The Emerging Role of Eicosapentaenoic Acid as an Important Psychoactive Natural Product: Some Answers but a Lot more Questions

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Abstract: Omega-3 polyunsaturated fatty acids play important roles in both the structure and communication processes of cells. Dietary deficiences of these fatty acids have been implicated in cardiac dysfunction, cancer and mood disorders. In the latter, clinical trials have strongly suggested that not all types of omega-3 PUFA are equally efficacious. In particular eicosapentaenoic acid (EPA) appears to be the most useful in ameliorating the symptoms of major depressive disorder. The mechanism by which omega-3 PUFA have these effects, and why EPA is apparently more effective in this role than the much more abundant brain lipid docosahexaenoic acid, is unclear. The available data do suggest various biologically plausible mechanisms all of which are amenable to study using straightforward experimental approaches. To progress further, a better understanding of how EPA and other omega-3 PUFA effect neurophysiological and neurosignalling processes is required.

Background

Omega-3 (n-3) polyunsaturated fatty acids (PUFA) are nutritionally important molecules normally found incorporated into either triglycerides or membrane phospholipids. The smallest omega-3 PUFA, 18 carbon alpha linolenic acid (ALA) must be acquired nutritionally but its metabolites, 20 carbon eicosapentaenoic acid (EPA) and 22 carbon docosahexaenoic acid (DHA), can be synthesised by cells. The rate of conversion of ALA into longer omega-3 PUFA is, however, rather slow and nutritional intake plays a large part in determining the abundance of EPA and DHA in the membrane (Emken et al. 1994). The other major PUFA class are omega-6 (n-6), for example, 20 carbon arachidonic acid (AA). The number refers to the location of the first C = C double bond relative to the omega (terminal) carbon atom.

Psychoactive effects of omega-3 fatty acids

Omega-3 fatty acids are emerging as key nutritional components having important physiological and biochemical effects upon a variety of important biological processes including the inflammatory response, blood clotting, and the immune system (Siddiqui et al. 2004). They are most abundant in marine animals and some plants, and a lack of fish intake and omega-3 fatty acids in the diet has been linked to a variety of disorders including heart disease and several types of cancer (Calviello et al. 2007; Berquin et al. 2008; Bays et al. 2008; Lee et al. 2008). Although much progress has been made in recent years, the precise biochemical mechanism(s) by which omega-3 PUFA cause such physiological and pathological changes remains unclear. Emerging research has also indicated that omega-3 PUFA may play a role in modulating brain function in such a way as to elevate mood and ameliorate the symptoms of depression, with a number of randomised, placebo controlled trials showing that omega-3 supplementation is beneficial (reviewed in Ross et al. 2007). Indeed, patients with depression exhibit reduced blood levels of omega-3 PUFA but not omega-6 PUFA (reviewed in Ross, 2007), while epidemiological studies have linked low dietary intake of omega-3 PUFA with an increased risk of developing the disorder (Sanchez-Villegas et al. 2007; Astorg et al. 2008 (and references therein) but see Appleton et al. 2007). Clinical studies testing the effect of omega-3 PUFA therapy upon depression do not, however,

