Dietary Supplementation with Vitamin E Reverses the Age-related Deficit in Long Term Potentiation in Dentate Gyrus*

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Long term potentiation (LTP) in dentate gyrus is impaired in aged rats, and this has been associated with an age-related decrease in membrane arachidonic acid concentration. In this study, we considered whether the trigger for this age-related decrease in arachidonic acid might be increased lipid peroxidation stimulated by the proinflammatory cytokine, interleukin-1β. Groups of aged and young rats were fed on a control diet or a diet supplemented with α-tocopherol and assessed for their ability to sustain LTP. Aged rats fed on the control diet exhibited an impaired ability to sustain LTP and analysis of tissue prepared from these rats exhibited increased interleukin-1β, increased lipid peroxidation, and decreased membrane arachidonic acid concentration compared with young rats fed on either diet. Aged rats fed on the supplemented diet sustained LTP in a manner indistinguishable from young rats, and the age-related increases in interleukin-1β and lipid peroxidation and the decrease in membrane arachidonic acid concentration were all reversed. We propose that interleukin-1β may be the trigger that induces these age-related changes and may therefore be responsible for the deficit in long term potentiation in aged rats. The observation that α-tocopherol reverses these changes is consistent with the hypothesis that some age-related changes in hippocampus might derive from oxidative stress.

Aging is associated with compromised neuronal activity, perhaps typified by a deficit in cognitive function. One particular age-related impairment in neuronal function in the rat is a decrease in ability to sustain long term potentiation (LTP); Refs. 1–5, which is a putative biological substrate for cognitive function and a model for learning and/or memory. Evidence suggests that a cascade of biochemical changes, initiated by activation of the N-methyl-D-aspartate subtype of glutamate receptor is required to support this form of synaptic plasticity in the dentate gyrus (6). Although the underlying cause of the age-related decrease in ability to sustain LTP is not known, down-regulation of several components of the biochemical cascade has been identified in aged rats. Thus, N-methyl-D-aspartate receptor activity is compromised with age (e.g. Ref. 7), whereas down-regulation of other factors that play a role in induction of LTP, like calcium handling by cells (1) and activation of enzymes such as phospholipase A2 (3) and protein kinases (8), has been reported. Similarly, factors that play a role in maintenance of LTP, like increased glutamate release, have also been shown to be compromised in hippocampus of aged rats (3, 5, 9). In addition, the more persistent aspects of LTP, which rely on increased synthesis of new proteins (10–12) and morphological changes (13), are also impaired in the hippocampus of aged rats (12, 14). Despite this immense body of data, the underlying cause of the impairment in ability of aged rats to sustain LTP is not known.

One unifying cause of the age-related changes in biochemical parameters might be a change in membrane composition. Thus, one might predict that, if membrane fluidity is altered, the activation of membrane proteins like receptors, ion channels, and membrane-associated enzymes might also be altered. Membrane arachidonic acid concentration has been shown to be markedly decreased with increasing age (3, 5); this is likely to decrease membrane fluidity (see Ref. 15) and is therefore one change that might explain significant changes in activity of the hippocampal membrane-associated proteins that impact on expression of LTP.

In the context of the change in membrane arachidonic acid concentration and the presumed increase in membrane rigidity, it is important to consider the growing body of evidence that supports the original hypothesis of Harman (16) that free radicals contribute to age-related changes in the central nervous system. One hallmark of free radical damage is lipid peroxidation; most reports indicate that lipid peroxidation increases with increasing age (17, 18), and this is supported by the observation that lipofuscin, one characteristic reaction product, accumulates in the aged brain (18). With specific reference to the hippocampus, recent evidence from this laboratory has indicated that there is an age-related increase in lipid peroxidation in the hippocampus of aged rats (19), supporting earlier findings (Ref. 20; see also Ref. 21). It has been acknowledged for many years that lipid peroxidation will profoundly affect membrane composition since it results in depletion of polyunsaturated fatty acids (22); therefore, the increase in lipid peroxidation in hippocampus may be the underlying cause of the age-related decrease in membrane arachidonic acid concentration (3, 5).

