

The role of polyunsaturated fatty acids in restoring the aging neuronal membrane

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Abstract

In addition to a gradual loss of neurons in various brain regions, major biochemical changes in the brain affect the neuronal membrane that is the “site of action” for many essential functions including long-term potentiation (LTP), learning and memory, sleep, pain threshold, and thermoregulation. Normal physiological functioning includes the transmission of axonal information, regulation of membrane-bound enzymes, control of ionic channels and various receptors. All are highly dependent on membrane fluidity, where rigidity is increased during aging. The significantly higher level of cholesterol in aging neuronal membrane, the slow rate of cholesterol turnover, and the decreased level of total polyunsaturated fatty acids (PUFA) may result from poor passage rate via the blood–brain barrier, or from a decreased rate of incorporation into the membrane, or a decrease in the activities of delta-6 and delta-9 desaturase enzymes. The added oxidative stress, which leads to an increase of free radicals leading to a decrease in membrane fluidity, may respond to a restricted diet, and thereby overcome the damaging effects of the free radicals. A central focus of this review is that a specific ratio of n-3/n-6 PUFA can restore many of these age-related effects.

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1. Introduction

Brain aging is a complex process involving many factors. Some are independent and others are inter-related. The aging brain is associated with many biochemical, physiological and behavioral deficiencies including, but not limited to, reduction of long-term potentiation (LTP), learning and memory loss, sleep disturbance, pain threshold alteration, and disturbed thermoregulation.

To better understand the aging process, the structural approach has been proposed, in which major structural changes occurring during this period are studied, e.g. the gradual loss of neurons in various brain regions. However, the course of the progression of these changes has not yet been established. While we know that it is a long and slow process, we do not know the appropriate statistic model to best predict the rate or form of the decline in either structure or function. The ability of the brain to create new synapses (synaptic genesis) is diminished during this period for rea-

sons that are not understood. Concurrently, there are major biochemical changes in the brain that affect the *neuronal membrane*, that is the “site of action” for many essential functions. Such functions include the conduction of neuronal information along the axon, regulation of membrane bound enzymes, control of the ionic channels structure and activity, and maintenance of various types of receptors. During aging, the level of cholesterol in the neuronal membranes as well as the level of the toxic metabolite of cholesterol (24-OH-cholesterol) is greatly increased, and the corresponding rigidity of the neuronal membrane is significantly increased ([3]; see also Section 14 of this paper). The normal physiological functioning of the neuronal membrane is highly dependent on its structure, and while many factors can influence the membrane fluidity index, one of the major factors is the lipid composition of the membrane, where cholesterol reduces the membrane fluidity, and polyunsaturated fatty acids (PUFA) increase it. The brain can obtain long chain PUFA (LC-PUFA) directly from the diet, or it can use supplemented essential fatty acids (linoleic and alpha-linolenic) and convert them to longer chain fatty acids.

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This review will attempt to provide a better appreciation of the factors responsible for neuronal membrane rigidity, and thereby to suggest interventions that may be introduced to offset the unwanted outcomes. For example, given that the level of total PUFA is decreased in the aged, it may be attributed to (a) poor passage rate via the blood–brain barrier, or (b) decreased rate of incorporation into the membrane, or (c) a decrease in the activities of delta-6 and delta-9 desaturase enzymes. Each of these possibilities deserves examination.

In addition to PUFA, oxidative stress, which increases the level of free radicals [23], and which in turn induces a decrease in membrane fluidity [58], is yet another factor relevant for normal membrane composition. The incorporation of a restricted diet may overcome the damaging effects of the free radicals, and may offer a useful clinical intervention. Finally, previous studies have shown that dietary supplementation by a particular ratio of a mixture of n-3/n-6 PUFA exerts many beneficial effects, such as a reduced cholesterol level and an increased level of PUFA in the neuronal membrane [129]. It is our thesis in this report that this specific ratio of n-3/n-6 PUFA can restore many of the undesirable above-mentioned age-related effects. To support this hypothesis, we present a summary of relevant studies that have been confirmed experimentally and address this issue.

