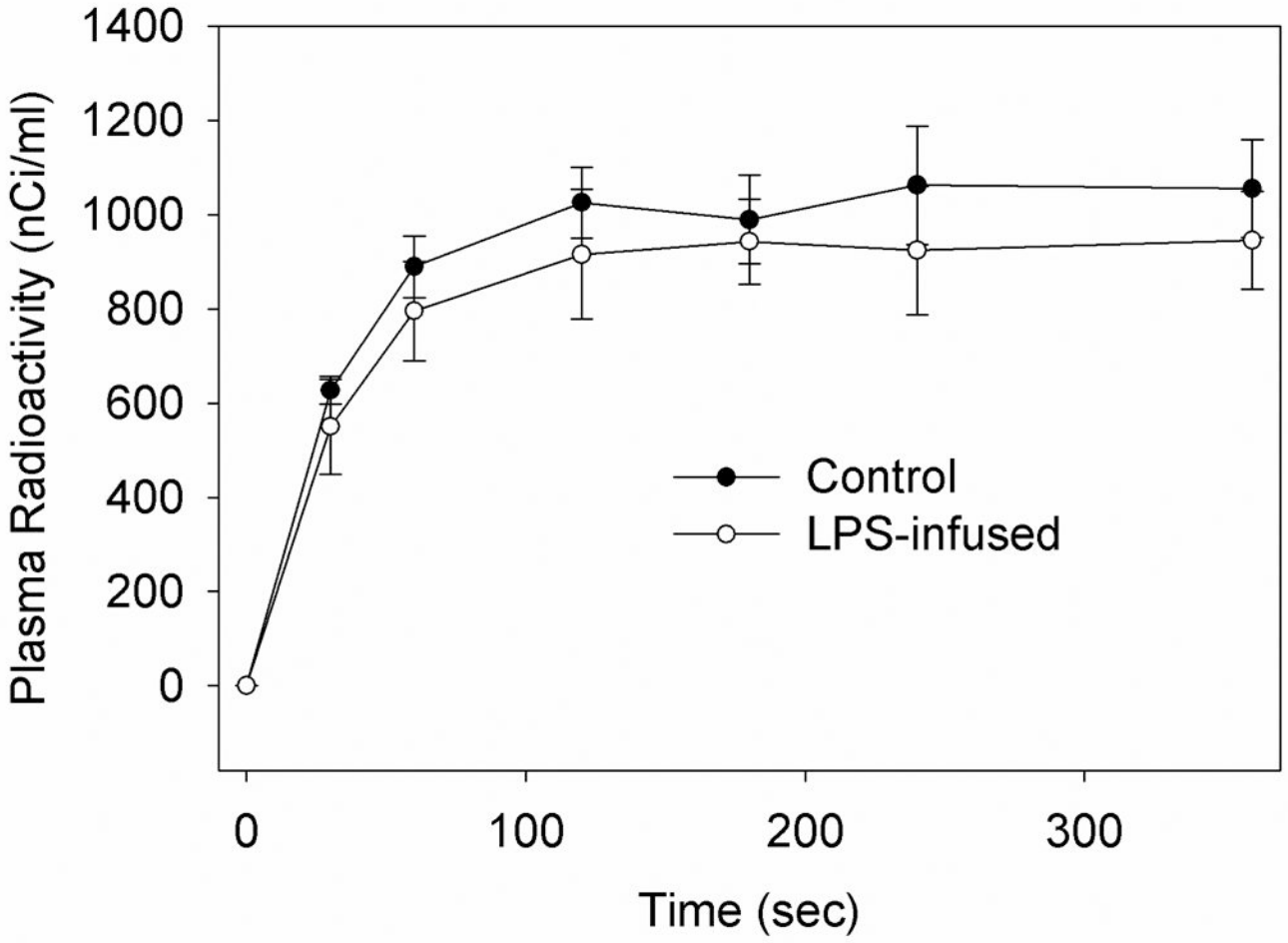

Figure 2.
Plasma radioactivity in control and LPS-treated rats during the intravenous infusion of [1-^{14}C]DHA. Values represent the means ± SD (n=7).

