Altered Fatty Acid Composition of Dopaminergic Neurons Expressing α-Synuclein and Human Brains with α-Synucleinopathies*

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α-Synuclein (αS) is an abundant neuronal protein that accumulates in insoluble inclusions in Parkinson’s disease (PD) and the related disorder, dementia with Lewy bodies (DLB). A central question about the role of αS in the pathogenesis of PD and DLB concerns how this normally soluble protein assemble into insoluble aggregates associated with neuronal dysfunction. We recently detected highly soluble oligomers of αS in normal brain supernatants and observed their augmentation in PD and DLB brains. Further, we found that polyunsaturated fatty acids (PUFAs) enhanced αS oligomerization in intact mesencephalic neuronal cells. We now report the presence of elevated PUFAs in PD and DLB brain soluble fractions. Higher PUFAs were also detected in the supernatants and high-speed membrane fractions of neuronal cells over-expressing wild-type or PD-causing mutant αS. This increased PUFAs content in the membrane fraction was accompanied by increased membrane fluidity in the αS overexpressing neurons. In accord, membrane fluidity and the levels of certain PUFAs were decreased in the brains of mice genetically deleted of αS. Together with our earlier observations, these results suggest that αS-PUFA interactions help regulate neuronal PUFAs levels as well as the oligomerization state of αS, both normally and in human synucleinopathies.

α-Synuclein (αS), a 140-residue protein implicated in both familial and sporadic Parkinson’s disease (PD), occurs principally as a cytoplasmic polypeptide in neurons and is localized in part to presynaptic terminals (1). In general, brain αS is highly soluble, and purified recombinant αS is reported to have a disordered random coil structure in solution (2). A wide array of factors has been found to induce the aggregation of αS under experimental conditions. These include PD-causing missense mutations in αS (3–5), mitochondrial inhibitors and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (6, 7), proteosome inhibitors (8, 9), heavy metals (10, 11), oxidative stress (12, 13), αS phosphorylation (14), and fatty acids, whether within phospholipids (15) or as free fatty acids (16). Interestingly, some of the factors that can induce αS aggregation experimentally have been found to be associated with PD, e.g. αS mutations, mitochondrial complex I dysfunction, proteosomal dysfunction, and oxidative stress. Other factors, including free fatty acid composition, have not been comprehensively studied in the brains of patients with PD and other synucleinopathies.

We recently obtained evidence that αS can directly bind fatty acids (17) and can form highly soluble oligomers in response to the treatment of cultured neurons with polyunsaturated fatty acids (PUFA) (16). Further, we observed a pool of soluble αS oligomers that is detectable only after biochemical treatments that remove fatty acids and other lipids (16, 17). These highly soluble, lipid-associated oligomers were detected in mesencephalic neuronal (MES) cells expressing human αS, in the brains of normal and human αS transgenic mice, and in the brains of humans with PD and the related disorder, dementia with Lewy bodies (DLB). We also showed that the formation of soluble αS oligomers is directly regulated by fatty acids in intact cells, with specific enhancement of the oligomers by PUFA and inhibition by saturated FAs (16).

These findings have now led us to search systematically for evidence of a correlation between αS oligomerization and endogenous PUFA composition. First, we find that the relative amounts of PUFA in the oligomer-rich soluble fraction of human brains with PD and DLB are higher than in those of age-matched normal brains. Second, higher PUFA levels are detected in the cytosols of normal mouse than αS-null mouse brain and in the cytosols of MES cells stably expressing human αS than in parental non-transfected cells. Third, the cytosolic PUFA accumulation in the human αS-transfected MES cells is accompanied by PUFA enrichment in membrane fractions containing synaptic vesicles. Fourth, we document higher membrane fluidity in membranes of αS-expressing MES cells than in the non-transfected cells and in the membranes of normal than αS-null mouse brains.

