Apoptotic Changes in the Aged Brain Are Triggered by Interleukin-1β-induced Activation of p38 and Reversed by Treatment with Eicosapentaenoic Acid*

Darren S. D. Martin, Peter E. Lonergan, Barry Boland, Marie P. Fogarty, Marcella Brady, David F. Horrobin‡, Veronica A. Campbell, and Marina A. Lynch§

From the Department of Physiology, Trinity College Institute of Neuroscience, Trinity College, Dublin 2, Ireland and Laxdale Research Ltd., Stirling FK7 9JQ, Scotland, United Kingdom

Among the several changes that occur in the aged brain is an increase in the concentration of the proinflammatory cytokine interleukin-1β that is coupled with a deterioration in cell function. This study investigated the possibility that the treatment with the polyunsaturated fatty acid eicosapentaenoic acid might prevent interleukin-1β-induced deterioration in neuronal function. Assessment of four markers of apoptotic cell death, cytochrome c translocation, caspase-3 activation, poly-(ADP-ribose) polymerase cleavage, and terminal dUTP nick-end staining, revealed an age-related increase in each of these measures, and the evidence presented indicates that treatment of aged rats with eicosapentaenoate reversed these changes as well as the accompanying increases in interleukin-1β concentration and p38 activation. The data are consistent with the idea that activation of p38 plays a significant role in inducing the changes described since interleukin-1β-induced activation of cytochrome c translocation and caspase-3 activation in cortical tissue in vitro were reversed by the p38 inhibitor SB203580. The age-related increases in interleukin-1β concentration and p38 activation in cortex were mirrored by similar changes in hippocampus. These changes were coupled with an age-related deficit in long term potentiation in perforant path-granule cell synapses, while eicosapentaenoate treatment was associated with reversal of age-related changes in interleukin-1β and p38 and with restoration of long term potentiation.

Increased expression of the proinflammatory cytokine interleukin-1β (IL-1β) has been linked with neurodegenerative disorders like Down’s syndrome, Alzheimer’s disease, and Parkinson’s disease (1, 2). Consistent with the view that IL-1β plays a role in deterioration of cell function are the findings that IL-1β expression is increased, in parallel with cell damage, in experimental models of ischemia (3), excitotoxicity (4), and traumatic lesions (5). Indeed, IL-1β has been shown to trigger cell death in primary cultures of human fetal neurons (6) and inhibition of caspase-1, which leads to formation of active IL-1β, and blocks lipopolysaccharide-induced changes in cell morphology, which are consistent with cell death (7).

IL-1β has been shown to stimulate the mitogen-activated protein kinases p38 and c-Jun NH2-terminal kinase (8, 9), and activation of both c-Jun NH2-terminal kinase (10, 11) and p38 (12–16) has been closely linked with apoptotic cell death. Significantly, an increase in p38 activity has been coupled with apoptotic changes in Alzheimer’s disease (17, 18). Concomitant increases in IL-1β concentration and p38 activity have been reported in the aged rat brain (19–21); in hippocampus these changes are correlated with compromised synaptic function and with an age-related impairment in long term potentiation (LTP) (19–22), while consistent with the high expression of IL-1β and IL-1RI in hippocampus is the finding that the cytokine depresses LTP in dentate gyrus (8, 19, 20, 23, 24). Significantly, we have recently reported that the age-related increases in IL-1β concentration and c-Jun NH2-terminal kinase activity, as well as the decrease in LTP, are reversed by treatment with the n-3 polyunsaturated fatty acid docosahexaenoic acid (22).

In this study we have attempted to identify the downstream consequences of the coupled age-related increases in IL-1β concentration and p38 activation in neuronal tissue. In particular, we have focused on assessing whether these changes might trigger apoptotic changes in neuronal tissue as it does in other tissues and have analyzed the effect of the ethyl ester of the n-3 fatty acid eicosapentaenoic acid (EPA) on age-related changes in cortex and hippocampus. The data indicate that dietary manipulation reversed several changes in the aged cortex that are indicative of apoptotic cell death as well as age-related changes in IL-1β concentration, p38 activation, and LTP in hippocampus.

