Vulnerability of Synaptosomes from ApoE Knock-Out Mice to Structural and Oxidative Modifications Induced by Aβ(1−40): Implications for Alzheimer's Disease[†]

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ABSTRACT: Apolipoprotein E (apoE) plays an important role in the response to central nervous system injury. The e4 allele of apoE and amyloid β-peptide (Aβ) are associated with Alzheimer's disease (AD) and may be central to the pathogenesis of this disorder. Recent studies demonstrate evidence for neurodegeneration and increased lipid peroxidation in transgenic mice lacking apoE (KO). In the current study, synaptosomes were prepared from apoE KO mice to determine the role of apoE in synaptic membrane structure and to determine susceptibility to oxidative damage by Aβ(1−40). ApoE KO mice exhibited structural modifications to lipid and protein components of synaptosomal membranes as determined by electron paramagnetic resonance in conjunction with lipid- and protein- specific spin labels. Incubation with 5 μM Aβ(1−40) resulted in more severe oxidative modifications to proteins and lipids in apoE KO synaptosomes as measured by protein carbonyls, an index of protein oxidation, and TBARs and protein-bound 4-hydroxynonenal (HNE), markers of lipid oxidation. Together, these data support a role for apoE in the modulation of oxidative injury and in the maintenance of synaptic integrity and are discussed with reference to alterations in AD brain.

Apolipoprotein E (apoE)[1] is the predominant lipoprotein in the central nervous system (CNS) and has three human isoforms designated ϵ2, ϵ3, ϵ4. Inheritance of the ϵ4 allele of apoE is associated with an increased risk of the development of Alzheimer's disease (AD) (*1*). Increased oxidative stress may also play a role in the pathogenesis of AD. Reactive oxygen species (ROS) mediate much of the neuronal injury induced by oxidative stress including protein, lipid, and DNA oxidation, all of which are increased in AD (*2*). Further, oxidative damage in the AD brain has been reported to be apoE allele-dependent, with increases in lipid oxidation correlating to the presence of the ϵ4 allele (*3*−*6*).

ApoE plays an important role in the response to CNS injury (*7*, *8*). Mice lacking apoE (KO) have increased markers of oxidative stress under basal conditions and display evidence for increased neurodegeneration (*9*). ApoE KO mice display greater basal levels of tyrosine nitration and isoprostane formation (*10*−*12*) while being more vulnerable to stroke and head injury (*13*, *14*). Mice that have been genetically modified to express only the human apoE ϵ3 or ϵ4 allele respond to injury in an allele-specific fashion. Brain lesions induced by ischemia or kainic acid are less severe in mice that express the ϵ3 allele of apoE than in mice that express the ϵ4 allele of apoE (*15*, *16*). These findings correlate with studies that suggest allele-specific (ϵ3 > ϵ4) antioxidant and 4-hydroxy-2-*trans*-nonenal (HNE) scavenging abilities (*17*, *18*).

The synapse is suggested to be a critical locus of neuronal damage in AD (*19*, *20*). This report investigates oxidative modifications in synaptosomes isolated from apoE KO mice. Oxidation of proteins and lipids lead to structural alterations that can be detected by electron paramagnetic resonance (EPR) analysis of synaptosomal membranes in conjunction with protein- and lipid-specific spin labels. In wild type (WT) and apoE KO synaptosomes, basal levels of protein carbonyls, a marker of protein oxidation (*21*), and levels of protein-bound HNE, a toxic product of lipid oxidation that covalently modifies proteins (*22*), were determined by slot blotting methods. In addition, because apoE KO mice are more vulnerable to injury and noting that amyloid β-peptide (Aβ) is associated with oxidative stress (*23*), we also analyzed the susceptibility of WT and apoE KO synaptosomes to Aβ-(1−40)-induced oxidation.