2. Essential fatty acids

The popular press routinely reports medical advisories urging the public to dramatically reduce the amount of fat they consume, in order to combat risks associated with cardiovascular disorders, diabetes, and other chronic disorders. Paradoxically, deficiencies in fat intake are likely to contribute to health hazards, including increased risk of infection, dysregulation of chronobiological activity, and impaired cognitive and sensory functions (especially in infants) [128]. Recently, Joseph et al. [60] reviewed the two conflicting roles of cholesterol, e.g. the “good” role of cholesterol and the “bad” role. A consensus from recent research suggests that it is not so much the *amount* of fat we eat, as the balance of the different types of fats, that is significant. The type of dietary fat affects how well the cell can perform its vital functions and its ability to resist disease.

Both linoleic and alpha-linolenic acids, the two PUFAs, are necessary for good health. They are called “essential fatty acids” (EFA) because the body cannot manufacture them or synthesize them. They must be provided by nutritional intake. Essential fatty acids are involved in energy production, the transfer of oxygen from the air to the bloodstream, the manufacture of hemoglobin, and are essential for normal nerve impulse transmission and brain function. They are also involved in growth, cell division and nerve function and are found in high concentrations in the brain. EFAs have beneficial effects when available in moderation.

Excesses of the otherwise beneficial fatty acids may, however, exert harmful effects, with high intakes of saturated and hydrogenated fats being linked to an increase in a number of health risks, including degenerative diseases, cardiovascular disease, cancer and diabetes.

3. PUFA: polyunsaturated fatty acids

Linoleic acid is a member of the family of omega-6 (n-6) fatty acids, while alpha-linolenic acid is an omega-3 (n-3) fatty acid. These terms refer to characteristics in the chemical structure of the fatty acids. Other omega-6 fatty acids, such as gamma-linolenic acid (GLA), dihomo-gamma-linolenic acid (DHGLA) and arachidonic acid (AA), can be manufactured in the body using linoleic acid as a starting point. Similarly, other omega-3 fatty acids that are manufactured in the body, using alpha linolenic acid as a starting point, include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Among the significant components of cell membranes are the phospholipids, which contain fatty acids. The types of fatty acids in the diet determine the types of fatty acids that are available to the composition of cell membranes. A phospholipid made from a *saturated* fat has a different structure and is less fluid than the one that incorporates an *essential fatty acid*. In addition, linoleic and alpha-linolenic acids per se have an effect on the neuronal membrane fluidity index. They are able to decrease the cholesterol level in the neuronal membrane, which would otherwise decrease membrane fluidity, which in turn would make it difficult for the cell to carry out its normal functions and increase the cell’s susceptibility to injury and death. These consequences for cell function are not restricted to absolute levels of FAs alone, rather it appears that the *relative* amounts of omega-3 fatty acids and omega-6 fatty acids in the cell membranes are responsible for affecting cellular function.

At least six categories of PUFA effects on brain functions have been noted and discussed elsewhere [128], namely: (a) modifications of membrane fluidity; (b) modifications of the activity of membrane bound enzymes; (c) modifications of the number and affinity of receptors; (d) modifications of the function of ion channels; (e) modifications of the production and activity of neurotransmitters; and (f) signal transduction, which controls the activity of neurotransmitters and neuronal growth factors.

Included among the symptoms of essential fatty acid deficiency are fatigue, dermatological problems, immune problems, weakness, gastrointestinal disorders, heart and circulatory problems, growth retardation, and sterility. In addition to these symptom conditions, a lack of dietary essential fatty acids has been implicated in the development or aggravation of breast cancer, prostate cancer, rheumatoid arthritis, asthma, preeclampsia, depression, schizophrenia and attention deficit and hyperactivity disorders (ADHD) [128]. This list is neither exhaustive nor conclusive.