An increase in lipid peroxidation will be triggered by an accumulation of reactive oxygen species, and this arises from a compromise in antioxidant defenses. Neuronal tissue is particularly vulnerable to oxidative insults; the vulnerability results from high oxygen consumption of brain tissue, coupled with modest antioxidant defense (23), high concentrations of iron, particularly in hippocampus (24), and high concentrations of polyunsaturated fatty acids, which are target substrates for reactive oxygen species (25). Accumulation of oxidative damage is readily observed in neuronal tissue (26, 27), and there is

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1 The abbreviations used are: LTP, long term potentiation; ANOVA, analysis of variance; IL-1β, interleukin-1β; PBS, phosphate-buffered saline; MDA, malondialdehyde; HPLC, high performance liquid chromatography; EPSP, excitatory postsynaptic potential.
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substantial evidence that expression of the proinflammatory cytokine interleukin-1β (IL-1β) and its receptor are increased in brain tissue exhibiting oxidative damage (28).

The importance of a-tocopherol, one naturally occurring antioxidant, for normal neuronal cell function has been defined most clearly by studies that assess the consequences of vitamin E deficiency. Thus, vitamin E deficiency in patients has been noted, among other problems, neurological deficiencies (29), whereas prolonged vitamin E deficiency in rats leads to demyelination of axons and gliosis (30). Maintenance of rats on a vitamin E-deficient diet markedly reduced the vitamin concentration in whole brain (31) or cortex (32), and this was associated with increased lipid peroxidation and increased susceptibility to oxidative stress (17, 31, 32), whereas protection against oxidative damage was offered by vitamin E administration (31). In parallel, vitamin E-deficient rats had impaired ability to sustain LTP (33), whereas a-tocopherol induced synaptic potentiation (34).

In light of these observations, we considered that the impaired ability of aged rats to sustain LTP might be due to a decrease in membrane arachidonic acid. Our hypothesis is that this change is triggered by increased lipid peroxidation, which in turn is triggered by IL-1β, perhaps through formation of reactive oxygen species. To test this hypothesis, aged and young rats were fed on either a control diet or a diet enriched in α-tocopherol and l-ascorbate (to limit oxidation of α-tocopherol). At the end of this period, rats were analyzed for their ability to sustain LTP in dentate gyrus in vivo and these data were interpreted in parallel with analyses performed on tissue prepared from hippocampus obtained from these rats at the end of the electrophysiological recording period. We report that dietary supplementation with antioxidant vitamins reversed the age-related decrease in α-tocopherol concentration and concomitantly restored ability of these rats to sustain LTP. The data presented are consistent with the view that an age-related increase in endogenous concentrations of IL-1β may be the underlying cause of certain neuronal impairments in hippocampus of aged rats.

EXPERIMENTAL PROCEDURES

Animals—Groups of male Wistar rats (average ages: 4 months and 22 months) were used in these experiments. Animals were housed in groups of 2–4 under a 12-h light schedule. Ambient temperature was constant at 22 and 23 °C. Food and water was freely available, and intake was measured daily for 2 weeks before commencement of the experiment and throughout the experiment. Aged (22 months) and young (4 months) rats were then randomly subdivided into two groups. One group received normal laboratory chow with added dl-α-tocopheryl acetate (250 mg/rat/day dissolved in corn oil; Beeline Healthcare, Dublin, Ireland) and drinking water to which l-ascorbic acid (250 mg/rat/day; Beeline Healthcare) was added. l-Absorbic acid was included since it has the ability to recycle vitamin E by reducing the oxidized form (35, 36). The second group received normal laboratory chow with corn oil added to ensure isocaloric intake with the first group. Food and water intake did not vary between groups, and there was no significant difference in daily food and water intake before and after dietary modifications were made. Rats were fed on the respective control or supplemented diet for 3 months; at the end of this time, ability of animals to sustain LTP was assessed. Animals were housed in the BioResources Unit at Trinity College, Dublin under veterinary supervision for the duration of this experiment.

Induction of LTP in Vivo—Animals were anesthetized by intraperitoneal injection of urethane; the absence of a pedal reflex was used as the indicator of adequate anesthesia. Young animals initially received 1.5 g/kg urethane, and an additional increment of up to 0.5 g/kg if required; the mean dose of urethane administered to 4-month-old rats was 2.07 g/kg (± 0.11; S.E.). In the case of 22-month-old animals, the initial dose of urethane was reduced to 1.0 g/kg and topped up if necessary; the mean dose of urethane given to 22-month-old rats was 1.63 g/kg (± 0.10).