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[1] Abbreviations: Alzheimer's disease (AD); apolipoprotein E (apoE); wild type (WT), knock-out (KO); amyloid-β (Aβ); electron paramagnetic resonance (EPR); 4-hydroxynonenal (HNE); 2,2,6,6-tetramethyl-4-maleimidopiperidin-1-oxyl (Mal-6); 5-nitroxide stearate (5-NS); reactive oxygen species (ROS); thiobarbituric acid reactive substances (TBARS).

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MATERIALS AND METHODS

Materials. Aβ(1−40) was purchased from Bachem (Torrance, CA) or Anaspec (San Jose, CA). For all experiments, Aβ was dissolved in phosphate-buffered saline (PBS) and incubated at 37 °C for 4 h prior to incubation with synaptosomes. WT and apoE KO mice used for these experiments were purchased from Jackson Labs (Bar Harbor, ME). Protease inhibitors used in the isolation buffer were purchased from ICN (Aurora, OH). Western and slot blotting materials including apparatus, nitrocellulose (0.45-μm pore size), and transfer filter papers were purchased from Bio-Rad (Hercules, CA). The anti-apoE antibody was a generous gift from Dr. M. Kindy. All other reagents were purchased from Sigma (St. Louis, MO).

Synaptosomal Preparations. Cortical synaptosomes were prepared as described previously (*24*). Briefly, cortices were isolated from 1−3-month-old male C57BL/6J or C57BL/6J apoE$^{tm/Unc}$ (apoE-deficient) mice and homogenized in isolation buffer (0.32 M sucrose, 4 μg/mL leupeptin, 4 μg/mL pepstatin, 5 μg/mL aprotinin, 20 μg/mL trypsin inhibitor, 0.2 mM PMSF, 2 mM EDTA, 2 mM EGTA, and 20 mM HEPES) by 12 passes with a motorized Teflon pestle. The homogenate was centrifuged at 3 800 rpm (1500*g*) for 10 min at 4 °C. The supernatant was collected and centrifuged at 14 800 rpm (20000*g*) for 10 min at 4 °C. The resulting pellet was mixed in a small volume of cold isolation buffer and layered onto discontinuous sucrose gradients containing 10 mL each of 1.18, 1.0, and 0.85 M sucrose solutions each with 2 mM EDTA, 2 mM EGTA, and 10 mM HEPES (pH 8.0 for 0.85 and 1.0 M solutions, pH 8.5 for 1.18 M solution). The gradients were centrifuged in a Beckman L7-55 ultracentrifuge at 22 000 rpm (82500*g*) for 2 h at 4 °C. The resulting purified synaptosomal vesicles were removed from the 1.18 M/1.0 M interface and subsequently washed twice in PBS. Protein concentrations were determined by the Pierce BCA method and all samples were adjusted to a concentration of 3−4 mg/mL.

Western Blotting. Analysis of apoE immunoreactivity was performed by Western blotting methods as described previously (*24*). Aliquots of synaptosomes were solubilized and separated by electrophoresis on a 7.5% polyacrylamide gel. Proteins were then transferred to nitrocellulose, and incubated with an anti-apoE antibody (1:1000). ApoE immunoreactivity was detected upon exposure of film after nitrocellulose was incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody and a chemiluminescent substrate for HRP.

Spin Labeling and EPR. The assessment of lipid bilayer alterations in synaptosomal membranes isolated from WT and apoE KO mice was conducted with a lipid-specific spin probe, 5-nitroxide stearate (5-NS). 5-NS was prepared in disposable glass culture tubes from a 10 mg/mL stock concentration in chloroform. The probe was added to the culture tubes in chloroform at a ratio of 6 μg 5-NS/mg protein (*25*). Tubes were placed under vacuum to evaporate the chloroform, leaving only the spin probe as a thin film. Synaptosomes (3 mg/0.5 mL in PBS) were added to the 5-NS-coated tubes and incubated with gentle shaking for 1 h at room temperature in order to initiate labeling of the synaptosomal membrane lipid bilayer. The volume of each sample was adjusted to 1 mL with PBS and the samples were taken immediately for EPR analysis. EPR spectra were recorded for each sample before the addition of 5 μM Aβ-(1−40) with the peptide being added immediately following acquisition of the control spectrum. Subsequent spectra were taken 1 h after the addition of Aβ.