4. Prostaglandins

Essential fatty acids are a special class of unsaturated fatty acids, that also act as precursors of yet another types of fatty acids. Most of the prostaglandins are derivatives of AA (itself derived from n-6) and all of them have a high physiological, hormone-like, activity level. They are involved in numerous brain functions, such as regional blood flow and permeability of various biological membranes. It has been suggested that prostaglandins are also involved in the functional level of the activity of cAMP (a second messenger) in the cells [56]. The behavioral and physiological effects of a specific ratio of n-3/n-6 compound (in a ratio of 1:4) correlates with changes in the fatty acid profile and with changes in the cholesterol level [129]. It may well be that such a compound has an effect on the prostaglandin system as well and mediates the behavioral and biochemical changes that have been observed in the rat. There is evidence that prostaglandin D2 has a profound effect on sleep [37,38,43,95]. Prostaglandins enhance corticotropin-releasing factor (CRF) activity [10,67,116,121], and CRF induces release of prostaglandins [97]. Prostaglandins enhance thyrotropin-releasing hormone (TRH) release and stimulate the dopaminergic and norenergic receptor activity [88,123], while beta-endorphin inhibits prostaglandin synthesis [43].

5. Cholesterol and fatty acids

Cholesterol is a complex lipid that is involved with many functions of the membrane. It is well established that cholesterol decreases the membrane fluidity index, with consequences on the activity of ion channels and receptor functions and is involved in dopamine release. Moreover, cholesterol is a key molecule in the end product of the CRF–ACTH (adreno-cortico-tropic hormone) axis. Considering that steroids are derivatives of cholesterol it is of interest to find that various fatty acids have differential effects on cholesterol metabolism. Huang et al. [51] cite many studies that reliably confirm that the administration of n-6 fatty acids reduces the level of cholesterol in the blood serum. However, n-6 fatty acids and n-3 fatty acids differ in their mode of action in cholesterol reduction, such that n-6 fatty acids redistribute cholesterol while the n-3 fatty acids actually reduce the levels of cholesterol in the neuronal membrane [50]. This may explain why an increase in cholesterol level in the blood is found in humans who consume n-3 fatty acids supplements. It has been demonstrated that n-3 essential fatty acids are more effective in reducing cholesterol levels in macrophages than n-6 essential fatty acids, most probably by the differential effect on the enzyme acyl-coenzyme A activity. However, Horrocks and Harder [50] indicated that cholesterol-esterifying enzymes that incorporate free fatty acids into cholesterol esters without the participation of CoA, are also present in the rat brain.

The mechanism by which n-3 fatty acids are able to reduce the cholesterol level is still unclear, although several hypotheses have been proposed. For example, Bourre [14] claimed that alpha-linolenic acid controls the composition of nerve membranes, which implies an inverse relationship between alpha-linolenic acid and cholesterol level. Salem and Niebylski [106] proposed that DHA or (22:6, n-3) controls the level of cholesterol as well as the composition and function of the neuronal membrane. We recently reviewed a number of studies that provided support for reducing neuronal membrane cholesterol by dietary supplementation of an n-3/n-6 compound in a ratio of 1:4 [125]. It is possible that such a ratio optimizes uptake of PUFA into the brain and promotes fatty acid incorporation into the neuronal membranes.