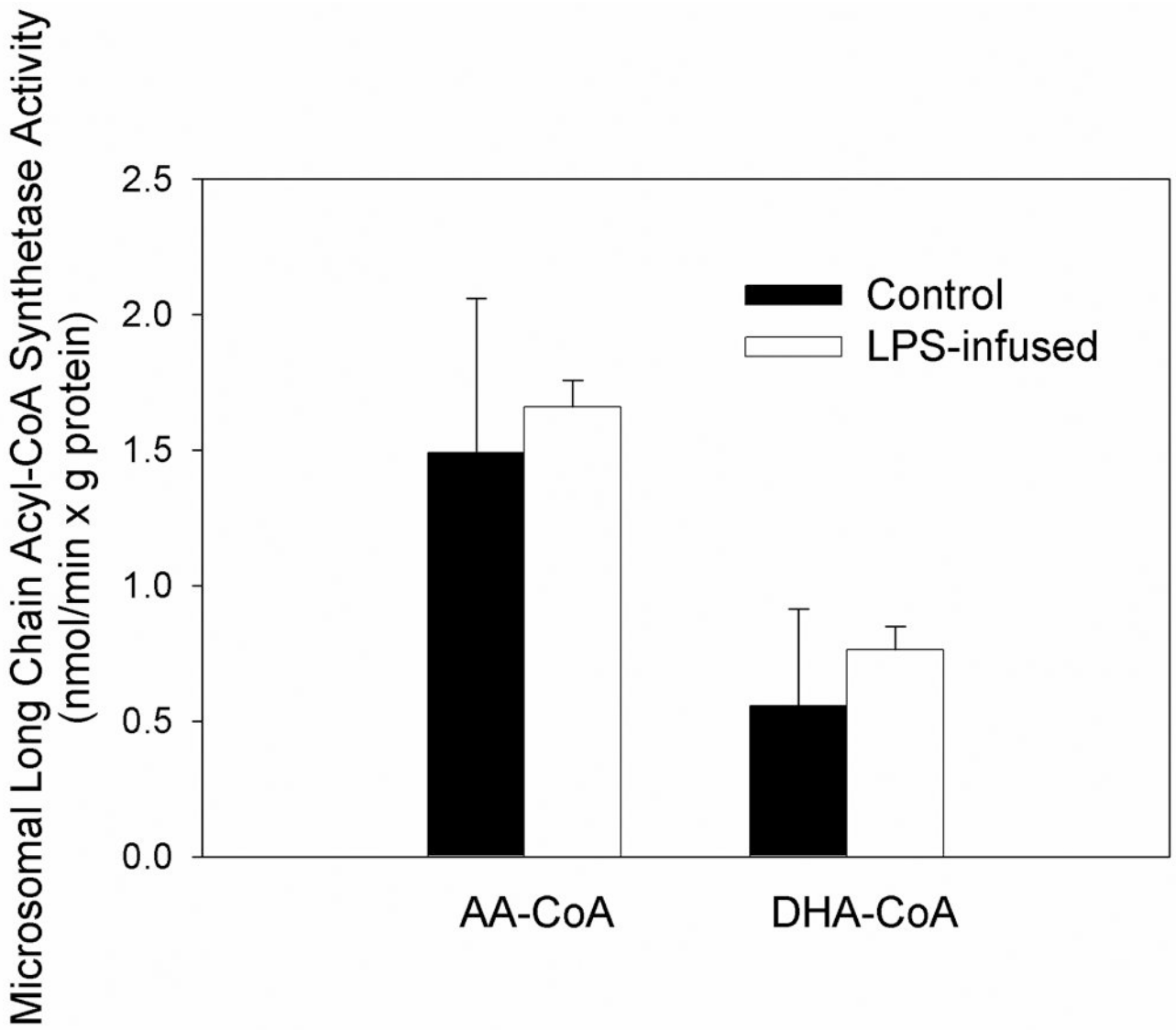

Figure 3.
Brain microsomal long-chain acyl-CoA synthetase activity using [^{14}C]AA or [^{14}C]DHA as substrate from control or LPS-treated rats. Values are the means ± SD (n=5). Abbreviations are; AA-CoA, arachidonoyl-CoA and DHA-CoA, docosahexaenoyl-CoA.