For protein conformational studies in synaptosomal membranes isolated from WT and apoE KO mice, a protein-specific spin label was used. Mal-6 (2,2,6,6-tetramethyl-4-maleimidopiperidin-1-oxyl) was dissolved in 100 μL of acetonitrile and diluted to a final concentration of 200 μM in 50 mL of lysing buffer (2 mM EDTA, 2 mM EGTA, 10 mM HEPES, pH 7.4). Samples were labeled by incubating 12.5 μg Mal-6/mg of protein (50 μM final concentration) overnight at 4 °C (*26*). Synaptosomes were pelleted in a refrigerated Eppendorf tabletop centrifuge, the supernatant discarded and replaced with fresh lysing buffer. After mixing, the cycle was repeated. Samples were washed six times to ensure complete removal of all unbound spin label before acquiring EPR spectra. To determine protein conformational changes induced by Aβ(1−40), synaptosomes were incubated with 5 μM of the peptide for 3 h at 37 °C. After incubation, samples were washed twice in PBS as indicated above and labeled with Mal-6.

EPR spectra were acquired on a Bruker EMX spectrometer with the following instrumental parameters: microwave power, 20 mW; microwave frequency, 9.77 GHz; modulation amplitude, 1.0 G; modulation frequency, 100 kHz; receiver gain, 1×10^5; time constant, 0.64 ms.

Slot Blotting. Levels of HNE and protein carbonyls were determined by slot blot analyses of WT and apoE KO synaptosomes before and after treatment with 5 μM Aβ(1−40). Proteins were filtered under vacuum onto a nitrocellulose membrane which was subsequently blocked with PBS containing 3% BSA. Detection of protein carbonyls required sample derivatization prior to filtration. Sample aliquots (30−50 μg protein) were incubated with 2,4-dinitrophenylhydrazine in the presence of SDS for 20 min at room temperature (rt) and quenched by the addition of a neutralization solution. The 2,4-dinitrophenyl hydrazone (DNP) adduct of the carbonyls is detected on the nitrocellulose paper using a rabbit antibody specific for DNP−protein adducts (1:150). HNE adducts were detected on the nitrocellulose membrane using a rabbit anti-HNE antibody (1:4000) as described previously (*27*). Following incubation with primary antibodies, nitrocellulose papers were incubated with an alkaline phosphatase-conjugated secondary antibody (goat anti-rabbit; 1:15000). SigmaFast was used as the colorimetric substrate for alkaline phosphatase. Blots were scanned into Adobe Photoshop and quantitated with Scion Image (PC version of Macintosh compatible NIH Image).

Thiobarbituric Acid Reactive Substances (TBARs). Levels of TBARs were determined in WT and apoE KO synaptosomes before and after treatment with 5 μM Aβ(1−40). Aliquots of 250 μg protein were taken from each sample and precipitated with 0.4 mL of ice-cold 10% TCA. After centrifugation for 5 min at 6000 rpm (3000*g*), 0.4 mL of the supernatant was incubated with 0.2 mL of thiobarbituric acid (0.335% TBA in 50% glacial acetic acid) for 1 h at 100 °C. Samples were allowed to cool to rt before addition of 0.4 mL of butanol. After mixing each sample with a pipet, the organic layer was allowed to separate it from the aqueous layer, and 100 μL was immediately removed from the top

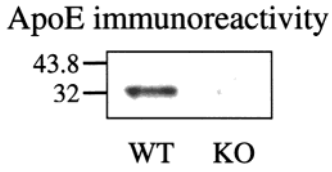

FIGURE 1: ApoE is present in synaptosomes prepared from WT but not in synaptosomes prepared from apoE KO mice. 50 μg of synaptosomal protein were separated by SDS−PAGE, transferred to nitrocellulose membranes, and incubated with an anti-apoE antibody. Molecular weight markers are indicated as 32 and 43.8 kDa.

organic phase and added to a 96-well plate. TBARs were detected by measuring the fluorescence with an λ_{ex} of 518 nm and an λ_{em} of 588 nm.