6. Specific fatty acid and the ratio between various fatty acids

Various fatty acids serve different roles in the nervous system and in the body and it has been suggested that the nervous system has an absolute molecular species requirement for proper function [106]. Studies in our laboratory offer a confirmation for this suggestion and provide an added qualifying requirement, viz. the need for a proper ratio between the essential fatty acids. We experimentally tested our hypothesis that the ratio of n-3 and n-6 may be a key factor in modulating behavioral and neuropharmacological effects of PUFA, and we then attempted to identify the optimal ratio [125]. To avoid the variations that occur in the composition of fatty acids in commercially prepared oils, and to exclude the possible confounding effects of other fatty acid or lipid admixtures, we used highly purified alpha-linolenic and linoleic acids. We tested a wide range of ratios of alpha-linolenic/linoleic acid (1:3, 1:3.5, 1:4, 1:4.5, 1:5, 1:5.5, 1:6 (v/v)) which were administered as dietary supplements. We found that a mixture of alpha-linolenic and linoleic acids with a ratio of 1:4 (which we referred to as SR-3) was the most effective in improving learning performance (as assessed by the Morris water maze), elevating pain threshold, improving sleep, and improving thermoregulation [125,128]. This compound was also able to correct learning deficits induced by the neurotoxins AF64A and 5,7-dihydroxytryptamine [127], and to provide protection from seizures induced by PTZ [126]. Also, SR-3 provides protection from bleoharospasm, which is induced by Ro4-1284 [129]. In addition, study showed that SR-3 administration exerts beneficial effects in rats given a diluted dose of the experimental allergic encephalomyelitis (EAE) toxin. The EAE rats showed learning and motor deficits as well as major changes in the fatty acids profile and the cholesterol level in frontal cortex synaptosomes. The SR-3 treatment was able to rehabilitate the changes induced by EAE to a significant degree, though not to completely reverse the deficits to the level of normal controls [129].

In addition, while old rats (22–24 months) showed a very poor performance in the Morris water maze, following pretreatment with SR-3 their level of performance was substantially improved.

The importance of the differentiation among the various types of fatty acids may be appreciated from noting their effects on immunological factors, viz. n-3 fatty acids suppress the synthesis of interleukin 1 (IL-1) and interleukin 6 (IL-6) and enhance the synthesis of interleukin 2 (IL-2), while n-6 fatty acids have the opposite effect. It should be recalled that both IL-6 and IL-1 (and to a lesser degree IL-2; [61]) promote CRF release via AA [17,18,101]. However, CRF inhibits the stimulating effect of IL-1 on prostaglandin synthesis [41,94].

7. Peptide interaction with the enzyme P450_{scc}, prostaglandins, cholesterol, and fatty acids

In light of the studies reviewed above, it seems that the various fatty acids and lipids play a major role in the syntheses, release, and function of several peptides, mainly the peptides that are concerned with releasing factors. As an example, in the analysis of the CRF–ACTH–steroids axis, P450 [73,135] and cholesterol metabolism have been shown to exert profound effects on dopamine at the beginning of the axis, and on steroids at the end of the axis. Prostaglandins are derivatives of fatty acids, but their metabolism is modulated by P450. Prostaglandins are involved in the metabolism and functions of all neurotransmitters (including dopamine), CRF, and steroids. The interaction between prostaglandins and ACTH is not yet clear. Cholesterol is a major molecule in the membrane, and an elevated level of cholesterol results in a decrease in the membrane fluidity and in a disturbance of the membrane function. In addition, steroids are derivatives of cholesterol. Many studies have demonstrated the role of specific fatty acids (mainly essential fatty acids of n-3 and n-6 groups) on all aspects of synthesis, release, and receptor functions of dopamine, CRF, ACTH, and steroids.

8. EFA ratio and stress

We have examined the effects of a mixture of fatty acids on cortisol and cholesterol level under laboratory conditions of stress [129]. A compound of free nonesterified unsaturated fatty acids alpha-linolenic and linolenic acids in a ratio 1:4 was administered for 3 weeks prior to the injection of cortisone (10 mg/kg) or prior to immersion of rats in a 10 °C saline bath. The results confirmed the expected elevation of cortisol and cholesterol level in stress, but more importantly the treatment prevented an elevation of blood levels of cortisol and cholesterol that were found in untreated control animals. Similarly, Morris water maze learning performance among the pretreated animals did not reflect deficits that

usually accompany such stressful conditions and that can be observed in the absence of the SR-3 pretreatment.