MATERIALS AND METHODS

αS-null Mice—An αS-targeted knock-out mouse line (αS+/−) in the C57Bl6 genetic background was purchased from Harlan Laboratories (Indianapolis, IN). Control C57Bl6 mice were obtained from Taconic Laboratories (Germantown, NY). Mice were bred routinely. To reduce experimental variability, we only used brains of mice that were born and raised in our animal facility rather than the founder mice that had been subjected to different husbandry.

Human Brains—We utilized 5 of the 7 brains in the PD, DLB, and control groups that we had studied in an earlier work (16) and added 2 more neuropathologically confirmed brains to each of these 3 diagnostic groups (total n = 7 in each group). Neuropathological diagnoses and specimen handling were as previously described (16).

Brain and Cellular Fractionation Protocol—Biochemical fractionation-
α-Synuclein Accumulation Alters PUFA Composition

We previously reported that αs can bind FAs in vitro (17), that PUFAAs promote whereas saturated FAs retard the formation of soluble αs oligomers both in vitro and in intact cells (16), and that the levels of soluble and insoluble αs oligomers are elevated in the brains of humans undergoing progressive αs accumulation, i.e. in PD and DLB (16). These findings led us to hypothesize that the increased levels of soluble αs oligomers in PD and DLB brains could be associated with changes in the composition of endogenous brain FAs. We further postulated that overexpressing αs in dopaminergic neuronal cells could alter their endogenous FA composition.

**Altered FA Composition of the High-Speed Soluble Fractions of PD and DLB Brains**—We assayed the FA composition of the very high-speed (post-370,000 × g) cytosols prepared from 7 brains each of the 3 diagnostic groups, PD, DLB, and age-matched controls. Five cases in each group had been previously tested for their levels of soluble αs oligomers, and these levels were invariably increased in the post-370,000 × g supernatants of the PD and DLB brains (16). SFAs, MUFAs, and PUFAs were quantified by GC in the high-speed soluble frac-

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**Materials and Methods**

**Quantitative FA Analysis**—Samples of high-speed cytosols or membranes (1–1.5 mg protein) were processed and analyzed for FAs using the Microbial Identification System at Microbial ID, Inc. (Newark, DE). The standard protocol begins with sample lyophilization and resuspension in methanol. The sample is then subjected to the following steps: saponification with sodium hydroxide (3.75 M); methylation with 3.25 M HCl in 46% methyl alcohol (incubation at 80 °C for 10 min); extraction in 50% hexane/50% methyl tert-butyl ether; and a basic wash with 0.25 M NaOH. The organic phase containing fatty acids is injected into a Hewlett-Packard 5890A gas chromatograph (GC) equipped with a hydrogen flame ionization detector, an automatic injector, a sample controller, and an electronic integrator controlled by a minicomputer. The GC used a fused silica capillary column with methylphenyl silicone (SE54) as the stationary phase. The computer-controlled operating parameters of the instrument were as follows: injector temperature, 250°C; detector temperature, 300°C; oven temperature, programmed from 170°C to 270°C at 5° per min. The FA methyl esters in each sample were identified by comparing retention times with known standards and by computer calculation of equivalent chain lengths.

**Size Exclusion Chromatography (SEC)**—SEC was performed on various high-speed cytosols, and the resultant fractions were collected and analyzed as described previously (16).

**Gas Chromatographic Mass Spectrometry (GC/MS)**—Following solid-phase extraction (18), samples were treated with diazomethane (20–30 min at 22°C) followed by incubation with BSTFA (N,O-bis[trimethylsilyl]trifluoroacetamide) for 8–12 h at room temperature in the dark. Analyses were performed on a Hewlett-Packard 6890 gas chromatograph using a mass selective detector (HP 5973) equipped with an MS Chemstation. The instrument was set to acquire and store data at 1.6 scans per second. The column was a HP Ultra (25 mm × 0.22 μm), and all injections were made in the splitless mode with materials suspended in BSTFA (~2 μl per injection). The GC temperature program was initiated at 150°C and reached 250°C at 10 min and 325°C at 20 min in a gradient fashion. Fatty acids (saturated and unsaturated) were identified according to the prominent ions present in their individual mass spectra and their retention times (see “Results”).