RESULTS

Alterations in ApoE KO Synaptosomal Membrane Structure. Because apoE KO mice have been reported to display increased basal levels of lipid peroxidation in the CNS (*11*, *12*, *24*, *28*−*30*), studies were conducted to investigate alterations in synaptosomal membrane structure with parameters that are known to change under oxidative conditions. Western blot analysis demonstrated that apoE KO synaptosomes were devoid of apoE, while WT synaptosomes exhibited a strong band of apoE immunoreactivity (Figure 1), suggesting that differences in membrane structure may be due to the absence of apoE. Structural alterations in synaptosomes with and without apoE were determined using EPR in conjunction with the lipid-specific spin probe, 5-NS, and the protein-specific spin label, Mal-6. EPR spectral changes are the result of changes in the motion of the nitroxide groups either covalently bound to synaptosomal membrane proteins (Mal-6) or intercalated within the synaptosomal lipid domain (5-NS). Thus, spectral changes represent structural alterations to synaptosomal protein or lipid microenvironments (*26*).

The half-width-at-half-height (HWHH) of the low-field ($M_I = +1$) resonance line of 5-NS (Figure 2A) is a measure of phospholipid order and motion that is collectively termed membrane fluidity (*26*). 5-NS undergoes anisotropic rotation around its long axis in the membrane bilayer as well as reorientation motion of the principal axis of the nitrogen π-orbital of the nitroxide between parallel and perpendicular orientations with respect to the major rotational axis. Similar to chemical exchange phenomena, as the reorientation rate increases, as would occur in a more fluid bilayer, the HWHH parameter increases (*26*). On the basis of numerous studies, the dynamic range of HWHH is from about 0−15% change. Analysis of EPR spectra from synaptosomes incubated with 5-NS suggests that apoE KO synaptosomes have a more fluid membrane bilayer than do WT synaptosomes. Measurement of the HWHH reveals a significant increase ($p < 0.006$, 8%) in the membrane fluidity of apoE KO synaptosomes compared to WT synaptosomes (Figure 2B).

Mal-6 binds mainly to protein thiol residues which can be classified into two main environments with respect to the motion of the spin label: weakly (W) and strongly (S) immobilized. The motion of Mal-6 bound to W sites is only weakly restricted, which is manifested as narrow lines in the EPR spectrum. In contrast, Mal-6 bound to S sites have strongly hindered motion, which is manifested as broadened