EXPERIMENTAL PROCEDURES

Animals—Groups of young and aged male Wistar rats (300–350 g), maintained at an ambient temperature of 22–23 °C under a 12-h light-dark schedule, were subdivided into those that were fed on a diet enriched in eicosapentaenoic acid (ethyl eicosapentaenoate, 10 mg/rat/day for 3 weeks and 20 mg/rat/day for 5 weeks; Laxdale Research Ltd.) or standard laboratory chow for 8 weeks. Daily food intake was assessed for 2 weeks prior to commencement of the treatment: mean values (±S.E.) were 21.25 ± 1.4 and 18.55 ± 0.6 g/day for 4- and 22-month-old rats, respectively. At this time the mean body weights of young rats assigned to control and experimental groups were 265.6 ± 1.4 and 250.2 ± 1.7 g, respectively; corresponding values in aged rats were 483.6 ± 9.8 and 481.2 ± 7.9 g, respectively. Diet was prepared fresh each day, and rats were offered 100% of their daily intake. Mean daily food intake in all groups remained unchanged throughout the 8-week treatment period, and at the end of this time mean body weights of
Rats were then killed by decapitation, and the hippocampus and cortex (three trains of stimuli; 250 Hz for 200 ms; intertrain interval, 30 s) commenced for 10 min before and 40 min after tetanic stimulation made before electrophysiological recording at test shock frequency (1/30 3.9 mm posterior to bregma) and perforant path, respectively (angular placed in the molecular layer of the dentate gyrus (2.5 mm lateral and

Vivo

4 and 22 months old. Rats were maintained under veterinary supervi-

sion for the duration of this experiment.

Induction of LTP in Perforant Path-Granule Cell Synapses in Vivo—At the end of the 8-week treatment, LTP was induced as described previously (19). Rats were anesthetized by intraperitoneal injection of urethane (1.5 g/kg), recording and stimulating electrodes were placed in the molecular layer of the dentate gyrus (2.5 mm lateral and 3.9 mm posterior to bregma) and perforant path, respectively (angular bundle, 4.4 mm lateral to lambda), and stable baseline recordings were made before electrophysiological recording at test shock frequency (1/30 s) commenced for 10 min before and 40 min after tetanic stimulation (three trains of stimuli; 250 Hz for 200 ms; intertrain interval, 30 s). Rats were then killed by decapitation, and the hippocampus and cortex were removed, cross-chopped into slices (350 x 350 μm), and frozen separately in 1 ml of Krebs’ solution (136 mM NaCl, 2.54 mM KCl, 1.18 mM KH₂PO₄, 1.18 mM MgSO₄·7H₂O, 16 mM NaHCO₃, 10 mM glucose, 1.13 mM CaCl₂) containing 10% dimethyl sulfoxide (22). For analysis, thawed slices were rinsed three times in fresh buffer and used as described below.

Analysis of IL-1β Concentration—IL-1β concentration was analyzed in homogenate prepared from cortex and hippocampus by enzyme-linked immunosorbent assay (ELISA). Antibody-coated (100 μl; 1.0 μg/ml) final concentration diluted in phosphate-buffered saline (PBS), pH 7.5; goat anti-rat IL-1β antibody) 96-well plates were incubated overnight at room temperature, washed several times with PBS containing 0.05% Tween 20, blocked for 1 h at room temperature with 300 μl of blocking buffer (PBS, pH 7.3 containing 5% sucrose, 1% bovine serum albumin, and 0.05% NaN₃), and washed. IL-1β standards (100 μl; 0–1,000 pg/ml in PBS containing 1% bovine serum albumin) or samples (homogenized in Krebs’ solution containing 2 mM CaCl₂) were added, and incubation proceeded for 2 h at room temperature. Secondary antibody (100 μl; final concentration, 350 ng/ml in PBS containing 1% bovine serum albumin and 2% normal goat serum; biotinylated goat anti-rat IgG antibody) was added and incubated for 2 h at room temperature, washed 3 times, and incubated for 1 h at room temperature with 0.1% horseradish peroxidase-conjugated streptavidin. Substrate solution (100 μl; 1:1 mixture of H₂O₂ and tetramethylbenzidine) was added and incubated at room temperature in the dark for 1 h after which time the reaction was stopped using 50 μl of 1 M H₂SO₄. Absorbance was read at 450 nm, and values were corrected for protein (25) and expressed as pg of IL-1β/mg of protein.