**Membrane Fluidity Measurements**—Samples of high-speed membranes (i.e membranes that sedimented between 10,000 and 370,000 × g) were kept at 4°C without freeze and thaw and used within 2–3 days from preparation. Membrane fluidity was determined using pyrene di-sulphonic acid (PDA), a fluorescent lipid reagent (Molecular Gene Technologies, product number 0271, Eugene, OR). Fluorescence was recorded using a 1420 Victor® fluorometer (PerkinElmer Life Sciences), and the assays were performed according to the manufacturer’s recommendations, with some modifications. Samples were excited at 330 nm with an 80 nm bandwidth. The pyrene monomer emission intensity was measured at 400 nm with 10 nm slits, and the pyrene eximer emission intensity was measured using a cut-off filter at 460 nm. Samples (100 μg protein) were incubated with 20 μM PDA in perfusion buffer (100 mM NaCl, 5 mM HEPES, pH 7.4, 2 mM MgCl₂, 5 mM KCl, 2 mM CaCl₂, 5 mM glucose, and 50 mM mannitol) in a final volume of 200 μl. Incubation was carried out at 30°C for 45 min with rotation. The membranes were sparged for 5 min, and washed twice in perfusion buffer, then re-suspended in fresh buffer and transferred to 96-well plates. Blank membranes from samples with no pyrene probe were subtracted, and the ratios of eximer (460 nm) to monomer (400 nm) signals were calculated. Values represent means ± S.E. For measurements in mouse brains, seven different experiments each consisted of different brain membrane preparations with each preparation comprising 2–3 brains. For measurements in cells, 6 different experiments each consisted of different high-speed membrane pellets.

**Statistical Analyses**—For individual FAs, we used exact Wilcoxon Rank Sum tests to compare the PD and DLB groups versus the control group of human brains, and the wt and A53T αs-transfected MES neuronal cells versus untransfected parental MES cells. We adjusted for multiple comparisons among fatty acid subgroups (i.e., saturated, mono-, di-, and polyunsaturated) using Bonferroni corrections. For the comparison of the FA subgroups (SFA, MUFAs, and PUFAs), exact Kruskal-Wallis tests were used to compare the levels of these summed fatty acids between diagnostic groups of subjects. As the total of the 3 types of fatty acids sum to 100% within each experiment, these three tests are dependent, and a Bonferroni-adjusted significance level of 0.053 = 0.017 was therefore used.
tions of frontal cortex (Fig. 1, a–c). We observed a highly consistent alteration in cortical PUFA composition in the PD and DLB brains: a decrease in linoleic acid (18:2) levels (with Bonferroni-adjusted p values of p = 0.055 for PD, p = 0.01 for DLB), an increase in docosahexaenoic acid (22:6) levels (adjusted p values of p = 0.003 for PD, p = 0.009 for DLB) and an increase in docosatetraenoic acid (22:4) levels for PD (p = 0.026) (the increase in 22:4 in DLB did not reach statistical significance (p = 0.129)). Other PUFAs (arachidonic acid, 20:4; eicosatetraenoic acid, 20:3) were not significantly changed. Importantly, no significant alterations were seen in the compositional profiles of MUFAs and SFAs in these same brain supernatants. These results demonstrate an association between PUFA accumulation in the soluble fractions of PD and DLB brains (Fig. 1c) and the accumulation of highly soluble αS oligomers documented in these same fractions (16).