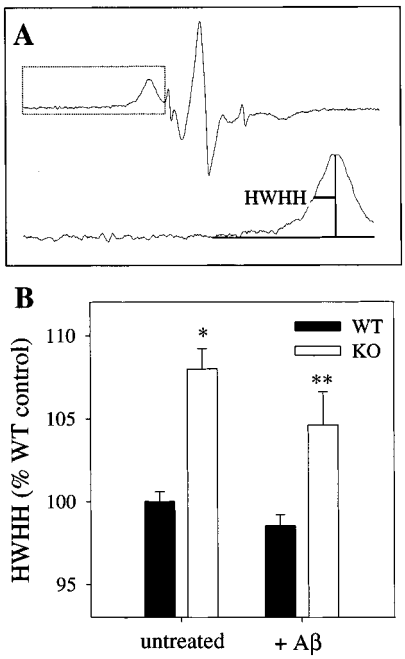

FIGURE 2: Synaptosomal membranes from apoE KO mice display membrane lipid structural alterations at basal levels and an increased vulnerability to lipid oxidation induced by Aβ(1−40). (A) Changes in the reorientation rate of the 5-NS spin probe within synaptosomal membranes are monitored by analyzing the half-width-at-half-height (HWHH) of the low-field EPR line. (B) At basal levels, 5-NS spectra from apoE KO synaptosomes have a greater HWHH [i.e., increased membrane lipid motion and decreased order (fluidity)] when compared to spectra from WT synaptosomes. During a 1-h incubation at 37 °C, 5 μM Aβ(1−40) induces a significant change in the HWHH of synaptosomal membranes isolated from apoE KO mice but not in WT synaptosomes. Data are the mean and SEM from 5−6 preparations (*$p < 0.006$ vs WT basal, **$p < 0.04$ vs KO basal; Student's *t*-test).

lines in the EPR spectrum. The resulting intensities of the respective W and S peaks of the $M_I = +1$ low-field resonance lines give rise to the W/S ratio, which is highly sensitive to protein conformational changes (*26*). The W/S ratio of apoE KO synaptosomes labeled with Mal-6 is decreased 5% ($p < 0.02$) when compared to WT synaptosomes (Figure 3). Decreased values of the W/S ratio arise from increased protein−protein interactions, protein cross-linking, or decreased segmental motion of spin-labeled proteins and invariably are lowered in synaptosomes following protein oxidation (*31*−*35*).

Susceptibility of ApoE KO Synaptosomal Membranes to Oxidative Damage. ApoE is thought to modulate response to neuronal injury (*7*, *8*). Accordingly, WT and apoE KO synaptosomes were incubated with 5 μM Aβ(1−40) to determine susceptibility to further oxidative damage. Aβ-peptides generate H_2O_2 and TBARs in solution (*36*, *37*) and induce oxidative modifications to protein and lipid components of cells (*38*−*42*) and synaptosomes (*20*, *43*−*45*) resulting in degeneration and death. As oxidation alters the physical state of synaptosomal membrane lipids and proteins, EPR analysis of synaptosomal lipid and protein structure after treatment with 5 μM Aβ(1−40) was performed. Further, to determine the extent of Aβ-induced oxidation to synaptosomes, the levels of protein oxidation, HNE modification, and TBARs were measured.

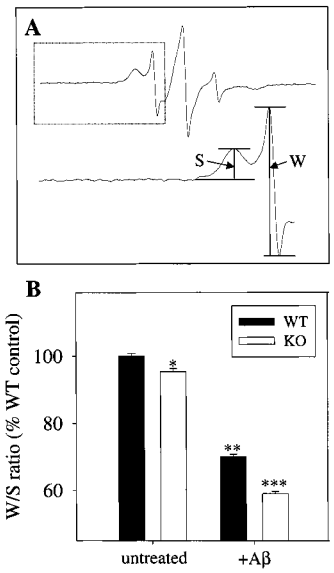

FIGURE 3: Synaptosomal membranes from apoE KO mice have altered protein conformations and are susceptible to further change induced by treatment with Aβ(1−40). (A) Changes in the motion of Mal-6 spin labeled proteins are assessed by changes in the W/S ratio and are indicative of protein conformational changes. (B) Indicated by a decrease in the W/S ratio, apoE KO synaptosomal membrane proteins have altered conformations under basal conditions. After a 3-h incubation at 37 °C with 5 μM Aβ(1−40), a decrease in the W/S ratio occurs in apoE KO synaptosomal proteins that is greater than the decrease observed in WT synaptosomal proteins. Data are the mean and SEM from 4−6 preparations (*p < 0.02 vs WT basal, **p < 0.001 vs WT basal, ***p < 0.001 vs KO basal and WT treated; Student's t-test).