9. PUFA and the immune system

Repeated demonstrations that PUFA can modify the production and activity of various components of the immune system have left unexplained the mode of action by which it exerts its effects. Several mechanisms had been proposed, including *membrane fluidity* (changes that might effect the capability of cytokines to bind to their respective receptors on the cell membrane); *lipid peroxidation* (decrease in free radical-induced tissue damage); *prostaglandin production* (an indirect mechanism whereby prostaglandins, which are derivatives of PUFA, modify cytokine activity); and *regulation of gene expression* (PUFA influences on the signal transduction pathways and modified mRNA activity). The role of PUFA in immune function is complicated by the fact that n-3 and n-6 have differential effects on various immune components. In a recent review, Singer and Richterheinrich [108] indicated that n-3 fatty acids induce a decrease in lymphocyte proliferation in humans and rats, a decrease in IL-1 production, and a decrease in IL-2 production in both humans and animals. In addition, n-3 FA decreases TNF_{alpha} production in humans but increases it in mice macrophages, and also decreases natural killer (NK) cell activity. On the other hand, n-6 increases the production of IL-2 in mice and decreases production of TNF_{alpha} production and NK cell activity. Still other studies have shown that linoleic acid (n-6) decreases the activity of IL-2 [129], and increases IL-1 production and tissue response to cytokines, while n-3 generally decreases IL-1 production and activity [44]. Despite some disagreement among studies, it seems that n-3 fatty acids (alpha-linolenic acid, DHA, EPA) decrease the production and activity of the pro-inflammatory cytokines (IL-1, IL-6, TNF_{alpha}) [13,20,52,124] and that n-6 family has the opposite effect [19,44,54]. The ability of n-3 PUFA to reduce pro-inflammatory cytokines and prostaglandin [21] lead to the proposal for the use of fish oil to relieve pain. Fish oil, rich in n-3 PUFA, has been shown to decrease IL-6, IL-10, IL-12, TNF_{alpha}, and PG E2 [29].

Increasingly, the salutary effects of PUFA are being examined not only with respect to their absolute level in diet, supplementation, or serum and tissue content, but also with respect to their proportional relationship to other FAs. One example of the critical nature and importance of a proper ratio can be seen in the level of anti-inflammatory IL-2 production that is increased following treatment by a mixture of n-3 to n-6 FA in a ratio of 1:3 [129], together with an increase in n-3 in the tissue [54].

10. Stress

In psychology and biology, the term “stress” is applied to describe a strain or interference that disturbs and jeopardizes

the functioning of an organism. Organisms, both animals and humans, respond to physical and psychological stress with behavioral and physiological defenses. If the stress is too powerful, too prolonged, or is perceived as too threatening, or if the defenses are inadequate, then a somatic or comparable dysfunction may be expressed.

Outside the laboratory, stress is accepted as an unavoidable effect of living and is an especially complex phenomenon in the modern technological society. While many may profess to thrive in a stressful environment, there is little doubt that an individual's success or failure in controlling stressful situations (real or perceived) can have a profound effect on the ability to function. The ability to "cope" successfully with stress has figured prominently in anxiety and psychosomatic research. Stress has figured prominently in discussions of Health Psychology or Behavioral Medicine. Reports of a statistical link between coronary heart disease and individuals with a particular personality profile that is characterized by a behavioral pattern that manifests a life style of impatience, a sense of time urgency, hard-driving competitiveness, and a preoccupation with vocational and related deadlines ("Type A personality") has been reported numerous times. Similar correlations with other behavior profiles have suggested potential links to cancer, diabetes, and other chronic medical conditions. While different types of stress can be identified, the following discussion will refer only to psychological stress.