Analysis of p38 Phosphorylation, Cytochrome c Translocation, and PARP Cleavage—p38 phosphorylation was analyzed in samples of homogenate prepared from hippocampus and cortex; cytosolic cytochrome c expression of 116-kDa PARP were analyzed in cortical tissue. p38 activity was also assessed in freshly prepared hippocampal and cortical tissue that was incubated for 20 min in the absence or presence of IL-1β (3.5 ng/ml), while cytochrome c translocation was assessed in vitro following incubation of slices of cortex with IL-1β (3.5 ng/ml) in the presence or absence of IL-1ra (350 ng/ml) or SB203580 (50 μM). For analysis of p38 in all experiments and also for PARP, homogenate was diluted to equalize for protein concentration, and aliquots (10 μl, 1 mg/ml) were added to 10 μl of sample buffer (0.5 mM Tris-HCl, pH 6.8, 10% glycerol, 10% SDS, 5% β-mercaptoethanol, 0.05% (w/v) bromphenol blue), boiled for 5 min, and loaded onto 10% SDS gels. In the case of cytochrome c, cytosolic fractions were prepared by homogenizing slices of cortex in lysis buffer (20 mM nEPES, pH 7.4, 10 mM KCl, 1.5 mM MgCl₂, 1 mM EGTA, 1 mM EDTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 5 μg/ml pepstatin A, 2 μg/ml leupeptin, 2 μg/ml aprotinin), incubating for 20 min on ice, and centrifuging (15,000 g for 10 min at 4 °C). The supernatant (i.e. cytosolic fraction) was suspended in sample buffer (150 mM Tris-HCl, pH 6.8, 10% (v/v) glycerol, 4% (w/v) SDS, 5% (v/v) β-mercaptoethanol, 0.002% (w/v) bromphenol blue) to a final concentration of 300 μg/ml, boiled for 3 min, and loaded (6 μg/lane) onto 12% gels. In all cases proteins were separated by application of 30 mA constant current for 25–30 min, transferred onto nitrocellulose strips (225 mA for 75 min), and immunoblotted with the appropriate primary and secondary antibodies. In the case of p38, anti-phospho-p38 (Santa Cruz Biotechnology; 1:500 in phosphate-buffered saline-Tween (101% Tween 20) containing 2% nonfat dried milk) and peroxidase-linked anti-mouse IgM (1:1,000; Amersham Biosciences) were used. In the case of PARP, we immunoblotted with an antibody (1:2,000) raised against the epitope corresponding to amino acids 764-1014 of poly(ADP-ribose) polymerase of human origin (Santa Cruz Biotechnology), and immunoreactive bands were detected using peroxidase-conjugated anti-rabbit IgG (Sigma) and ECL (Amersham Biosciences). To assay cytochrome c, a rabbit polyclonal antibody raised against recombinant protein corresponding to amino acids 1-104 of cytochrome c (Santa Cruz Biotechnology) was used. In addition to loading equal amounts of protein, some blots were reprobed for analysis of total (rather than phosphorylated) p38, and in other cases blots were probed with an anti-actin