Expression of Human αS in Dopaminergic Neurons Increases Endogenous PUFA Levels in Both Cytosolic and Membrane Fractions—Because increased αS oligomer levels were associated with increased PUFA levels in the soluble fractions of PD and DLB brains, we asked whether αS overexpression could itself induce alterations of PUFA composition. High-speed cytosols and membrane pellets were prepared from MES dopaminergic neuronal cells that were either untransfected or stably transfected with wt or A53T human αS. Quantitative analyses of FA composition were carried out at the peak stage of αS oligomer accumulation in these cells, i.e., between 85 and 130 days post-DNA transfection (16) (Fig. 2, a–c). These GC analyses of SFAs, MUFAs, and PUFAs were conducted in parallel with Western blots that confirmed the presence of αS oligomers in the high-speed cytosols (Fig. 3). We observed a selective accumulation of PUFAs in the cytosols of the αS-overexpressing cells. In non-transfected, parental MES cells, several PUFAs were undetectable by our assay: 20:5, 22:4, 22:5, and 22:6 (Fig. 2c). However, upon αS overexpression, these long chain PUFAs accumulated to readily detectable levels. In contrast, the levels of individual saturated and monounsaturated FAs were not significantly altered in the same samples (Fig. 2, a and b).

In a previous study (16), we showed that treating αS-expressing MES neurons with medium supplemented with PUFAs induced αS oligomerization and aggregation. In view of the above results indicating an association between αS oligomer accumulation and cytosolic PUFA levels, we now asked whether PUFA molecules themselves are associated with the αS oligomerization, or rather whether a PUFA-derived metabolite or other hydrophobic molecule is responsible. We analyzed the high-speed cytosols of wt human αS-transfected (versus untransfected) MES neurons by GC/MS at the peak stage of oligomer accumulation. We found that MES cells conditioned in medium supplemented with α-linolenic acid (18:3) (Fig. 4) or docosahexaenoic acid (22:6, not shown) accumulated these specific PUFAs in their cytosols in the case of the αS-overexpressing neurons (Fig. 4a), but not in the parental MES neurons (not shown). In an extensive GC/MS screen for αS-associated alterations in other hydrophobic molecules, we identified only elevations in the cellular levels of those FAs that had been enriched in the conditioning medium of the neurons. This result suggests that enhancement of soluble αS levels in a living neuronal cell leads to specific intracellular sequestration of those fatty acids enriched in the extracellular milieu of the neuron.

We previously utilized a centrifugal fractionation protocol to obtain high-speed membrane fractions (sedimenting between 10,000 and 370,000 × g) from mouse brains that were then analyzed by quantitative Western blotting for αS content.
About 10% of total mouse brain αS was found in this high-speed membrane fraction (17). We therefore performed GC analyses of PUFA composition in the high-speed membrane fractions of the MES cells. This revealed a striking accumulation of several PUFAs in the membranes of the wt and A53T αS-transfected MES neurons versus the parental untransfected cells (Fig. 5), in association with the large rise in soluble αS oligomers in the cytosols of the former lines (Fig. 3) (16). Specifically, we observed increases in the following: 18:2 (wt p < 0.005 and A53T p < 0.01, versus untransfected MES); 20:4 (wt p = 0.055 and A53T p = 0.055); and 20:3 (present in detectable amounts in both wt and A53T but undetectable in untransfected cells) (Fig. 5c). Interestingly, the levels of the MUFA, oleic acid (18:1), were significantly reduced in the wt and A53T overexpressing cells (p = 0.04 versus untransfected cells) (Fig. 5b). Other MUFAs detectable by our assay were unchanged, as were SFAs.

In addition to this analysis of individual FAs, we compared the summed values of all FAs in a group (i.e. total SFAs, total MUFAs, and total PUFAs) across our 3 cell lines (untransfected MES, wt human αS MES, and A53T human αS MES). We observed significant −7−8% increases in total cytosolic PUFA content in both the wt and A53T αS expressing cells (Bonferroni-corrected p values of 0.008 and 0.016, respectively). This was accompanied by significant −10−12% decreases in total cytosolic MUFA content (Bonferroni-corrected p values of 0.008 and 0.016, respectively). There were no significant changes in SFA content. These results clearly indicate that the expression of human αS in mesencephalic neurons results in relative increases in PUFA relative levels at the expense of MUFA relative levels. No differences were detected in the total amount of FAs in transfected αS cells compared with the parental MES cells.