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all show a beneficial effect of supplementation compared to placebo. Specifically, supplementation with EPA (or predominantly EPA) has been shown to be efficacious in trials of adults with major depressive disorder (also known as clinical depression) and bipolar disorder (BD) (although there has been one negative study involving BD patients), and childhood depressive disorder (Peet et al. 2002; Nemets et al. 2002, 2007; Su et al. 2003; Stoll et al. 1999; Frangou et al. 2006; Keck et al. 2006). On the other hand DHA is consistently is found to be ineffective for alleviating the symptoms of depression (Marangell et al. 2003; Silvers et al. 2005; Rogers et al. 2008). These findings are supported by recent meta-analyses of the clinical trials data which suggest that only EPA exhibits clinically useful antidepressant activity, while DHA and ALA are apparently inactive (Freeman et al. 2006; Ross et al. 2007; Lin and Su, 2007; Richardson 2008). Such a finding is rather unexpected given that EPA is a relatively minor brain fatty acid (approximately 0.2% of fatty acids), with the predominant brain omega-3 PUFA being DHA (approximately 15% of fatty acids) (Phibrick et al. 1987). Given that omega-3 PUFA are being studied for their use in other mental illnesses such as anxiety and attentional deficits it is important to understand which fatty acid is likely to be efficacious and why (Richardson and Puri, 2002; Yehuda et al. 2005; Richardson and Montgomery 2005; Buydens-Branchey et al. 2008; Johnson et al. in press). Furthermore, a presumed selective benefit of EPA upon mood has lead to the marketing of EPA-rich dietary supplement made by augmenting fish oils with EPA concentrates. A differential effect of EPA vs. DHA has not been absolutely proven, however, given that a clinical trial comparing the effects of different omega-3 PUFA upon the symptoms of depression, whether by a parallel or crossover design, has not been conducted, a serious deficiency in the literature. Nevertheless, a recent report indicates that EPA is approximately as efficacious for the treatment of major depressive disorder as the SSRI fluoxetine, and that coadministration of the two compounds produces an additive effect (Jazayeri et al. 2008). Such results are supportive of EPA being a clinically significant antidepressant, although it should be noted that the study included utilised a double dummy design with no double placebo group. As such, the accumulated evidence is sufficient to consider the question of whether a differential effect of EPA compared to DHA on depression is biologically plausible.

Possible Explanations for the Mood Altering Effects of EPA

Potentiation of conventional antidepressant action

Various possibilities present themselves, the simplest being that the beneficial effect of EPA supplementation is due to it altering the pharmacokinetics e.g. absorbance or elimination rate, or the pharmacodynamics of conventional antidepressants. The implication of such a hypothesis is that administered alone, omega-3 PUFA would have no effect upon mood. Most clinical trials so far performed have utilised omega-3 PUFA in a role adjunctive to existing medication and hence it is difficult to rule out such a possibility (Ross et al. 2007). Recently, however, a beneficial effect of omega-3 supplementation in the absence of conventional antidepressants has been reported to alleviate the symptoms of depression in children (Nemets et al. 2006). While requiring of replication as well as extension to the adult population, when taken in the context of the epidemiological data linking depression to omega-3 PUFA intake, such data does not support a simple modulatory role of EPA upon conventional antidepressant action.

Non central nervous system effects

The low levels of EPA to be found in the brain does suggest the possibility that EPA alters mood by means of an indirect effect occurring outside of the central nervous system. Currently there is no evidence in support of such a hypothesis although investigation of this possibility has not been extensive. For example, it is well known that hypothyroidism can lead to depression, however, omega-3 fatty acid supplementation has no effect upon thyroid status in an experimental animal model (Morcos and Camilo, 2001; Davis and Tremont, 2007), although human subject studies appear warranted. Furthermore, although there may be an association between depression and obesity and it is established that omega-3 PUFA can effectively reduce circulating triglyceride levels (Lewis et al. 2004), there is no evidence

that hypertriglyceridemia *per se* is a risk factor for the development of depression (Lehto et al. in press). Conversely, although elevated homocysteine levels have been associated with depression (Kim et al. 2008), omega-3 PUFA are reported to either have no effect upon or to slightly increase homocysteine levels (Piolot et al. 2003; Beavers et al. 2008). These studies utilised normolipodemic non-depressed subjects or end-stage renal patients and their results may not be generalisable to persons with depression, however, and hence further investigation is required to rule out an omega-3/homocysteine connection in the disorder. Low levels of vitamin B12 and folate have also been linked to depressed mood (Murakami et al. 2008; Lehto et al. in press) but it is presently unknown as to whether omega-3 PUFA supplementation can alter circulating and cellular levels of these compounds. On the other hand one study has shown a correlation between DHA and folate levels in aggressive human subjects a finding which merits further investigation in a supplementation trial (Umhau et al. 2006). Lastly, although some controversial studies have linked reduced cholesterol levels with depressed mood and suicide (Huffman and Stern, 2007), the effect of omega-3 PUFA on LDL and HDL cholesterol abundance is heterogenous between individuals and does not present a clear mechanism for the mood altering effects of omega-3 PUFA (Balk et al. 2006; Bays et al. 2008). Nevertheless, given the apparent or unknown interaction between omega-3 PUFA abundance and that of other compounds which have been linked to the development of depression, future clinical studies may usefully examine such potential mechanisms in more detail, including any differential effects of EPA and DHA.