11. EFA and stress

As early as 1964, Back and Bogdanoff [8] reported elevations of free fatty acids and cholesterol among stressed people. Rosenman [102] summarized many years of research on the increased level of cholesterol among Type A behavior subjects. Subsequent studies confirmed the correlation between stressful situations and an increased level of cholesterol and free fatty acids [4,16,24,83]. It is not surprising, therefore, that dietary intake of soybean oil and fish oil has stress reduction properties [117]. A striking exception is the report that stressed medical students were found to exhibit lower levels of linoleic and AA (n-6), with no change in n-3 fatty acids [93,122]. Stress was shown to modify several key steps in fatty acid and lipid metabolism [75,82]. It is of interest to note that the hormones, which are released during stress (both catecholamines and glucocorticoids), serve as strong inhibitors of the first desaturase reaction, which converts linoleic and alpha-linolenic acids to longer fatty acids. Mills et al. [82] reported this finding in psychosocial stressed rats. One way to overcome the blocked biochemical step, is to administrate gamma-linolenic acid (which bypasses the blocked step in the n-6 essential fatty acids pathway) to stressed patients in order to reduce the elevated blood pressure and the elevated catecholamine level [83]. On the other hand, administration of linoleic and alpha-linolenic acids reduce the elevated cortisol level [131].

In addition, during stress, the cardiac uptake of free FA was reduced [9]. Administration of DHA (an n-3 derivative) improved cardiac response to stress [103], decreased the level of aggression [48,107], decreased stress responses [47,104,107,108], and decreased the level of prostaglandin E2 [31,103].

12. Membrane fluidity

The fluidity of the membrane is dependent on the lipid composition of the membrane. The protein component is very stable, but the lipid component has a high turnover rate. More specifically, the fluidity depends on (1) the transition temperature (i.e. the temperature where the membrane is converted from fluid to gel state) and (2) tight packing (where unsaturated fatty acids lower the transition temperature, and cholesterol abolishes the sharp transition temperature and disturbs packing by membrane insertion). It seems that the critical transition temperature may change during aging, along with the increase in cholesterol. The membrane fluidity index can also be regulated by the neuron in any one of a number of ways, such as: (1) desaturation of fatty acids; (2) transferring fatty acids between molecules for phospholipids with two unsaturated fatty acids (3) producing more unsaturated fatty acids, and (4) changing the tail length.

In addition to cholesterol, which decreases the membrane fluidity [15,35,72,109] there are some molecules that fluidize the membrane. Prominent among them is alcohol (e.g. [25,100,112,136]). It is interesting to note that pretreatment with an n-6 PUFA diet prevents the alcohol effect [81]. Local anesthetics [65] and several peptides [46] can also fluidize the membrane. Finally, behaviors such as REM sleep deprivation and stress are also able to induce rigidity in the neural membrane [74].

13. Membrane fluidity in aging—general

Several studies indicate that the changes in the membrane fluidity index do not affect all brain regions uniformly. One study [96] identified the hippocampus, cerebellum and cortex as the most affected areas, while another study [96,114] found the cortex, hippocampus, striatum and hypothalamus as areas that are most vulnerable to change. While the reason for this selectivity is not known, two unrelated studies might be able to provide a hypothesis. One of them [7] found that the protein composition in those areas is different from the protein composition in other brain areas. The other [3] found that these areas (hippocampus and cortex) differ from other brain areas in the percentage of cholesterol.

14. Membrane fluidity in aging—cholesterol

Numerous studies have shown that a high level of cholesterol is correlated with a decrease in membrane fluidity and

that the level of cholesterol is increased during aging, (e.g. [3,5,33–36,76,85,89,90,96,111,134]). Of particular interest are those studies that show that not only an increase in the level of cholesterol, but also in the level of the toxic metabolite, 24S-hydroxycholesterol (cerebrosterol) [32,64]. These studies seem to indicate that the level of cerebrosterol is even higher in patients with Alzheimer disorder [68,69,71]. The lipid lowering drugs (Lavastatine) [34,35], piracetam [86], and *Ginkgo biloba* [111] improve membrane fluidity by decreasing the level of cholesterol, that would otherwise inhibit the nicotine-cholinergic G-protein alpha subunit and thereby interfere with the coupling/uncoupling process [30].