FIG. 1. The age-related increases in IL-1β concentration and p38 activity in cortex are abolished by EPA. a, IL-1β concentration was significantly enhanced in cortical tissue prepared from aged rats fed on the control diet (n = 10) compared with young rats fed on either diet (*, p < 0.05, ANOVA; n = 6), but this change was not evident in tissue prepared from aged rats fed on the EPA-enriched diet (n = 10). b, p38 activity was significantly enhanced in cortical tissue prepared from aged rats fed on the control diet (lane 3) compared with young rats fed on either diet (lane 1) or EPA (lane 2) diet (*, p < 0.05, ANOVA), but this change was not evident in tissue prepared from aged rats fed on the EPA-enriched diet (lane 4). c, IL-1β (lane 2) significantly enhanced p38 activity in cortical tissue in vitro (*, p < 0.05, Student’s t test for paired values; n = 6). Con, control.
EPA Reduces Age-related Neuronal Death

34241

Figs 2 and 3. Cortical slices (350 × 350 μm) were incubated at 37 °C for 30 min in an EPA-enriched Krebs’ solution (145 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 1 mM MgSO₄, 1 mM KH₂PO₄, 10 mM glucose, 30 mM HEPES at pH 7.4) containing trypsin (1 mg/ml), DNase (1,600 kilounits/liter), protease X (1 mg/ml), and protease XIV (1 mg/ml). Slices were washed, triturated, and passed through a nylon mesh filter. Cells were centrifuged (1,000 rpm for 1 min), resuspended in HEPES-buffered Krebs’ solution, plated onto coverslips, fixed in 4% paraformaldehyde in PBS (v/v) for 30 min, and permeabilized in 0.1% Triton in PBS (v/v) for 15 min. Cells were incubated in normal goat serum in PBS (v/v) to block nonspecific binding, treated with anti-α-phosphospecific p38 antibody (1:100; Santa Cruz Biotechnology) or anti-caspase-3 (1:500; BioSource), and incubated overnight at 4 °C. Cells were washed and incubated in the dark for 2 h in either fluorescein isothiocyanate-labeled goat anti-rabbit IgG and IgM (1:100; BioSource) or R-phycocyanin-labeled goat anti-rabbit-IgG (1:100; BioSource) to visualize labeling with p38 and caspase-3, respectively. Following a further wash, slides were mounted using 2 mg/ml p-phenylenediamine in 50% glycerol in PBS (v/v) and sealed. Fluorescence was analyzed using the Bio-Rad MRC-1024 laser scanning confocal imaging system in which the fluorochromes were excited by laser light emitted at 565 and 494 nm and detected at 578 and 520 nm, which measured bound R-phycocerythrin and fluorescein isothiocyanate, respectively. Cells were analyzed at ×63 magnification under oil immersion with the laser at 100% power. The images were analyzed using the Bio-Rad software, and the Kalman filter was used to decrease background. In this system R-phycocerythrin-labeled cells are stained red, and fluorescein isothiocyanate-labeled cells are stained green. In separate series of experiments, cultures of aged and young rats were killed and brains were rapidly removed, coated in OCT compound, immersed in an isopentane bath over liquid nitrogen, and used to prepare sections for analysis of phosphorylated p38 as described above.

RESULTS

IL-1β concentration and p38 activity were both significantly increased in cortical tissue prepared from aged rats fed on the control diet compared with young rats (p < 0.05, ANOVA; Fig. 1, a and b), but EPA suppressed these age-related changes so that the values in tissue prepared from EPA-treated rats were not significantly different from control values. In vitro analysis revealed that IL-1β significantly enhanced p38 activity in cortical tissue (Fig. 1c). In parallel with this observation, we found that cytochrome c translocation was significantly increased in cortical tissue prepared from aged rats fed on the control diet compared with tissue prepared from either group of young rats (p < 0.05, ANOVA; Fig. 2a). A causal relationship between the age-related change in cytochrome c translocation and IL-1β is suggested by the finding that cytochrome c translocation was significantly enhanced by IL-1β (p < 0.05, ANOVA; Fig. 2b) and that this action relied on IL-1RI activation since the IL-1β-induced change was inhibited by IL-1ra. Fig. 2b also demonstrates that the IL-1β-induced change was inhibited by SB203580 suggesting that the effect was mediated by activation of p38.
One downstream consequence of cytochrome c translocation is activation of caspase-3, therefore we analyzed enzyme activity in tissue prepared from aged and young rats fed on either diet (*, p < 0.05, ANOVA), but this change was not evident in tissue prepared from aged rats fed on the EPA-enriched diet. In an effort to establish whether the change in caspase-3 activation was coupled with the increases in IL-1β concentration and p38 activation, a series of in vitro experiments were undertaken that revealed that IL-1β significantly enhanced caspase-3 activity (*, p < 0.05, Student's t test for paired means); values were normalized with reference to expression of glyceraldehyde-3-phosphate dehydrogenase (lower bands). Con, control; SB, SB203580.