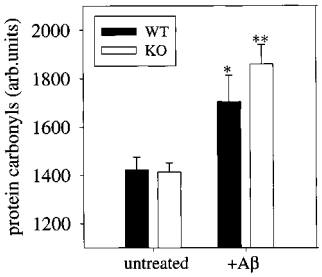

FIGURE 4: Synaptosomes from apoE KO mice are susceptible to increases in protein oxidation induced by Aβ(1−40). Treatment of synaptosomes with 5 μM Aβ(1−40) for 3 h at 37 °C resulted in increased protein carbonyl levels that are significantly greater in apoE KO synaptosomes as compared to WT synaptosomes. Data are the mean and SEM from duplicates of 5 preparations (*p < 0.02 vs WT untreated, **p < 0.001 vs KO untreated, **p < 0.04 vs WT treated; Student's t-test).

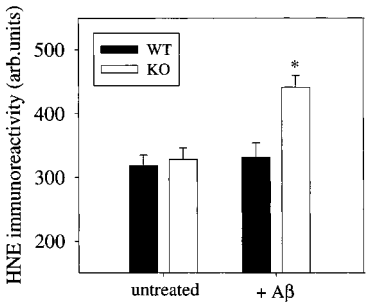

FIGURE 5: Protein-bound HNE is increased in apoE KO synaptosomes after treatment with Aβ(1−40). Treatment of synaptosomes with 5 μM Aβ(1−40) for 3 h at 37 °C resulted in a significant increase of covalent protein modifications by HNE in apoE KO membranes but not in WT membranes. Data are the mean and SEM from duplicates of 5 preparations (*p < 0.008 vs untreated KO and WT, treated WT; Student's t-test).

Both lipid and protein structural alterations induced by Aβ were more severe in apoE KO synaptosomes. There is a significant (p < 0.04, 3.4%) increase in membrane rigidity (or decrease in HWHH) in apoE KO synaptosomes after a 1-h treatment with 5 μM Aβ(1−40), while the change in WT synaptosomes was insignificant (Figure 2B). Aβ also induced changes in synaptosomal protein conformation after 3 h, but these changes were significant in both WT (p < 0.001, 30%) and apoE KO (p < 0.001, 41%) membranes. However, the overall decrease in W/S in apoE KO synaptosomes is significantly different (p < 0.001) from the change observed in WT synaptosomes (Figure 3B).

Similarly, the absence of apoE increases the vulnerability of synaptosomes to oxidative damage as measured by overall increases in oxidative markers. Protein carbonyls are markers of protein oxidation (*21*) and increase in various paradigms of oxidative injury (*32*−*35*, *38*, *39*, *46*, *47*). HNE and TBARs are formed by free radical oxidation of unsaturated lipids (*22*) and are increased after CNS injury (*5*, *48*, *49*) or by the addition of Aβ to cultured neurons or synaptosomes (*44*, *50*, *51*). HNE covalently modifies proteins by reacting with cysteine, histidine, and lysine residues of the protein backbone (*21*, *22*). Although the analysis of protein carbonyls and protein-bound HNE indicates that there is no difference between untreated WT and apoE KO synaptosomes, a 3-h incubation with 5 μM Aβ(1−40) causes increases in both markers that are significantly greater in apoE KO synaptosomes. Aβ induces significant increases in protein carbonyls in both WT (p < 0.02, 20%) and apoE KO (p < 0.001, 32%) preparations; however, the increase in apoE KO synaptosomes is significantly different (p < 0.04) from the increase observed in WT membranes (Figure 4). Interestingly, after a 3-h incubation with Aβ, protein-bound HNE is significantly increased (p < 0.008, 35%) in synaptosomes lacking apoE, while WT synaptosomal proteins remain unchanged (Figure 5). Measurement of TBARs suggests that there is a significant difference (p < 0.025, 31%) between untreated WT and apoE KO synaptosomes, while a 3-h incubation with 5 μM Aβ(1−40) results in an overall increase in TBARs in KO synaptosomes that is significantly higher than the increase in TBARs formed when apoE is present (Figure 6).