LTP was induced in perforant path-granule cell synapses as described previously (5). Briefly, rats were placed in a head holder in a stereotaxic frame and a window of skull was removed to allow placement of a bipolar stimulating electrode in the perforant path (4.4 mm lateral to lambda) and a unipolar recording electrode in the dorsal cell body region of the dentate gyrus (2.5 mm lateral and 3.9 mm posterior to lambda). The depths of the stimulating and recording electrodes were adjusted to maximize the slope of the excitatory postsynaptic potential (EPSP) and the stimulating intensity was adjusted to produce a population spike amplitude of about 1 mV. Test shocks were delivered unilaterally at 30-s intervals and after a 10-min control period, three trains of stimuli (250 Hz for 200 ms) were delivered with an intertrain interval of 1 min (Fig. 1). Evaluations indicated that this stimulation paradigm produced saturation of LTP in perforant path-granule cell synapses. Recording at test shock frequency then resumed for 40 min. Data were stored on disk for later analysis. At the end of the recording period, rats were killed by cervical dislocation. The dentate gyri from the ipsilateral (i.e. tetanized) and contralateral (i.e. untetanized) sides were cross-chopped (350 μm x 350 μm) and frozen separately in 1 ml of Krebs solution (composition of Krebs in mM: NaCl 136, KCl 2.54, KH₂PO₄ 1.18, MgSO₄, 7H₂O 1.18, NaHCO₃ 16, glucose 10, CaCl₂ 1.13) containing 10% dimethyl sulfoxide as described previously (37). The remaining hippocampal (i.e. whole hippocampus minus dentate gyrus) tissue was treated in the same way, and all samples were stored at 80 °C until required. For analysis, slices were thawed rapidly (1.5–2 min) and rinsed four times in oxygenated Krebs solution. Analysis of IL-1β, lipid peroxidation, and arachidonic acid were made in samples prepared from untetanized dentate gyrus. Formation of reactive oxygen species was analyzed in stored contralateral (untetanized) hippocampal tissue (minus dentate gyrus).

Analysis of IL-1β Expression—We used an enzyme-linked immunosorbent assay for analysis of IL-1β (DuoSet ELISA Development System, Genzyme Diagnostics). Slices of dentate gyrus prepared from aged and young animals were homogenized in Tris buffer (50 mM; 250 μl, pH 7.4) containing 1 mM EDTA, 5 mM dithiothreitol, 0.1 mM mg/ml trypsin inhibitor, 50 μM leupeptin, and 50 μM aprotinin. For analysis of IL-1β, 96-well plates were coated with 100 μl of capture antibody (2.0 μg/ml final concentration, diluted in 0.1 mM sodium carbonate buffer, pH 9.5; monoclonal hamster anti-mouse IL-1β antibody) and incubated overnight at 4 °C. Wells were washed several times with phosphate-buffered saline (PBS) containing 0.05% Tween 20 and then blocked for 2 h at 37 °C with 250 μl of blocking buffer (PBS, pH 7.3; 0.1% w/v bovine serum albumin). Blocking buffer was aspirated, and aliquots (100 μl) of samples or IL-1β standards (0–1000 pg/ml) were added to each well, and incubated for 1 h at 37 °C. Plates were washed with secondary antibody (100 μl; final concentration 0.8 μg/ml in PBS containing 0.05% Tween 20 and 1% bovine serum albumin; biotinylated polyclonal rabbit anti-mouse IL-1β antibody) was added to each well, and incubation continued for 1 h at 37 °C. The plates were then washed, 100 μl of detection agent (horseradish peroxidase-conjugated streptavidin; 1:1000 dilution in PBS containing 0.05% Tween 20 and 1% bovine serum albumin) was added to each well, incubated for another 15 min at 37 °C, and plates were again washed. Aliquots of substrate (100 μl; tetramethylbenzidine liquid substrate; Sigma) were added, and the plate was incubated at room temperature for 10 min. Absorbance was read at 450 nm within 30 min. Protein concentrations were determined (38), and results were expressed as nanograms of IL-1β/mg of tissue corrected for protein concentration.