In an effort to consolidate these findings, which suggested that caspase-3 activation is closely coupled with p38 activation, we observed that IL-1β significantly increased caspase-3 mRNA in cultured cortical cells (Fig. 3c). In contrast, several cells prepared from aged rats fed on the control diet stained positively for p38, but no evidence of colocalization with caspase-3 was observed. These findings support the idea that caspase-3 activation is closely coupled with p38 activation. In addition to the stimulatory effect of IL-1β on caspase-3 activity, we observed that IL-1β significantly increased caspase-3 mRNA in cultured cortical cells (Fig. 3c).
TUNEL-positive cells (*, p < 0.05, ANOVA) and mimicked by IL-1β neurons in the presence of IL-1β/H9252.

In an effort to explore the synaptic changes that might occur as a consequence of IL-1β-induced cell death, we turned to analysis of changes in hippocampus and first assessed age-and diet-related changes in IL-1β concentration and p38 activation. Fig. 6a shows that, in parallel with the age-related findings in cortical tissue, IL-1β concentration was significantly increased in hippocampal tissue prepared from aged rats fed on the control diet compared with young rats fed on either diet (p < 0.05, ANOVA). There was no evidence of a similar age-related increase in aged rats fed on the EPA-enriched diet. Analysis of p38 activation revealed a similar pattern; thus there was a significant age-related increase in p38 activity (p < 0.05, ANOVA), aged rats fed on the control diet versus young rats; Fig. 6b), which was not observed in tissue prepared from aged rats fed on the EPA-enriched diet. A likely causal relationship between IL-1β concentration and p38 activation is suggested by the finding that IL-1β significantly increased p38 activity in hippocampus in vitro (Fig. 6c). In a separate series of experiments, in which no dietary manipulation was made, cryostat sections of tissue were prepared from young and aged rats. We observed that there was a marked increase in p38 staining in hippocampus of aged compared with young rats; sample sections are demonstrated in Fig. 6d.

Analysis of LTP in dentate gyrus was undertaken in the same rats in which biochemical analyses were performed. Fig. 7 demonstrates that LTP was successfully induced in both groups of young rats (Fig. 7a) but was impaired in aged rats fed on the control diet (Fig. 7b); in the latter group of rats the mean percent changes in population excitatory postsynaptic potential slope in the 2 min immediately following tetanic stimulation (Fig. 7c) and in the last 5 min of the experiment (Fig. 7d; compared with the mean value in the 5 min prior to the tetani) were 136.5 ± 3.92 and 111.8 ± 0.86, respectively. In contrast, the corresponding values in aged rats fed on the EPA-enriched diet were 181.3 ± 3.12 and 149.1 ± 0.82, respectively. These latter values were similar to those observed in young rats fed on the control (190.5 ± 4.83 and 147.48 ± 1.31, respectively) and EPA-enriched (184.0 ± 3.24 and 154.8 ± 0.92, respectively) diets, indicating that EPA treatment restored the ability of aged rats to respond to tetanic stimulation.