αS Expression Increases Membrane Fluidity in Cultured Dopaminergic Cells—Membrane fluidity is controlled by the balance among SFAs, MUFAs, and PUFAs in cell membranes (19). Relative increases in the amounts of PUFAs lead to increases in membrane fluidity, whereas relative increases in SFAs decrease membrane fluidity (19–21). In light of the consistent shift in PUFA composition we observed in the membranes of αS-transfected MES neurons and human synucleinopathy brains (see Figs. 1, 2, and 5) as well as the documented interaction of αS with vesicle membranes (17, 22–24), we reasoned that cellular αS content should directly affect membrane fluidity. To address this hypothesis, high-speed membrane pellets were again prepared from untransfected and human αS-transfected MES cells cultured under identical basal conditions in conditioned medium containing 5% adult plus 5% fetal bovine serum. To analyze membrane fluidity, we used a pyrene probe and measured its eximer formation after excitation at 330 nm. We quantified the emissions at 400 nm (pyrene monomers) and 460 nm (pyrene eximers). The ratio of eximer to monomer emission values serves as an indicator of relative membrane fluidity (see “Materials and Methods”). Pyrene eximer/monomer ratios were significantly higher, increasing by 49% (p = 0.002; n = 6 different measurements) in the membranes of the wt human αS-expressing MES cells versus those of the parental untransfected cells (Table I).

Altered Cytosolic Fatty Acid Composition and Reduced Membrane Fluidity in αS-null Mouse Brain—To validate in vivo the apparent effects of αS content on PUFA composition and membrane fluidity, we analyzed a C57Bl6 mouse line genetically...
α-Synuclein Accumulation Alters PUFA Composition

We first observed that pure recombinant αS is capable of binding FAs in vitro and that a pool of highly soluble αS oligomers exists normally in cultured dopaminergic neurons and the brain and can be revealed by procedures that bind fatty acids (17). Next, we showed that the assembly of αS into soluble oligomers is enhanced by the exposure of intact neurons to physiological levels of PUFAs, whereas disassembly of the oligomers is favored by exposure of the cells to SFAs (16). These regulatory effects of FAs on αS assembly state may be direct, as PUFAs consistently enhanced the oligomerization of pure, recombinant αS in vitro. Here, we extend these findings by showing that in the pathological state of a human α-synucleinopathy, the accumulation of αS, including as soluble oligomers, is associated with alterations in brain PUFA composition. Specifically, we demonstrate the accumulation of certain long-chain PUFAs in the high-speed soluble fraction of brains with PD and DLB, the same fraction in which we previously found accumulation of soluble αS oligomers in these disorders (16). We observed a significant accumulation of docosahexaenoic acid (22:6) in PD and DLB brain cytosols and also of docosatetraenoic acid (22:4) in PD cytosol, compared with simultaneously measured levels in age-matched control brain cytosol. In parallel, there was a significant reduction in the shorter chain PUFA, linoleic acid (18:2), in PD and DLB brains. To attempt to correlate the altered PUFA composition specifically with αS accumulation, we examined FA composition in mesencephalic neurons containing αS oligomers, we detected higher relative amounts of high-speed membranes. *, p < 0.017 (see "Materials and Methods").

Table I
Membrane fluidity values for parental and αS-overexpressing MES cells

| Cell type     | Eximer/monomer ratio ± S.E. | Relative fluidity | Statistical significance |
|---------------|-----------------------------|-------------------|--------------------------|
| MES           | 3.57 ± 0.31                 | 100               |                          |
| Wt-αS-MES     | 5.23 ± 0.39                 | 149 ± 4           | p = 0.002                |