It is interesting to note that one of the early studies in old rats found that complex learning per se resulted in a decrease in both cholesterol level and in membrane rigidity (hippocampus and frontal cortex) [62]. These results should be considered in the context of understanding the salutary effects of an enriched environment as a treatment option.

15. Membrane fluidity in aging—caloric restriction

Caloric diet restriction (reduced caloric intake with added nutritional maintenance) is one method available to improve the status of rigid membrane fluidity in the aged. Studies have shown that caloric restriction during aging increases membrane fluidity [42,55]. Mattson et al. [76] compared caloric restriction with the effect of an enriched environment, and concluded that caloric restriction is a very effective method (more effective than exercise [63]) that not only improved membrane fluidity, but also increased life span. Possible explanations to account for the effectiveness of caloric restriction include (a) the reduction of lipid peroxidation [131], (b) the decrease in the level of free radicals [131], and (c) the modification in the level of Ca^{2+} [39].

16. Role of fatty acids in aging neuronal membrane

PUFAs are major molecules responsible for regulating cellular differentiation and apoptosis [105]. Most of the studies on aging report a significant decrease in the level and turnover of PUFA [40,98,117,130], especially in the hippocampus, cortex, striatum and hypothalamus. During aging, there is a significant change in the transition temperature (see above), a change which is more profound in Alzheimer patients [46]. This change causes the membrane to be more rigid. The most studied fatty acids, in this respect, are the DHA and AA. While the level of both fatty acids is very low in the neuronal membrane of aged hippocampus in rats, treatment with n-3 fatty acids improved the membrane status [79,80]. Basically, there are two ways to explain the low level of PUFA in the aging brain, viz. the low rate of penetration of PUFA from the blood into the brain and an impaired biochemical machinery that would be expected to incorpo-

rate and elongate the fatty acid. These two alternatives are directly related to their respective parent issues: the problem of the blood brain barrier and the dynamics of FA brain metabolism

16.1. Blood–brain barrier

Recently, Rapoport et al. [99] provided a detailed discussion of the complex mechanism of delivery of essential PUFA as it progresses from the blood into the brain. They also considered the effects of dietary supplements as a tool to treat states of PUFA imbalance, but they did not include aging in their review. However, structural changes in the blood–brain barrier complex, in aging and in Alzheimer patients, were reported [28,45]. Despite the knowledge about structural changes, the knowledge about functional changes is quite limited. Most of the studies did not find changes in the rate of penetration of PUFA during aging (e.g. [112,113]). Essentially, these studies showed that there is no difference between young and old rats with respect to the penetration of AA into the brain. This is in contrast to a different study that found less AA reaching the old brain [1]. It has been suggested without evidence that saturated fatty acids, but not PUFA, need a carrier to cross the blood–brain barrier, and that linoleic acid inhibits palmitic acid passage, and palmitic acid then increases linoleic passage into the brain [6]. Other studies indicated that while there is no difference in the rate of penetration of PUFA into the old brain, the difference between the young and old brain is attributed to the biochemical machinery that is different for each age group [114,115].

16.2. Fatty acid metabolism in the aging brain

An increase in cholesterol level in the brain leads to selected functional modifications in the blood–brain barrier. Recent studies showed that various parts of the fatty acid metabolism pathways are malfunctioning in the aging brain. For example, the level of fatty acid incorporation into the membrane is inhibited and the turnover rate is very slow [115]. The activity level of the desaturase enzymes, such as delta-9 (which transfers saturated fatty acids to monounsaturated fatty acids) and other elongating enzymes, is very low [66]. In addition, the pathway to phospholipids is blocked [53]. Taken together, the combination and interaction of these various activities results in a decreased membrane fluidity index.