**DISCUSSION**

We present evidence demonstrating that treatment with EPA prevents neuronal cell death in aged rats and show that suppression of the age-related coupled increases in IL-1β concentration and p38 activity is the key to inhibiting the cascade of cellular events that leads to apoptosis. The age-related increase in IL-1β concentration in cortical and hippocampal tissue, which confirms previous findings (19–21), is coupled with increased TUNEL staining in vivo. The implicit suggestion that endogenous IL-1β induces cell death is supported by the finding that IL-1β also enhances TUNEL staining in cultured cortical cells, although a comparison of data from two such different experimental conditions must be made with caution. Significantly, treatment with EPA, which has been shown to have anti-inflammatory properties (27, 28), prevented the age-related increases in IL-1β concentration and TUNEL staining in cortex. These data support the previous finding that fish oils, which contain EPA, reduce production of proinflammatory cytokines in circulating cells (29–32) and chondrocytes (33) and suppress the lipopolysaccharide-induced increase in circulating IL-1β (34).
Increased activation of p38 accompanied the age-related increase in IL-1β concentration consistent with previous observations in hippocampal cells (8, 19–21) and in other cell types (35–39). The evidence presented here pinpoints IL-1β-induced increased activation of p38 as a pivotal event in triggering changes that are characteristic of apoptotic cell death, for example cytochrome c translocation and caspase-3 activation. This suggestion concurs with previous findings. Thus inhibition of p38 by SB203580 has been shown to prevent singlet oxygen-induced apoptosis in HL-60 cells (40), while another p38 inhibitor, SB2390663, prevents caspase cleavage in thiol-oxidant-induced apoptosis in forebrain neuronal-enriched cell cultures (41). That inhibition of p38 proffers protection is further supported by the finding that its activation spared dopaminergic neurons deprived of serum (42) and markedly reduced infarct size induced by ischemia (16). In parallel with the effect of dietary manipulation on IL-1β concentration, the data indicate that EPA treatment blocked the age-related increase in p38 activation in hippocampus and cortex.

The data from in vitro analysis suggested that IL-1β, through an action on IL-1RI and mediated through activation of p38, stimulates cytochrome c translocation in cortical tissue. Since IL-1β concentration and p38 activation were enhanced in cortical tissue prepared from aged rats, it was predicted that translocation of cytochrome c might also be a feature of the aged brain; the present data indicate that there was an age-related increase in cytochrome c translocation that paralleled the increases in IL-1β concentration and p38 activation. Sig-

**FIG. 6.** The age-related increases in IL-1β concentration and p38 activity in hippocampus are abolished by EPA. a, IL-1β concentration was significantly enhanced in hippocampal tissue prepared from aged rats fed on the control diet (n = 10) compared with young rats fed on either diet (*, p < 0.05, ANOVA; n = 6), but this change was not evident in tissue prepared from aged rats fed on the EPA-enriched diet (n = 10). b, p38 activity was significantly enhanced in hippocampal tissue prepared from aged rats fed on the control diet compared with young rats fed on either control or EPA diet (*, p < 0.05, ANOVA), but this change was not evident in tissue prepared from aged rats fed on the EPA-enriched diet (lane 4). c, IL-1β significantly enhanced p38 activity in hippocampal tissue in vitro (*, p < 0.05, Student's t test for paired values; n = 6). d, expression of phosphorylated p38 is markedly increased in cryostat sections prepared from aged compared with young rats. Con, control.
EPA Reduces Age-related Neuronal Death

Consequently, this increase was absent in cortical tissue prepared from EPA-treated aged rats. Disruption of the mitochondrial transmembrane potential, which is accompanied by cytochrome c translocation from the mitochondria to the cytosol (43), is a relatively early event in apoptotic cell death. It has been shown that among the stimuli that lead to these events is oxidative stress (43); indeed it is possible that enhanced reactive oxygen species accumulation, which we have shown to accompany increased IL-1β concentration in other studies (19–21) and in the present one (data not shown), is responsible for the observed age-related increase in cytochrome c translocation.