deleted of αS, in comparison to C57Bl6 control mice (Fig. 6a). High-speed cytosols of wild-type (+/+ ) and αS-deleted (−/−) brains were subjected to quantitative analyses of FA composition. In agreement with our results in human brains and MES neurons containing αS oligomers, we detected higher relative PUFA levels in the presence of monomeric and oligomeric αS (+/+ brains) than in their absence (−/− brains). Docosahexaenoic acid (22:6) and α-linolenic acid (18:3) were detected in the cytosols of αS +/+ but not in αS −/− brains, and therefore were significantly different between the two groups (Fig. 6b). Decreases in certain other PUFAs (20:3, 22:4, and 22:5) in the −/− mice did not reach statistical significance. To strengthen this correlation between the presence of αS and selective PUFA accumulation, we next conducted SEC on the high-speed cytosols of both wild-type and αS−/− mouse brains and analyzed the FA composition of those SEC fractions found to contain αS oligomers in the +/+ mice (fractions 22–26 combined, of 54 fractions collected) (see Fig. 6c). The results revealed a direct correlation between the presence of αS and the presence of certain PUFAs: 18:3 and 22:6 were detected only in the SEC fractions containing αS (wt) and not in the corresponding fractions of the αS−/− mouse brains (Fig. 6d). Certain other PUFAs, such as 20:3, 22:4, and 22:5, that were present in low amounts in the +/+ total brain cytosols and undetectable in the −/− total brain cytosols were not detectable in the SEC fractions. We detected no significant alterations of MUFA or SFAs in either the total brain cytosols or the SEC fractions (data not shown).

We then compared the brain membrane fluidity of the αS +/+ and −/− mice. High-speed brain membranes (sedimenting between 10,000 and 370,000 × g) were prepared in parallel from mice of closely similar ages (± 2 weeks) between 3 and 10 months old and subjected to the pyrene membrane fluidity assay (Table II). Lower fluidity values for the eximer/monomer ratios were consistently obtained in the αS−/− brain membranes, with the mean decrease being 37 ± 2.6% (p < 0.005, n = 7 different experiments). This result is in accord with the higher membrane fluidity we observed in MES cells over-expressing αS (Table I). However, when we measured the FA composition of the mouse brain membrane fractions, there was no reduction in the relative amount of PUFAs in the αS−/− mice, in contrast to the increased PUFA content observed in the αS-overexpressing MES cells. On the other hand, we observed an increase of ~25% in the total amount of fatty acids per mg protein in the high-speed membranes of αS−/− mouse brains compared with wild-type membranes (data not shown).

**DISCUSSION**

In a series of recent studies (16, 17), we have documented different aspects of the interaction of neuronal α-synuclein with fatty acids under normal and pathological circumstances. In agreement with our results in human brains and MES neurons containing αS oligomers, we detected higher relative PUFA levels in the presence of monomeric and oligomeric αS (+/+ brains) than in their absence (−/− brains). Docosahexaenoic acid (22:6) and α-linolenic acid (18:3) were detected in the cytosols of αS +/+ but not in αS −/− brains, and therefore were significantly different between the two groups (Fig. 6b). Decreases in certain other PUFAs (20:3, 22:4, and 22:5) in the −/− mice did not reach statistical significance. To strengthen this correlation between the presence of αS and selective PUFA accumulation, we next conducted SEC on the high-speed cytosols of both wild-type and αS−/− mouse brains and analyzed the FA composition of those SEC fractions found to contain αS oligomers in the +/+ mice (fractions 22–26 combined, of 54 fractions collected) (see Fig. 6c). The results revealed a direct correlation between the presence of αS and the presence of certain PUFAs: 18:3 and 22:6 were detected only in the SEC fractions containing αS (wt) and not in the corresponding fractions of the αS−/− mouse brains (Fig. 6d). Certain other PUFAs, such as 20:3, 22:4, and 22:5, that were present in low amounts in the +/+ total brain cytosols and undetectable in the −/− total brain cytosols were not detectable in the SEC fractions. We detected no significant alterations of MUFA or SFAs in either the total brain cytosols or the SEC fractions (data not shown).