DISCUSSION

The present study demonstrates that synaptosomes isolated from apoE KO mice are structurally different than synaptosomes isolated from WT mice. For example, synaptosomal membrane lipid dynamics from apoE KO mice are increased. EPR studies reveal that apoE KO synaptosomes have increased membrane fluidity, monitored by increased anisotropic motion of the paramagnetic spin probe, 5-NS. Our results are consistent with previous reports of increased membrane fluidity in apoE KO synaptic membranes (*24*, *52*). ApoE KO mice display increases in oxidized cholesterol (*28*), which has been found to increase membrane dynamics (*53*). ApoE facilitates the transport of cholesterol in to and out of cells (*54*), and in the absence of apoE, oxidized cholesterol may accumulate and perturb membrane structure. The increased synaptosomal membrane bilayer fluidity in apoE KO synaptosomes is, however, unlikely to be due to

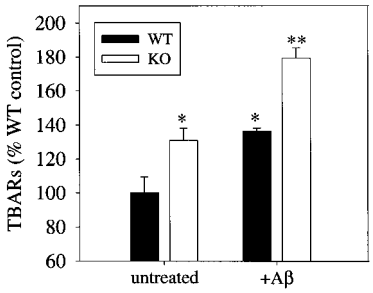

FIGURE 6: ApoE KO synaptosomes are vulnerable to Aβ(1−40)-induced increases in TBARs. Untreated synaptosomes from apoE KO mice have increased levels of TBARS when compared to untreated synaptosomes from WT mice. Synaptosomes treated with 5 μM Aβ(1−40) for 3 h at 37 °C resulted in the increased formation of TBARS from both WT and apoE KO membranes; however, apoE KO synaptosomes displayed significantly higher levels after treatment, indicating a vulnerability to oxidative insult by Aβ. Data are the mean and SEM from duplicates of 4−5 preparations (*p < 0.025 vs untreated WT, **p < 0.0003 vs untreated KO and treated WT; Student's t-test).

alterations in the cholesterol/phospholipid ratio, since this ratio is reportedly unchanged in the absence of apoE (*52*). A redistribution of cholesterol between the two phospholipid leaflets of the membrane bilayer does exist in apoE KO synaptic membranes (*52*), which, together with increased oxidized cholesterol, may contribute to the increased membrane fluidity observed in our study (*55*). In this study, we also demonstrated that Aβ(1−40) increases the rigidity of the synaptosomal membrane in the absence of apoE, supporting the role of apoE in the modulation of oxidative damage. These findings are consistent with previous studies of the effects of Aβ on membranes (*56*) and are likely due to the oxidative properties of the Aβ-peptides (*23*, *36*, *37*). Therefore, on the basis of Aβ-induced oxidative modifications to membranes resulting in decreased membrane fluidity, it is likely that the initial increases in membrane fluidity observed are due to altered cholesterol transport in the absence of apoE.

Synaptosomes isolated from apoE KO mice also have significantly altered membrane protein conformations, as assessed by the W/S ratio of Mal-6. This parameter has been reported to consistently decrease in various oxidative stress paradigms (*31*−*35*). Motion of the covalently bound spin label, Mal-6, is sensitive to protein conformational changes that result from increased protein−protein interactions and protein carbonyl formation, both of which can be induced by oxidative treatment (*23*). Modification of proteins byproducts of lipid oxidation such as HNE also decreases the W/S ratio (*57*). These covalent modifications may account for the protein structural changes reported in untreated apoE KO membranes. However, since basal levels of both protein carbonyls and HNE are not different in the presence or absence of apoE, the reported structural damage to lipids in apoE KO synaptosomes may induce the basal structural changes in these proteins.