Analysis of Lipid Peroxidation—Formation of malondialdehyde (MDA) was used to assess lipid peroxidation as described previously (39). Aliquots (10 μl) of homogenate prepared from dentate gyrus were incubated at 37 °C for 60 min, at which time 8.1% (w/v) SDS (30 μl), 20% acetic acid (pH 3.5 with NaOH; 225 μl), and 0.8% (w/v) thiobarbituric acid (225 μl) were added. The volume was then adjusted with H₂O to 600 μl, and the samples were incubated for another 60 min at 95 °C, cooled at room temperature, and absorbance assessed at 532 nm with reference to a standard curve made using 1,1,3,3-tetramethoxypropane. In some experiments, IL-1β (3.5 ng/ml) or hydrogen peroxide (5 mM) were added to analyze its effect on lipid peroxidation; the effect of antioxidants (± a-tocopherol phosphate (disodium salt; 200 μM) or melatonin (5 mM) on IL-1β- or hydrogen peroxide-induced lipid peroxidation was assessed as described previously. Results were expressed as nanomoles of MDA/mg of protein for the in vitro experiments. Since we observed that values for protein/mg of tissue were different in hippocampal preparations obtained from aged and young rats, data from these experiments were expressed as nanomoles/mg of tissue corrected for protein concentration.

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reactive oxygen species was assessed in hippocampal tissue (minus dentate gyrus) as described previously (40). The method relies on oxidation of the non-fluorescent probe, 2',7'-dichlorofluorescein diacetate, by reactive oxygen species, to the highly fluorescent 2',7'-dichlorofluorescein. To assess reactive oxygen species production, either homogenate or the synaptosomal pellet, \( P_s \), was resuspended in 1 ml ice-cold 40 mM Tris buffer (pH 7.4). Aliquots (1 ml) of synaptosomes or homogenate were incubated with 2',7'-dichlorofluorescein diacetate (10 \( \mu l \); final concentration 5 \( \mu M \); from a stock solution of 500 \( \mu M \) in methanol) at 37 °C for 15 min. Analysis was performed in hippocampal preparations under control conditions (i.e. no additions), in the presence of IL-1 \( \beta \) (3.5 ng/ml) or in the presence of IL-1 \( \beta \) (3.5 ng/ml) and (+)-\( \alpha \)-tocopherol phosphate (disodium salt; 200 \( \mu M \)). In a separate group of experiments, we analyzed reactive oxygen species production in tissue prepared from aged and young rats that were fed on the control diet, and aged and young rats that were fed on the experimental diet. To terminate the reaction, the dye-loaded synaptosomes were centrifuged at 13,000 \( \times \) g for 8 min. The pellet was resuspended in 3 ml of ice-cold 40 mM Tris buffer, pH 7.4. Aliquots of resuspended synaptosomes (750 \( \mu l \)) were incubated in the presence or absence of IL-1 \( \beta \) (3.5 ng/ml). Fluorescence was monitored at a constant temperature of 37 °C immediately before and after addition of the sample. Aliquots (1 ml) of synaptosomes or homogenate were incubated in the presence or absence of IL-1 \( \beta \) (3.5 ng/ml) for 8 min. The pellet was resuspended in 3 ml of ice-cold 40 mM Tris buffer, pH 7.4. Aliquots of resuspended synaptosomes (750 \( \mu l \)) were incubated in the presence of IL-1 \( \beta \) (3.5 ng/ml). Fluorescence was monitored at a constant temperature of 37 °C immediately before and after addition of the sample. Al.

**RESULTS**

The mean pretreatment EPSP slope was similar in 4-month-old and 22-month-old rats (1.13 mV/ms ± 0.11 (S.E.) and 1.11 mV/ms ± 0.09, respectively). Dietary supplementation with (+)-\( \alpha \)-tocopherol and ascorbate significantly increased mean EPSP slope to 1.40 mV/ms (± 0.10 S.E.; \( p < 0.05 \); Student’s \( t \) test for independent means) in young rats but not aged rats (mean value, 1.03 mV/ms ± 0.06). There was an age-related decrease in mean amplitude of the response; the mean values were 3.51 ± 0.35 mV and 2.18 ± 0.17 mV in 4-month-old and 22-month-old rats fed on the control diet (\( p < 0.01 \); Student’s \( t \) test for independent means). This was partially reversed by dietary supplementation; the corresponding mean values were 3.10 ± 0.21 mV and 2.59 ± 0.18 mV. The stimulus strength required to induce a response at which the spike became evident was slightly, although not significantly, greater in aged (4.29 ± 0.54 V) compared with young (3.57 ± 0.21 V) rats.