Correction of a brain omega-3 PUFA deficiency

An obvious explanation for the effect of EPA upon mood is that supplementation with this omega-3 PUFA corrects a relative deficiency in this fatty acid, whether caused by a metabolic insufficiency or reduced dietary intake, which has resulted in depressed mood. Evidence is rather lacking however for such a simple mechanism. Although omega-3 PUFA levels in patients with major depressive disorder are consistently found to be reduced compared to healthy controls this deficiency is not limited solely to EPA, with ALA and DHA also being diminished, and in a manner which is heterogeneous between different studies (reviewed in Ross, 2007). Indeed, although most studies have measured fatty acids levels in various blood compartments, one post mortem brain study reports that it is DHA levels which are reduced in the frontal cortex of patients with depression and bipolar disorder, although due to the very low abundance of EPA in brain the levels of this omega-3 PUFA were not assessed (McNamara et al. 2007, 2008). While a predominantly DHA involvement may suggest that omega-3 PUFA deficiency and antidepressant efficacy are uncorrelated, it cannot presently be ruled out that the brain may be much more sensitive to EPA deficiency than to that of the other omega-3 PUFA species. The question of whether EPA supplementation treats a deficiency state or not is an important one since the answer predicts whether those patients with depression who have normal omega-3 PUFA abundance will benefit from EPA therapy. Further clinical trials which relate baseline omega-3 PUFA abundance and change in abundance during the trial to antidepressant action would go some way to helping answer this question.

Neurophysiological mechanisms

How then might EPA be affecting the functioning of the brain? A clue to the neurophysiological mechanisms which may be occurring can be derived from clinical studies indicating that omega-3 PUFA can ameliorate the symptoms of mood disorders, attentional disorders and anxiety disorders but not schizophrenia and other psychotic disorders (Freeman et al. 2006; Ross et al. 2007; Lin and Su, 2007). For all disorders exhibiting efficacy the potentiation of dopaminergic and/or serotonergic neurotransmission by conventional pharmacotherapies is clinically usefull, while the same potentiation can actually worsen psychosis (Stahl, 2000). As such it is reasonable to hypothesise that EPA may act by a similar mechanism, having a greater effect than that of DHA (see below for potential molecular mechanisms). Such a question is amenable to study using animal models but unfortunately comparative studies of different omega-3 PUFA species are lacking, with most investigators utilising omega-3 PUFA mixtures. With the ready availability of purified fatty acids at a reasonable cost such experiments become

feasible. For example, neurotransmitter release following supplementation with different omega-3 PUFA can be investigated using in vivo dialysis in animal models, while PET imaging could be employed to test various hypotheses in human subjects. Structural neuroimaging is also warranted as an investigational tool given that depression is associated with morphological and potentially neurodegenerative changes in the brain (reviewed in Kanner, 2004). Given that omega-3 PUFA intake is reported to be correlated with cortical and limbic grey matter volume a neurotrophic effect can also be postulated which could underlie any anti-depressant activity (Conklin et al. 2007). Such a possibility is supported by observations in experimental animals that omega-3 PUFA deficiency can reduce brain levels of brain derived neurotrophic factor (BDNF), and that EPA supplementation increases BDNF levels after traumatic brain injury (Wu et al. 2004; Rao et al. 2007). BDNF plays an important role in the neuroplasticity which occurs in the brain in response to environmental changes, while altered brain levels and genetic polymorphism of the BDNF gene have been associated with both bipolar and major depressive disorders (reviewed in Castrén and Rantamäki, 2008). Furthermore, polymorphisms of the BDNF gene have been linked to hippocampal volume in patients with depression and healthy controls, a brain anatomical factor related to the incidence of mood disorders (Frodl et al. 2008). As such, the use of MRI to monitor brain volume changes which occur during the treatment of depression with omega-3 PUFA would be of great interest. Importantly, a case study of a patient with schizophrenia has indicated that treatment with EPA can increase cerebral volume (Puri et al. 2000). Until further such investigations are carried it will remain unclear how omega-3 PUFA effect the brain (and hence how they effect behaviour), and why EPA would be more effective than other omega-3 PUFA in doing so.