Because Aβ-peptides generate ROS (*23*, *36*, *37*), treatment of synaptosomes with Aβ(1−40) resulted in an overall increase in oxidative modification. Specifically, Aβ induced increases in TBARs, HNE, and protein carbonyls as well as changes in membrane protein and lipid structure in both WT and apoE KO synaptosomes. However, the observed changes were consistently greater in synaptosomes lacking apoE. This suggests that apoE plays a role in the modulation of oxidative damage, while the absence of apoE renders synaptosomes susceptible to oxidative injury. Given the role of apoE in lipid trafficking (*54*), apoE may be particularly important in the modulation of lipid oxidation and may be relevant in the explanation of our findings. By covalent modification, products of lipid oxidation (e.g. HNE) induce changes in protein conformation (*57*), introduce carbonyl groups, and directly raise HNE immunoreactivity. Thus, increased lipid oxidation induced by Aβ(1−40) may account for the greater observed changes in the W/S ratio, carbonyl formation, TBARs, and HNE levels in apoE KO synaptosomal proteins relative to the WT samples. Consistent with increased HNE in apoE KO, but not in WT, synaptosomes after oxidation by Aβ(1−40) presented in the current study, apoE has been demonstrated to scavenge HNE (*18*), promoting the role of apoE in the clearance of lipid oxidation products.

ApoE has been implicated in the modulation of oxidative injury in the brain by prevention and/or repair of damage. Synthesis of apoE is upregulated, and although synthesized in astrocytes, apoE immunoreactivity is detected in neurons in response to ischemic injury (*7*, *8*). ApoE exhibits antioxidant properties and protects cells against ROS toxicity (*17*), while mice deficient in apoE display increased neurodegeneration (*9*, *16*). The data in the current study support these findings and indicate an increased vulnerability of apoE KO synaptosomes to oxidative modification of proteins and lipids. In this study, protein and lipid modifications that may result from unrepaired oxidative damage in the absence of apoE are reported. Increased membrane permeability as a consequence of increased membrane fluidity (*58*) may alter Ca^{2+} homeostasis (*59*) and may explain Aβ-induced increases in ROS and mitochondrial dysfunction reported in synaptosomes deficient in apoE (*24*). Further, protein structure is critical for proper function, and changes in protein conformations in the absence of apoE may result in altered function of apoE KO synaptosomal proteins. This may be particularly relevant at the synapse where transporter and receptor functions are critical. In particular, glutamate transporters and ion motive ATPases (e.g. Na^+/K^+ ATPase) play critical roles in the maintenance of synaptic homeostasis (*44*, *60*), and the loss of apoE may contribute to the impaired function of these proteins.

A major risk factor for AD is the inheritance of the ϵ4 allele of apoE (*1*). Oxidative damage is also believed to play a role in the pathlology of AD (*2*, *23*). There is a reported apoE allele specificity (ϵ3 > ϵ4) in antioxidant properties (*17*), HNE scavenging (*18*), and protection against ischemia- and kainic acid-induced lesions (*15*, *16*). Furthermore, there is evidence that oxidative damage in AD patients is also apoE allele-dependent. Montine et al. have demonstrated that HNE immunoreactivity in AD brain is associated with the inheritance of the ϵ4 allele of apoE (*3*, *4*). Additional oxidative damage as well as increases in antioxidant enzymes in the hippocampus of AD brains has also been reported to be ϵ4-dependent (*5*, *6*). Also an apoE isoform dependence (ϵ4 > ϵ3) on Aβ deposition in a mouse model of AD has been reported (*61*). These data suggest that the apoE genotype may play a critical role in the neuropathology of AD.

The current report demonstrates that mice deficient in apoE exhibit synaptosomal membrane structural alterations and an increased susceptibility to oxidative damage. Synaptosomal

membrane structure may likely be critical to synaptic function while increased vulnerability to oxidative injury may be detrimental to neuronal tissue. These findings support the hypothesis that apoE may play a role in the modulation of oxidative damage in the brain and that synaptic integrity is altered in the absence of apoE.

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