Dietary supplementation with vitamins had little effect on induction of LTP in either 4-month-old or 22-month-old rats. In contrast, a significant effect on maintenance of LTP, particularly in aged rats, was observed; in this study, we compared mean EPSP slopes and mean amplitude of the responses in the last 5 min of the experiment with the values in 5 min immediately before tetanic stimulation. In 4-month-old rats, the mean percentage changes in EPSP slopes (± S.E.) were 134.27% (2.67) in rats fed on control diet compared with 126.88% (1.83) in rats fed on the experimental diet; these values were not significantly different (Fig. 1A). The corresponding values for mean amplitude of the response were 142.76% (3.56) and 128.55% (1.70) in the groups fed on control and experimental diets, respectively; thus mean amplitude of the response was significantly decreased in rats fed on the experimental diet (\( p < 0.05 \); Student’s \( t \) test for independent means; data not shown).

Fig. 1B indicates that LTP, as measured by the change in EPSP slope, was impaired in aged rats fed on the control diet compared with young rats, but that this impairment was reversed in aged rats fed on the experimental diet. The mean percentage changes (± S.E.) in EPSP slope in the last 5 min of the experiment compared with the 5 min immediately before tetanic stimulation were 107.99% (2.92) in aged rats fed on control diet compared with 124.07% (1.89) in aged rats fed on the experimental diet. The corresponding values for the mean percentage change in the amplitude of the response were 109.01% (2.47) and 131.51% (2.56) for aged rats fed on the control and experimental diets, respectively (data not shown).

We analyzed \( \alpha \)-tocopherol concentration in hippocampal homogenate prepared from young and aged rats in both dietary groups; data are presented for all samples analyzed, i.e. 11 young rats and 12 aged rats. Analysis revealed that there was an age-related decrease in \( \alpha \)-tocopherol (\( p < 0.05 \); ANOVA; Fig. 2A). Our results indicate that, although dietary supplementation with \( \alpha \)-tocopherol and ascorbate did not significantly affect mean \( \alpha \)-tocopherol concentration in hippocampus of young rats, it significantly increased it in aged rats and completely reversed the age-related decrease. Analysis of the correlation between \( \alpha \)-tocopherol concentration and ability of rats to sustain LTP in dentate gyrus indicated a marked age-related difference. We found that, although there was no significant correlation between these parameters in young rats (Fig. 2B; \( r = 0.013; \ p = 0.97 \)), a significant correlation was observed in aged rats (Fig. 2C; \( r = 0.65; \ p = 0.022 \)).

Fig. 3C indicates that there was no significant effect of dietary manipulation on membrane arachidonic acid concentration in dentate gyrus. However, mean arachidonic acid concentration was significantly decreased in tissue prepared from aged rats fed on the control diet, compared with young rats fed...
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We analyzed lipid peroxidation in aliquots of the same tissue used for analysis of arachidonic acid, since an increase in lipid peroxidation might account for the decreased membrane arachidonic acid concentration. Fig. 3B indicates that there was an age-related increase in lipid peroxidation in rats fed on the control diet (p < 0.01; ANOVA). Although dietary manipulation had no significant effect on lipid peroxidation in 4-month-old rats, dietary supplementation with α-tocopherol and ascorbate reversed the age-related increase in this measure. The mean value was significantly lower in aged rats fed on the experimental diet compared with that in aged rats fed on the experimental diet (p < 0.05; ANOVA).

Previous evidence indicated that one trigger for lipid peroxidation was IL-1β; therefore, we analyzed the concentration of this cytokine in aliquots of the same tissue used in the analyses described above. Fig. 3A indicates that IL-1β concentration was significantly increased in aged rats fed on the control diet compared with young rats fed on either diet (p < 0.01; ANOVA). Dietary supplementation with α-tocopherol and ascorbate reversed the age-related increase in IL-1β concentration in dentate gyrus; the mean value in aged rats fed on the experimental diet was similar to those observed in young rats and significantly greater than that in the group of aged rats fed on the experimental diet (p < 0.01; ANOVA).