Molecular mechanisms

A molecular understanding of why EPA may act differently from DHA is more advanced. Omega-3 PUFA are known to have a wide range of biochemical interactions including modulating the activity of sodium, potassium and calcium ion channels (Honoré et al. 1994; Xiao et al. 1997; Leaf 2001; Doolan et al. 2002; Jude et al. 2003; Guizy et al. 2005; Matta, 2006; Blondeau et al. 2007) and phospholipases C and D (Estes et al. 1997; Diaz et al. 2002). In addition omega-3 PUFA exert regulatory effects upon the levels of neurotransmitter receptor and transporter levels (Dyall et al. 2007; du Bois et al. 2008; Kuperstein et al. 2008), part of a wide array of gene expression changes (Sampath and Ntambi, 2004), as well as having an effect upon the protein kinase C signalling system (McNamara et al. 2006) in addition to the potential neurotrophic roles (Conklin et al. 2007). While such findings may well explain the mechanism of action of omega-3 PUFA upon mood, they frequently demonstrate that different types of omega-3 PUFA have a similar magnitude and potency of effect and hence are unlikely to underlie the differential effects of EPA and DHA. Importantly, however, most investigations do not set out to compare the effects of each omega-3 PUFA type in a detailed manner with many reports utilising just one form, commonly DHA. As such it would be beneficial to revisit some of these findings in order to carefully compare the effects ALA, EPA and DHA. Indeed the tendency of past research reports to treat the various omega-3 PUFA types as functionally equivalent might usefully be replaced by an assumption that they are non-equivalent unless proven otherwise by experiment. Not surprisingly therefore, clear differential effects of the various omega-3 PUFA types are infrequently reported. Dissimilar effects of EPA and DHA have been noted in several systems, however, for example the modulation of hepatocyte fatty acid oxidation and triglyceride synthesis, and the intracellular calcium ion levels of astrocytes (Sergeeva et al. 2005). The biochemical mechanisms underlying such differential activity are, presently, unclear, although altered phospholipid metabolism and fatty acid dependent signalling has been postulated (Berg et al. 1999). Besides playing an important role as cell structural components, PUFA are precursor molecules for many intracellular signalling systems which play an important role in many major physiological processes and body systems (Curtis-Prior, 2004). The signalling cascade is initiated by lipases releasing free PUFA from membrane phospholipids which then act either directly as intramembrane messengers or following their controlled oxidation by cyclo- and lipoxygenases. These enzymes catalyse the synthesis of an array of bioactive metabolites including the prostaglandin, leukotriene and HETE classes of signaling molecules (Curtis-Prior, 2004). Indeed,