Table 1

Concentration of plasma and brain non-esterified fatty acids, and brain acyl-CoA in control and LPS-treated rats

	Plasma Non-esterified Fatty Acid (nmol/ml)		Brain Non-esterified Fatty Acid (nmol/g)		Brain Acyl-CoA (nmol/g)	
Fatty Acid	**Control**	**LPS**	**Control**	**LPS**	**Control**	**LPS**
Palmitate (16:0)	266.1 ± 95.6	283.1 ± 53.7	25.9 ± 10.3	26.8 ± 5.7	29.0 ± 6.7	34.6 ± 10.0
Stearate (18:0)	72.2 ± 42.6	81.0 ± 21.3	77.7 ± 12.3	60.9 ± 17.7	5.0 ± 3.8	8.4 ± 4.7
Oleate (18:1n-9)	221.4 ± 82.8	214.3 ± 51.0	62.0 ± 10.1	65.2 ± 9.1	27.6 ± 8.0	30.4 ± 8.7
Linoleate (18:2n-6)	277.2 ± 62.6	296.3 ± 77.3	6.2 ± 2.7	8.3 ± 3.0	10.4 ± 3.0	14.3 ± 10.2
Arachidonate (20:4n-6)	20.9 ± 7.3	26.2 ± 7.0	4.6 ± 0.5	*15.8 ± 5.4	1.8 ± 0.8	1.3 ± 1.0
Docosahexaenoate (22:6n-3)	9.4 ± 4.6	9.1 ± 3.9	4.2 ± 0.8	5.3 ± 1.2	1.5 ± 0.8	2.1 ± 0.7

Values are means ± SD (n = 7).

* $p < 0.05$, differs from control mean.

Table 2

Incorporation coefficients (k_i^*), net incorporation rates from brain docosahexaenoyl-CoA ($J_{FA,i}$), and fractional turnover rates ($F_{FA,i}$) of DHA in different rain phospholipid pools in control and LPS-treated rats

	Incorporation Coefficient		Incorporation Rate		Fractions Turnover Rate	
	k_i^* (ml/g × s, ×10^{-5})		$J_{FA,i}$ (nmol/g × s, × 10^{-3})		$F_{FA,i}$ (%/h)	
Phospholipid	**Control**	**LPS**	**Control**	**LPS**	**Control**	**LPS**
PtdEtn	4.5 ± 1.3	4.9 ± 0.6	14.2 ± 4.0	14.9 ± 1.8	3.5 ± 1.0	3.6 ± 0.5
PlsEtn	1.1 ± 0.4	1.4 ± 0.9	3.5 ± 1.3	4.3 ± 2.7	0.9 ± 0.3	1.1 ± 0.7
PtdIns	2.7 ± 0.6	2.5 ± 0.7	8.6 ± 1.8	7.7 ± 2.1	16.3 ± 3.5	14.7 ± 3.9
PtdSer	1.5 ± 0.5	1.3 ± 0.4	4.7 ± 1.6	4.0 ± 1.3	1.1 ± 0.4	1.0 ± 0.3
PtdCho	4.4 ± 1.2	4.5 ± 0.9	13.8 ± 3.9	13.5 ± 2.6	8.6 ± 2.4	8.4 ± 1.6
PlsCho	0.7 ± 0.3	0.5 ± 0.2	2.0 ± 0.9	1.6 ± 0.5	6.4 ± 2.9	5.2 ± 1.5

Values are means ± SD (n = 7).

Abbreviations: PtdEtn, phosphatidylethanolamine; PlsEtn, plasmenylethanolamine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; PtdCho, phosphatidylcholine; PlsCho, plasmenylcholine.