In parallel with the coupled age-related increases in IL-1β and lipid peroxidation in vivo, our data revealed that IL-1β significantly increases lipid peroxidation in hippocampal homogenate prepared from 4-month-old rats in vitro (p < 0.01; ANOVA; Fig. 4A). The data indicate that this effect was mimicked by hydrogen peroxide (p < 0.01; ANOVA), and both the IL-1β-induced increase and the hydrogen peroxide-induced increases were inhibited by α-tocopherol and the antioxidant, melatonin (Fig. 4A). These results suggested that IL-1β induced its effect by formation of reactive oxygen species. This supported by the demonstration that IL-1β induced a significant increase in reactive oxygen species production in hippocampus prepared from 4-month-old rats in vitro (p < 0.05; ANOVA; Fig. 4B); we observed that this effect was inhibited by α-tocopherol. Consistent with the in vitro findings, we report that there was a significant age-related increase in reactive oxygen species production in hippocampus prepared from rats fed on the control (Fig. 4C; p < 0.05; ANOVA), but this effect was reversed in tissue prepared from aged rats that were fed on the experimental diet; thus, mean reactive species production was significantly lower in 22-month-old rats fed on the diet supplemented with α-tocopherol and ascorbate, compared with that in 22-month-old rats fed on the control diet (p < 0.05; ANOVA).

**DISCUSSION**

We addressed the hypothesis that the impaired ability of aged rats to sustain LTP might be due to decreased membrane arachidonic acid concentration triggered by oxidative stress. If this hypothesis is to be accepted, it must be predicted that reversing the cause of the oxidative stress will restore ability of aged rats to sustain LTP. The data presented indicate that dietary supplementation with the antioxidants α-tocopherol and ascorbate reversed the age-related decreases in α-tocopherol and arachidonic acid concentrations in dentate gyrus and also reversed the age-related impairment in LTP.

Aged rats exhibited an impaired ability to sustain LTP in perforant path-granule cell synapses, confirming previous find-
ings (1–5). Several age-related changes have been proposed as factors that might lead to this impairment in LTP (7–14); however, because many of the age-related changes involve membrane-associated functions, we have proposed that altered membrane composition, specifically a decrease in membrane arachidonic acid concentration might be the underlying cause (3, 5). In support of this hypothesis, we have demonstrated that, if membrane arachidonic acid concentration in hippocampus of aged rats is restored to levels observed in young rats reversed by dietary supplementation with arachidonic acid and its precursor γ-linolenic acid, the impairment in LTP is reversed (5).

**FIG. 2.** Analysis of the correlation between α-tocopherol concentrations and responses to tetanic stimulation. A, α-tocopherol concentration in hippocampus was significantly decreased in aged rats compared with young rats (p < 0.05 ANOVA). Dietary manipulation with α-tocopherol and ascorbate had no significant effect on α-tocopherol concentration in hippocampus of young rats, but it significantly increased it in aged rats (↑ p < 0.05; ANOVA). B and C, analysis of the correlation between α-tocopherol concentration in dentate gyrus and EPSP (epsp) slope in the last 5 min of the experiment in young (B) and aged (C) rats. Regression analysis revealed that there was a statistically significant correlation between α-tocopherol concentration and the mean percentage change in EPSP slope in the last 5 min of the experiment in aged, but not young, rats. Con, control; Exp, experimental.