Cytochrome c translocation triggers a cascade of reactions initiated by interaction with apoptosis protease-activating factor-1 and propagated by activation of caspase-9 and subsequently other caspases that eventually culminates in cell death (44). The importance of the role of cytochrome c translocation in this cascade was underscored by the demonstration that injection of active cytochrome c into cells led to apoptosis (45). The present findings, which show parallel changes in cytochrome c translocation and caspase-3, are consistent with the view that caspase-3 activation is triggered by cytosolic cytochrome c (46, 47). Our data also indicate that caspase-3 mRNA, as well as enzyme activity, was increased by IL-1β in vitro and, furthermore, that caspase-3 activation was dependent on interaction of IL-1β with IL-1RI and mediated through the subsequent activation of p38. The observation that caspase-3 colocalized with activated p38 was a further indication of a coupling between these parameters. This finding is strikingly similar to data reported in a recent study that showed that SB203580 diminishes caspase activity and protects SH-Sy5Y neuroblastoma cells and cultured cortical neurons from NO-induced cell death (48). EPA reversed the age-related increases in p38 activation, cytochrome c translocation, and caspase-3 activity, and we view this concurrence as strong evidence of a causal relationship between the parameters.

Our data indicate that the age-related increases in IL-1β concentration and p38 activation observed in cortical tissue were also observed in the hippocampus; these findings support our previous observations (19, 20). However, we report that no such changes were observed in hippocampus of aged rats that were fed on the EPA-enriched diet. LTP in perforant pathway-granule cell synapses was markedly depressed with age, confirming data from several previous studies (19–22, 51); significantly, this age-related impairment in LTP was completely absent in rats fed on the EPA-enriched diet. The negative correlation between the IL-1β concentration and p38 activation and the expression of LTP together with the observation that the IL-1β-induced inhibition of LTP was suppressed by p38 inhibition provides strong evidence of a causal relationship between these measures. The question of how polyunsaturated fatty acid, specifically EPA, uptake into neuronal tissue is achieved should be considered. It has been shown that circulating fatty acids cross the blood-brain barrier, and the rate of incorporation is proportional to plasma concentration; evidence indicates that transport is mainly by diffusion, although a facilitated process may also contribute (52). The question of the underlying cause of the decrease in polyunsaturated fatty acids in aged rats remains to be fully resolved, but it appear that fatty acid uptake into brain tissue is not altered with age (53).

Our working hypothesis is that aging is coupled with a significant increase in IL-1β concentration in neuronal tissue that is likely to exert multiple effects including activation of p38. We propose that increased p38 causes mitochondrial mem-

![Graph](http://www.jbc.org/)

**FIG. 7.** The age-related impairment in LTP in dentate gyrus is suppressed by EPA. a and b, tetanic stimulation (time 0) resulted in an immediate and sustained increase in the mean population excitatory postsynaptic potential (Epsp) slope in young rats (a) fed on either diet. A similar change was observed in aged rats (b) fed on the EPA-enriched diet, but aged rats that were fed on the control diet exhibited a marked attenuation in response to tetanic stimulation. c and d, analysis of the mean changes in the 2 min immediately following tetanic stimulation and in the last 5 min of the experiment (compared with the mean excitatory postsynaptic potential slope in the 5 min preceding the tetanus) revealed a significant decrease in excitatory postsynaptic potential slope in aged rats fed on the control diet compared with the other three groups (*, p < 0.01, ANOVA; n = 8 in the case of young and n = 12 in the case of aged rats). Con, control.
brane perturbation leading to translocation of cytochrome c. One consequence of these changes is an increase in caspase-3 activation; caspase-3 acts on its substrate, PARP, resulting in its cleavage. Apoptotic changes in cells occur since DNA repair is compromised as a result of this action. The evidence presented points to a pivotal role for IL-1β in triggering the cellular events that lead to an increase in cell death in the aged brain and identify activation of p38 as a key mediator. It is proposed that, although EPA abolished several age-related changes, the primary action of EPA may be to block the age-related increase in IL-1β expression.