metabolites of arachidonic acid are likely involved in the dopaminergic and serotonergic neurosignalling systems which may be dysfunctional in depression (Piomelli et al. 1991; Vial and Piomelli, 1994; Berg et al. 1998; Evans et al. 2001). Omega-3 PUFA are also involved in signaling cascades although, in general, they produce opposing effects to omega-6 derived products. For example, the omega-6 PUFA AA gives rise to a variety of inflammatory mediators while those of the omega-3 PUFA EPA are generally anti-inflammatory (Babcock et al. 2000), although recent results suggest that some omega-6 PUFA products are also anti-inflammatory (Serhan et al. 2008). As such, the abundance of different PUFA derived species, itself determined by various metabolic and dietary factors, has significant effects upon cellular and physiological function. Importantly, a lipid-dependent signalling basis of conventional mood altering drugs has been proposed (Rapoport and Bosetti, 2002). Specifically, the selective serotonin reuptake inhibitors and antidepressant fluoxetine increases the turnover of arachidonic acid in the brain, while the mood stabilisers lithium and valproate downregulate arachidonic acid turnover, phospholipase A_2 activity and eicosanoid synthesis via cycloxygenase (Rapoport and Bosetti, 2002; Bosetti et al. 2002; Qu et al. 2003; Gherlardoni et al. 2004; Lee et al. 2007). Such observations are complimentary to the mood elevating effects of omega-3 PUFA and suggestive of mechanistic links between conventional drugs and this class of lipids. Our knowledge of the mechanism by which omega-3 PUFA alter signaling processes is, however, still poor. One hypothesis suggests that omega-3 and omega-6 fatty acids are competitive inhibitors of each others metabolism by cyclo- and lipoxygenases (Ringbom et al. 2001). Simple antagonism of omega-6 PUFA dependent signaling by omega-3 PUFA as their mechanism of action is, at best, an incomplete explanation since although EPA and DHA are equally potent inhibitors of the cyclooxygenation of omega-6 PUFA (Ringbom et al. 2001), as I have described the effects of each omega-3 PUFA are not equivalent. A more likely explanation for such differential effects is that EPA and DHA give rise to different bioactive metabolites possessing different biological functions (Serhan et al. 2008). For example, it has been known for some time that the enzyme cyclooxygenase can utilise EPA to produce the 3-series prostaglandins, a form of eicosanoid, although their rate of synthesis by cyclooxygenase is slow and the biological significance of these reactions remains unclear (Smith, 2005). More recently, it has been reported that both EPA and DHA can give rise to an array of potent metabolites including neuroprotectins and resolvins (Serhan et al. 2008). These compounds are formed in the brain and have potent anti-inflammatory and immunomodulatory effects, appearing to act mainly as agonists at G-protein coupled receptors. Importantly, EPA gives rise to different mediators compared to those derived from DHA, a fact which may underlie their apparently varying effects on mood (Serhan et al. 2008). The anti-inflammatory effect of these molecules is intriguing given that some evidence supports the notion that depression is associated with a mild but chronic inflammatory response (Maes, 2008), and that induction of inflammation in animals results in depression-like behaviours which are antagonised by supplementation with EPA (Song et al. 2007). Furthermore, omega-3 PUFA derived compounds can downregulate levels of tumour necrosis factor α (TNF-α), a key component of the inflammatory cascade. TNF-α levels are elevated in the bloodstream of patients with bipolar disorder and it has been argued that TNF-α represents a new target for drug discovery in mental illness (Arita et al. 2005; Brietzke and Kapczinski, in press). A link between mental illness and inflammation is an intriguing possibility and provides a clear mechanism by which omega-3 PUFA, including EPA, could ameliorate the symptoms of the disorder. No studies have been performed, however, as to what the behavioural effects of the resolvins and protectins may be and whether they can act to modulate the activity of the neural pathways and transmitter systems most relevant to mood. Interestingly, the drug aspirin can induce the synthesis of epimers of the resolvins and protectins which may allow a pharmacological route to test hypotheses regarding their behavioural effects in both clinical and animal models (Serhan et al. 2008). Experiments in which different omega-3 PUFA are administered along with low doses of aspirin would be of interest.