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neuronal cells that stably overexpress human aS (either wt or A53T). Intriguingly, long-chain PUFAs, including 22:4 and 22:6, accumulated specifically in the cytosol of MES cells expressing human aS, compared with simultaneously analyzed parental MES cells expressing low levels of endogenous aS. In this cellular model, the accumulation of certain PUFAs was substantial; for several long-chain PUFAs, the levels in the cytosol of parental MES cells was below detection by GC, but upon human aS expression, these PUFAs became readily detectable and accumulated (Fig. 2). A specific correlation between altered PUFA composition and aS monomers and oligomers was further supported by our FA analyses of aS-containing SEC fractions of the cytosols. That is, the specific FA composition of the five SEC fractions (of 54) from aS+/− brain cytosol that contained aS monomers and oligomers and that of the corresponding SEC fractions of aS−/− brain cytosol is very similar to the FA composition of the respective whole cytosols (Fig. 6). This result suggests that a major portion of cytosolic PUFAs is associated with aS under native conditions. Furthermore, an increase in PUFA levels was also found in the high-speed membrane fraction of the aS-expressing MES cells. Finally, we predicted that membrane fluidity should accordingly be affected by aS, and indeed, increased membrane fluidity was observed in neuronal cells overexpressing aS, whereas decreased membrane fluidity were found in the brains of mice genetically deleted of aS.

These various findings lead us to propose a model in which neuronal FA content, particularly of PUFAs, is regulated in part by the oligomerization state of aS. Increases in the steady-state levels of soluble aS oligomers, as observed in the MES transfectants and in PD and DLB brains (16), can shift FA composition to increase the relative amounts of long-chain PUFAs. Conversely, primary rises in PUFA concentration can enhance the steady-state levels of soluble aS oligomers, as we observed in MES cells exposed to PUFAs (16). Therefore, aS-PUFA interactions appear to coordinate regulation of neuronal PUFA levels as well as the oligomerization state of aS. We postulate that this co-regulation represents a normal, physiological process, in that FA-associated soluble oligomers of endogenous aS exist in normal mouse brain and such oligomers also occur in healthy MES neurons expressing wt human aS (16, 17). On the other hand, this process can be abnormally augmented in pathological states such as brain aging (in the case of 18-month-old aS transgenic mice (16)) and human a-synucleinopathies (PD and DLB (16)). We assume that the altered PUFA composition we document in the high-speed cytosols of PD and DLB brains (Fig. 1) occurs secondary to whatever causes aS to oligomerize and accumulate in these disorders. On the other hand, we cannot currently exclude the less likely possibility that an alteration in brain PUFA composition precedes and contributes to the accumulation of aS oligomers. The latter possibility suggests that one should search for evidence of altered fatty acid composition in the plasma and cer-

**TABLE II**

Membrane fluidity values for wild-type and aS-null mouse brains

Membrane fluidity measurements as in Table I. Mean of n = 7.

| Mouse line | Eximer/monomer ratio ± S.E. | Relative fluidity | Statistical significance |
|------------|-----------------------------|-------------------|--------------------------|
| B6         | 3.10 ± 0.12                 | 100 ± 100        | p < 0.005                |
| aS−/−      | 3.28 ± 0.08                 | 73 ± 2.6          |                          |


A primary function of PUFAs in cellular membranes is believed to be the enhancement of membrane fluidity. Fatty acids having higher molecular volumes, e.g. via increased carbon chain length and/or decreased degree of saturation, can alter the highly organized structure of the lipid bilayer and make it more fluid. Membrane fluidity is a term that describes a physical property of cellular membranes that reflects the ability of a molecule to move laterally in the membrane (25). Therefore, it determines in part the permeability of membranes (26). Membrane fluidity can in turn regulate the positioning and motility of integral membrane proteins. As a result, diverse cellular functions such as receptor signaling, membrane transporter activation, ion channel conductance, and neurotransmitter release are all affected by the membrane fluidity state (reviewed in Ref. 19). Other membrane properties that are known to be affected by PUFA composition are membrane thickness (27), deformability (28), and curvature (29). It will now be interesting to determine whether αS-PUFA interactions affect one or more of these membrane properties, in addition to the clear effects on membrane fluidity we report here.