**FIG. 3.** The age-related increases in IL-1β concentration (A) and lipid peroxidation (B) and the decrease in membrane arachidonic acid concentration (C) in dentate gyrus are reversed by dietary supplementation with α-tocopherol and ascorbate. A, IL-1β concentration in dentate gyrus was significantly increased in aged rats compared with young rats (++, p < 0.01; ANOVA) when both were fed on the control diet. There was no significant effect of dietary manipulation in young rats (compare open and hatched bars), but IL-1β concentration in dentate gyrus of aged rats that were fed on the experimental diet was similar to the values observed in dentate gyrus of both young groups and significantly lower than that in the aged group fed on the control diet (++, p < 0.01; ANOVA). B, lipid peroxidation, which was assessed by formation of MDA, was significantly increased in dentate gyrus of aged rats compared with young rats (+, p < 0.01; ANOVA) when both were fed on the control diet. There was no significant effect of dietary manipulation in young rats (compare open and hatched bars), but MDA formation in dentate gyrus of aged rats that were fed on the experimental diet was similar to the values observed in dentate gyrus of both young groups and significantly lower than that in the aged group fed on the control diet (**, p < 0.01; ANOVA). C, arachidonic acid (AA) concentration in dentate gyrus was significantly decreased in aged rats compared with young rats (+, p < 0.05; ANOVA) when both were fed on the control diet. There was no significant effect of dietary manipulation in young rats (compare open and hatched bars), but arachidonic acid concentration in dentate gyrus of aged rats that were fed on the experimental diet was similar to the values observed in dentate gyrus of both young groups and significantly lower than that in the aged group fed on the control diet (*, p < 0.05; ANOVA). Con, control; Exp, experimental.
A number of reports have indicated that aging is associated with changes in membrane composition (15, 42, 43), and, although the underlying cause remains unclear, it has been accepted that lipid peroxidation, apparently triggered by reactive oxygen species, can lead to a decrease in membrane polyunsaturated fatty acids (23), which contributes to membrane rigidity (22, 44). We have found that lipid peroxidation is increased, whereas membrane arachidonic acid concentration is decreased, in hippocampal tissue prepared from aged rats2; these data, albeit obtained in separate experiments, suggested that arachidonic acid may be a target molecule for lipid peroxidation. The results of the present study develop this argument further, inasmuch as, in separate aliquots prepared from the same sample of dentate gyrus, we observed an age-related increase in lipid peroxidation coupled with an age-related decrease in membrane arachidonic acid concentration. Although this cannot be considered as conclusive proof of a causal relationship between these measures, it is consistent with the hypothesis that lipid peroxidation depletes membrane arachidonic acid. We also report here that there was an age-related increase in reactive oxygen species production in hippocampus, supporting the view that formation of free radicals contributes to changes associated with aging (45).

In theory, increased formation of reactive oxygen species will occur if there is down-regulation of the enzymatic defense strategies or a decrease in concentration of reactive oxygen species scavengers, like vitamins E or C or glutathione. Results from studies investigating antioxidant enzyme activities in aged brain are generally consistent with reduced ability to cope with free radicals (46–48), although there is no consensus about age-related changes in tocopherol concentration in brain, with some reports indicating an increase (27) and others reporting no change (49) depending on the species, brain area, and precise age. The results of the present experiment indicate a marked age-related decrease in tocopherol concentration in dentate gyrus. This compromise in one component of the antioxidant defense strategy might be sufficient to account for the increase in free radical formation described in the present study in the hippocampus and previously in other brain areas (26).

Our prediction was that, if the age-related increases in reactive oxygen species production and lipid peroxidation could be inhibited, then the age-related decrease in membrane arachidonic acid concentration and impairment in LTP would be reversed. The data presented demonstrate that aged rats which received a dietary supplement of tocopherol and ascorbate for 12 weeks sustained LTP in a manner indistinguishable from young rats. The age-related decrease in tocopherol concentration in dentate gyrus was also reversed, consistent with the previous observation that incorporation of the vitamin occurs after a relatively long period of dietary supplementation (29).

We observed that there was an increase in EPSP slope in young rats, but not aged rats, which received the experimental diet. One explanation for this finding might be that the action of tocopherol relies on increased release of arachidonic acid (50), which is attenuated in aged rats due to decreased availability.

The argument that the underlying cause of the age-related impairment in LTP was due to decreased tocopherol concentration is supported by the observation that there is a significant correlation between tocopherol concentration in hippocampus and ability of aged rats to sustain LTP. Interestingly, no correlation was observed in young rats. A significant role for vitamin E in generation of LTP in area CA1 in vitro has been suggested by previous studies. Thus rats fed on a vitamin E-deficient diet showed impaired ability to sustain LTP (33), whereas application of tocopherol induced a slowly developing form of potentiation (34).

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2 C. A. Murray and M. A. Lynch, unpublished data.
α-Tocopherol might have a direct effect on LTP, although it is more likely that its effect may be explained by its antioxidant action (22). This is supported by the observation that the impairment in LTP in vitamin E-deficient rats (33) is mimicked by application of free radicals (51). The data presented here provide strong evidence to further support this hypothesis; we observed that age was associated with a concomitant decrease in α-tocopherol and increases in reactive oxygen species and increase lipid peroxidation, all of which were reversed by dietary manipulation with antioxidant. The tight coupling of these parameters, in tandem with age-related and diet-related changes in LTP, are suggestive of a causal relationship between these parameters.