17. Oxidative stress—free radicals

Research indicates that the vulnerability to an oxidative stressor increases the deleterious effects of aging [57–59]. Oxidative stress at the cellular level results from many factors, including exposure to alcohol, medications, trauma, cold, toxins or radiation. In short, oxidative stress is a

condition of an increase in free radicals and a decrease in antioxidants.

Recent studies have shown that the oxidative stress increases the cholesterol level in the brain [29,57,118,132,133]. In young animals oxidative stress can raise the level of brain cholesterol to the level of aged rats [29]. One possible mechanism to the harmful effects of the oxidative stress is the finding that the stress induced an increase in lipid peroxidation by ATP via Ca^{2+} [22,26,120]. Some successful efforts to correct the effects of the stress via special food supplements have been reported [11,12,57–60,119], including PUFA supplements that can reverse the alcohol effects on the membrane [81], and increase the activity of certain membrane bound enzymes in different brain areas [110].

18. Oxidative stress and LTP

LTP is operationally defined as a long-lasting increase in synaptic efficacy, following high-frequency stimulation of afferent fibers. Since the first detailed description of the phenomenon in 1973, exploration of the mechanisms underlying LTP induction has been one of the most active areas of research in neuroscience. Of principal interest to those who study LTP, particularly LTP in the mammalian hippocampus, is its presumed role in the establishment of stable memories; a role consistent with “Hebbian” descriptions of memory formation. Other characteristics of LTP, including its rapid induction, persistence, and correlation with natural brain rhythms, provide circumstantial support for this connection to memory storage.

Oxidative stress, especially in older organisms, inhibits LTP (e.g. [79]) This effect of the oxidative stress is Ca^{2+} dependent [87,88,91,92,133,134]; a finding consistent with the fact that calcium inhibits synaptic plasticity. It is noteworthy that treatment with n-3 fatty acids can restore the LTP in aged rats [70,71,77–79]. Although a detailed account of the role of sodium, potassium, and calcium in the brain is beyond the scope of this review [2,49], it is worth noting that PUFA has also been shown to modulate sodium and calcium currents in the CA1 hippocampus neurons.

19. Hippocampus vulnerability and aging

The hippocampus is a major brain area, which is involved in spatial learning and memory. Decreased volume and functions of the hippocampus have been reported in aged organisms and among Alzheimer patients. There is sufficient data to conclude that chronic elevation of the corticosteroids levels might lead to hippocampal regeneration [27,84], since high levels of corticosterone and cortisol are toxic to the hippocampus, while estradiol protects it. The intact hippocampus plays a major role in overall inhibitory activity of the hypothalamus–pituitary–adrenal axis activi-

ties. Accordingly, it has a protective role in stress situations. In aging, the response to stress which is mediated via CRF, ACTH, corticosterone (or cortisol) is enhanced and prolonged, compared to young animals. In addition, the level of the involved molecules returned very slowly to normal level. Even without a stressor, the level of corticosterone in aged rats is elevated. de Kloet et al. [27] found a reduced number of mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) in aged hippocampus. He proposed that the normal feedback mechanism is disintegrated in the hippocampus of aged rats. Only MR-type receptors are involved in stress responses. A preliminary study from our laboratory showed that treatment with SR-3 can prevent structural changes in the hippocampus, decrease corticosterone level, and prevent a decrease in MR-type receptors in stressed young and old rats.

20. Concluding remarks

The aim of this review was to emphasize the role of essential fatty acids in the aging neuronal membrane. It is our position that the neuronal membrane is the prime site of action for most of the vital neuronal activities. In aging, two factors further debilitate the condition of the membrane, i.e. increased cholesterol level (and toxic cholesterol metabolites), together with a decreased level of PUFA, both of which render the membrane more rigid. A specific ratio of n-3/n-6 PUFA can protect the aged neuronal membrane against insults from many effects that would otherwise accompany aging.

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