An alternative, though complementary, hypothesis is that DHA and EPA differentially regulate phospholipid metabolism, in particular those phospholipids containing arachidonic acid residues and their dependent signalling cascades. Many studies have noted that omega-3 fatty acid supplementation alters omega-6 PUFA

abundance (for example see Healy et al. 2000); how this occurs is, however, unclear. In U937 hystiocytic lymphoma cells supplementation with EPA, but not DHA, significantly increased the stimulated release of AA from the cell membrane (Obajimi et al. 2005). Although the reason for such an interaction between EPA and AA, but not DHA, is unclear, a likely explanation is that the two 20 carbon fatty acids (EPA and AA) utilise the same metabolic pathways for their uptake and release which differ from that used for the 22 carbon DHA. Importantly, the *enhancement* of arachidonic acid release is not supportive of EPA being antagonistic to AA function, rather it playing a potentiating role. While attempting to extrapolate from cell culture models to in vivo can be misleading, some in vivo data are supportive of such an intriguing possibility. Although much data suggests that n-3 PUFA are able to reduce AA abundance in the membrane i.e. they are antagonistic to AA function, such experiments normally involve administration of fairly high doses. At lower doses (<0.1% total lipids), EPA leads to *increased* AA abundance in both experimental animals and human subjects (reviewed in Horrobin et al. 2002). Such findings are consistent with reports that lower doses of EPA can be more efficacious than high doses in the treatment of mental health disorders (Ross et al. 2007). The mechanism at play is unclear but likely involves EPA acting at multiple points along the phospholipid metabolic pathway. One probable target is regulation of the activity of lysophospholipid acyltransferase (LPAT) which esterifies coenzyme A fatty acid esters synthesised by fatty acid CoA ligase to lysophospholipid molecules. Many different types of LPAT exist in the cell although only limited characterisation of this enzyme family has been performed. While LPAT is a synthetic enzyme it plays a key role in removing the free fatty acids released by lipase such as phospholipase A_2, competing with the oxygenases which form the various signalling molecules. As such modulation of this pathway could have significant effects upon both omega-3 and omega-6 dependent signalling systems. Clearly, further investigation is required to determine the mechanism by which EPA and AA may undergo regulatory 'cross-talk', studies which may reveal new levels of complexity with regard to how different PUFA species interact metabolically. The application of techniques that allows the analysis of large numbers of lipids and related compounds simultaneously, known as lipidomics, could be usefully employed to answer these questions.

Besides a direct action of EPA either by acting as a precursor of various signalling molecules or by a direct action of phospholipid metabolism, PUFA can also exert effects via the activation of peroxisome proliferators-activated receptors (PPARα). PPAR are a family of nuclear receptors which bind to specific DNA sequences to regulate gene transcription along with various co-activators and co-supressors (Sampath and Ntambi, 2005). There are several different types of PPARs including α, β, and γ forms. PUFA are agonists of PPARα, while PPARγ is activated weakly by PUFAs and more strongly by oxygenated PUFA metabolites including HETEs (Sampath and Ntambi, 2005). Thus, the activation of PPARα by EPA offers a further mechanism by which neural processes may be altered. PPARα activation in itself is not sufficient to explain the differential effects of EPA and DHA given that both can act as agonists. This does not rule out a role for PPARα, however, given that in hepatocytes, although EPA can stimulate fatty acid β-oxidation via a PPARα dependent mechanism, DHA, which also activates PPARα, does not (Berge et al. 1999). Such findings indicate that PPARα activation may be a necessary but not sufficient event to modify some metabolic processes, and that EPA may act at multiple points in the signaling cascade. Further experiments utilizing specific PPARα agonists administered along with omega-3 PUFA may be useful in determining the possible involvement of this receptor class.

Conclusions

In summary, the emerging data supporting the hypothesis that omega-3 PUFA have psychoactive effects have also raised the possibility that EPA is more efficacious than other forms of omega-3 PUFA. While more clinical research is required to fully characterise and confirm this possibility, it is currently unclear as to why EPA could possess these properties, and how its effects on the brain and body differ from that of DHA. In particular, the role that EPA has upon neurophysiology and neurosignalling remains to be elucidated.

The techniques required to do so are, however, well established and mostly straightforward. Further molecular characterisation of the effects of omega-3 PUFA action upon the brain are also required although the recently developed fields of lipidomics and metabolomics should allow rapid progress in this area also.

Disclosure

The author reports no conflicts of interest.

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