The membranes of the αS−/+ mouse brain were exceptional in this regard. Here, membrane fluidity was reduced even though no reduction in the relative amount of PUFA was detected by our assay. On the other hand, an increased ratio of total FAs to protein was detected. This increase in FA to protein ratio may explain the reduced fluidity and may result from the function of additional FA-interacting proteins besides αS. A likely candidate is β-synuclein, a member of the synuclein family that we have observed to be elevated in the αS−/+ mouse brains. In the context of the predilection of αS accumulation for dopaminergic neurons in Parkinson’s disease, it is intriguing that a role for PUFAs in modulating dopamine levels and dopamine vesicles in neurons has been reported. A significant reduction in the number of dopaminergic synaptic vesicles occurred in the frontal cortex of rats fed a diet deficient in n-3 PUFAs (i.e. in FAs that have their first double bond after the third carbon atom, e.g. α-linolenic acid (18:3), eicosapentaenoic acid (20:5), and docosahexaenoic acid (22:6)) (30, 31). Furthermore, dopamine levels in the frontal cortex of rats fed a PUFA-deficient diet were also reduced (32–34). Importantly, the opposite trend (increased dopamine levels) was observed in rats fed a PUFA-rich diet (35). A potential role for αS in the formation and/or maintenance of synaptic vesicles has been shown in other studies (36, 37). Combining these results with our new findings, we hypothesize that αS is directly involved in proper maintenance of the neuronal membrane vesicle system through its interaction with PUFAs. αS-PUFA interactions of the sort we report here may thus be involved in the assembly of membrane vesicles, allowing a degree of membrane fluidity that is important for proper synaptic transmission.

Data from experimental models and postmortem human brain tissue implicate enhanced oxidative stress as a factor that contributes to the pathogenesis of PD (reviewed in Ref. 38). In particular, increased lipid peroxidation in the substantia nigra is one documented manifestation of PD (reviewed in Refs. 39–42). Based on our new findings, we hypothesize that one physiological role for αS is to enrich neuronal membranes with certain PUFAs. It is possible, therefore, that oxygen free radicals generated during the oxidative stress phenomena that occur in the substantia nigra of PD patients can attack PUFAs

enriched by interactions with αS and thus make these neuronal membranes more susceptible to further oxidative damage. In this regard, dopaminergic neurons, in which oxidative stress is intrinsically high (38), may be more vulnerable than other classes of neurons to αS-mediated enrichment of PUFAs. The oxidative stress occurring in dopaminergic neurons may act in an amplification cycle toward cell death. Continuous peroxidation of membrane PUFAs is known to cause decreased membrane integrity and the loss of selected cellular organelles (43, 44). Presumably, dopaminergic vesicles, along with other vesicles, will lose membrane integrity (19, 44), which could result in dopamine leakage out of the vesicle and increased cytosolic dopamine concentrations. These could, in turn, increase oxidative stress in the neuronal cytoplasm by exposing dopamine to oxidative reaction, resulting in the accumulation of DOPAC and hydrogen peroxide. The increased oxidative stress that results may reach a level that causes cell death.

In conclusion, the finding of altered PUFA composition in αS-expressing mesencephalic neurons and in PD and DLB brains is consistent with our earlier discovery of a pool of lipid-associated, soluble αS oligomers in these tissues and a positive role for certain PUFAs in enhancing the oligomerization of αS in intact, living cells. The recognition of physiologic and pathologic αS-PUFA interactions raises the possibility that therapeutic down-regulation of brain PUFA content could mitigate against αS oligomerization and perhaps oxidative stress, thereby stabilizing dopaminergic neurons prior to frank cell death.

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