REFERENCES

1. Griffin, W. S. T., Stanley, L. C., Ling, C. White, L., MacLeod, V., Perrot, L. J., White, C. L., III, and Arauz, C. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 7611–7615
2. Mogi, M., Harada, M., Kondo, T., Riedler, P., Inagaki, J., Minami, and M., Nagatsu, T. (1994) Neurosci. Lett. 180, 147–150
3. Boutin, H., LePeuvre, R. A., Horai, E., Asano, M., Iwakura, Y., and Rothwell, N. J. (2001) J. Neurosci. 21, 5528–5534
4. Panegyres, P. K., and Hughes, J. (1998) J. Neurol. Sci. 154, 123–132
5. Taupin, V., Toulmond, S., Serrano, A., Benavides, J., and Zavala, F. (1993) J. Neuroimmunol. 42, 177–185
6. Hu, S., Peterson, P. K., and Chao, C. C. (1997) Neurochem. Int. 30, 427–431
7. Vereker, E., O’Neill, E., and Lynch, M. A. (2000) J. Biol. Chem. 275, 26252–26258
8. Vereker, E., O’Neill, E., and Lynch, M. A. (2000) J. Neurosci. 20, 6811–6819
9. O’Neill, L. A. J., and Greene, C. (1998) J. Leucob. Biol. 63, 650–657
10. Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J., and Greenberg, M. E. (1995) Science 270, 1326–1331
11. Mielke, K., and Herdegen, T. (2000) Prog. Neurobiol. 61, 45–60
12. Kummer, J. L., Rao, P. K., and Heidenreich, K. A. (1997) J. Biol. Chem. 272, 20490–20494
13. Harada, J., and Sugimoto, M. (1999) Brain. Res. 842, 311–323
14. Castagne, V., and Clarke, P. G. (1999) Brain. Res. 842, 215–219
15. Barone, F. C., Irving, E. A., Ray, A. M., Lee, J. C., Kassis, S., Kumar, S., Badger, A. M., Legos, J. J., Erhardt, J. A., Ohlstein, E. H., Hunter, A. J., Harrison, D. C., Philpott, K., Smith, B. R., Adams, J. L., and Parsons, A. A. (1999) J. Biol. Chem. 274, 129–135
16. Zhu, X., Rottkamp, C. A., Boux, H., Takeda, A., Perry, G., and Smith, M. A. (2000) J. Neuroen. Exp. Neurol. 59, 880–888
17. Hensley, J., K. Floyd, R. A., Zheng, Y. N., Yael, H., Robinson, K. A., Nguyen, X., Pye, Q. N., Stewart, C. A., Geddes, J., Markesbery, W. R., Patel, E., Johnson, G. V., and Bing, G. (1999) J. Neurosci. 22, 2053–2058
18. Murray, C. A., and Lynch, M. A. (1998) J. Neurochem. 16, 2974–2981
19. Murray, C. A., and Lynch, M. A. (1998) J. Biol. Chem. 273, 12161–12168
20. O’Neill, E., Vereker, E., and Lynch, M. A. (2000) Eur. J. Neurosci. 12, 345–352
21. McCartney, M. P. (1999) Med. Hypotheses 53, 369–374
Apoptotic Changes in the Aged Brain Are Triggered by Interleukin-1β-induced Activation of p38 and Reversed by Treatment with Eicosapentaenoic Acid
Darren S. D. Martin, Peter E. Lonergan, Barry Boland, Marie P. Fogarty, Marcella Brady, David F. Horrobin, Veronica A. Campbell and Marina A. Lynch

J. Biol. Chem. 2002, 277:34239-34246.
doi: 10.1074/jbc.M205289200 originally published online June 28, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M205289200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 53 references, 19 of which can be accessed free at http://www.jbc.org/content/277/37/34239.full.html#ref-list-1