Lipid peroxidation was enhanced in dentate gyrus of aged compared with young rats; this observation confirms our previous data in whole hippocampus (19) and the previous data of others (Refs. 17, 18, and 20; but see Ref. 21). The parallel decrease in membrane arachidonic acid concentration 1 supports the finding that there is an increase in lipid peroxidation and 2 suggests that this polyunsaturated fatty acid might be one target lipid molecule for oxidation.

Previous observations have indicated changes in membrane composition, particularly in arachidonic acid concentration, with age (3, 19, 42, 43). Arachidonic acid modulates synaptic function (3, 5), and its membrane concentration correlates with ability of aged rats to sustain LTP (3); therefore, it was predicted that the age-related changes in arachidonic acid may underlie some of the impairments in neuronal function that accompany aging (e.g. Ref. 15). Significant support for this hypothesis was obtained when we established that reversing the age-related decrease in membrane arachidonic acid concentration reversed the age-related impairment in LTP (5). This is further supported by the results of the present study, which demonstrated that dietary supplementation with α-tocopherol and ascorbate reversed the age-related decrease in membrane arachidonic acid concentration and restored ability of aged rats to sustain LTP. We propose that this effect is secondary to its effect on reactive oxygen species production and lipid peroxidation.

We report that there was an age-related increase in IL-1β concentration in dentate gyrus. Although it must be considered that the procedures involved in tissue preparation contributed to the increase in IL-1β concentration and that this was exacerbated in tissue in which α-tocopherol concentration was decreased, the data presented support our previous observation in whole hippocampus (52, 53). The data also indicate a correlation between IL-1β concentration in hippocampus and ability to sustain LTP; thus, IL-1β was increased in hippocampus of aged rats maintained on the control diet (compared with young rats), and these rats showed impaired ability to sustain LTP, whereas the experimental diet reversed both the age-related increase in IL-1β and the age-related deficit in LTP. Thus, a causal relationship between the two parameters may exist, consistent with the findings that IL-1β inhibited full expression of LTP in perforant path-granule cell synapses in vitro (54) and in vivo (55). The mechanism by which IL-1β inhibits LTP might relate to the finding that IL-1β inhibits glutamate release and calcium influx in the hippocampus in vitro (52), but the molecular mechanism underlying its inhibitory effect remains to be established. It has been reported that IL-1β increased reactive oxygen species production in pancreatic islets (55), and we report here that IL-1β also increased reactive oxygen species production in hippocampus in vitro. This action of IL-1β might explain its inhibitory effect on LTP (53, 54), whereas the age-related increase in endogenous IL-1β together with the compromised antioxidant defenses in the aged brain might explain the age-related compromise in LTP (1–3), since reactive oxygen species inhibit LTP (51). This action might also couple two parallel changes reported here, i.e. the age-related increase in endogenous IL-1β and the impaired ability of aged rats to sustain LTP. Thus, IL-1β may trigger formation of reactive oxygen species in vivo, leading to increased lipid peroxidation and, in turn, to a decrease in membrane arachidonic acid concentration. The observation that the age-related increase in IL-1β concentration was reversed by the experimental diet, in parallel with the changes in the other parameters, supports this proposal; therefore, the data presented are broadly consistent with the “free radical hypothesis” of aging.

The mechanism by which dietary supplementation with α-tocopherol and ascorbate reverses the age-related increase in IL-1β concentration is unclear, but may be due to the antioxidant property of these vitamins since it has been reported that stress, including oxidative stress, induces IL-1β (28).

Current and previous (3, 5) data have indicated that one factor which contributes to the poor ability of aged rats to sustain LTP is a decrease in membrane arachidonic acid; the present data indicate that a decrease in α-tocopherol concentration in dentate gyrus also correlates with the deficit in LTP. When these decreases are reversed, as they were by dietary supplementation with α-tocopherol and ascorbate, ability of aged rats to sustain LTP is restored; these observations are consistent with the view that both rely on the ability of the hippocampus to sustain an adequate antioxidant defenses in age. We propose that the age-related increase in endogenous IL-1β in hippocampus may be the trigger leading to the biochemical deficits observed, but additional studies are clearly necessary to provide conclusive evidence to support this proposal.

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