Enhancement of endogenous Anandamide using natural bioactives in a nanoemulsion

Authors: Cherry, Tyrone; Uppal, Shivani

Contributing authors: Davidovits, Moises.

Abstract: The endogenous cannabinoid system is a complex cell signaling system containing CB1 receptors found in the brain, which are in large part activated by the lipophilic endogenous ligand Anandamide. The activation of CB1 receptors by anandamide stimulates multiple pathways, including: analgesia, antidepressant, well-being, appetite regulation, and inflammatory response. Anandamide also occurs naturally in the cacao beans of the theobroma cacao tree. The fatty acid amide hydrolase enzyme decreases the concentration of anandamide via hydrolysis to arachidonic acid. The present paper explores a method to prevent/delay the hydrolysis of anandamide by applying nanotechnology with natural bioactive compounds. Cacao beans contain N-oleolethanolamine and N-linoleoylethanolamine; and Macca extract contains numerous macamides (N-benzylstearamide, N-benzyloleamide, N-benzyloctadeca-9Z, 12Z-

dienamide, and N-benzyloctadeca-9Z,12Z,15Z-trienamide) exhibiting FAAH enzyme inhibitory effect. Due to lipophilic nature, anandamide exhibits low aqueous solubility and hence low bioavailability. In this article, we propose that a combination of natural bioactives in a nanoemulsion will act as CB1 agonist and commitant inhibitor of FAAH to increase anandamide retention time to a level sufficient to enhance well-being and relieve pain, through reduction of inflammation pursuant to its known action on CB1 receptors.

Introduction

Anandamide (AEA) (Fig. 1) is a fatty acid derived neurotransmitter chemically called N-arachidonoylethanolamine. It is an endogenous cannabinoid, isolated from pig brain by William Devane and coworkers in 1992 [1] and found in tissues of many mammals [2], and also occurring naturally in many plants, including the cacao beans of the theobroma cacao tree. Biologically, AEA acts similar to cannabinoids (isolated from Cannabis sativa or Cannabis indica), and is synthesized enzymatically in the areas of the brain that are important in memory, thought processes and control of movement [3]. The endocannabinoid system (ECS) is a complex cell signaling system discovered in 1990 by researchers, when they were exploring tetrahydrocannabinol, the primary psychoactive component of cannabis. It is a systemic neuro-modulatory system affecting numerous aspects of central nervous system (CNS) development and responses to certain exogenous cannabinoid type compounds. The ECS is an expansive and complex cell signaling network of receptors, endocannabinoids, and enzymes within our bodies and is directly involved in establishing and maintaining human health and homeostasis, remaining active full time, and is not dependent on exogenous cannabinoids. ECS research has revealed numerous functions including effects on sleep, learning and memory, motor

control, mood regulation, pain and inflammation, immune response, metabolism, appetite and digestion, stress, and neuroprotection and neuroplasticity [4].

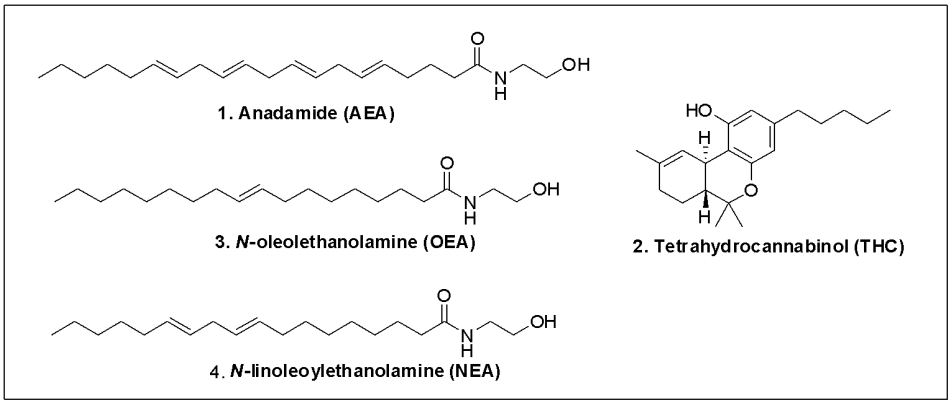

Figure 1: Structure of AEA (1), THC (2), OEA (3) and NEA (4).

The ECS can be divided into three main components: the endocannabinoids which are signaling molecules or ligands, the receptors they bind to, and the proteins and enzymes that break down the ligands following signaling. The ECS contains two key endocannabinoids: AEA and 2-arachidonoglycerol (2-AG) that are biosynthesized on-demand in the postsynaptic terminals and that act at the receptor sites [5]. AEA was the first endocannabinoid to be discovered and is one of the most well studied endogenous cannabinoids, while also being an exogenous cannabinoid found in some foods.

The main receptors associated with the ECS are of a specific type known as G-protein-coupled receptors and are named CB1 and CB2 for cannabinoid

receptors 1 and 2 respectively. These receptors are located throughout the body with CB1 found most densely in our central nervous system while CB2 receptors are more concentrated in the peripheral nervous system and immune cells [6, 7]. CB1 is one of the most abundant G protein-coupled receptors (GPCRs) in the human body. Endocannabinoids can bind to either CB1 or CB2. Examples include the binding action to CB1 receptors of spinal nerve cells resulting in pain relieving effects, and anti-inflammatory responses produced when cannabinoids bind to CB2 receptors of the ECS. Due to the shape, size and chemical residues of AEA, it maintains a higher affinity for the CB1 receptor (Ki = 89 nM) as compared to the CB2 receptor (Ki = 329 nM) [8]. AEA binding to CB1 results in blocked neurological signals for pain, resulting in an analgesic effect [Fig. 2] [9]. Tetrahydrocannabinol, the principal psychoactive constituent of cannabis, like AEA, also binds primarily to CB1 receptors [10, 11]. CB1 receptors are also affected by other non-psychoactive components of cannabis and numerous other endogenous ligands that can provide physiological benefits [12, 13].

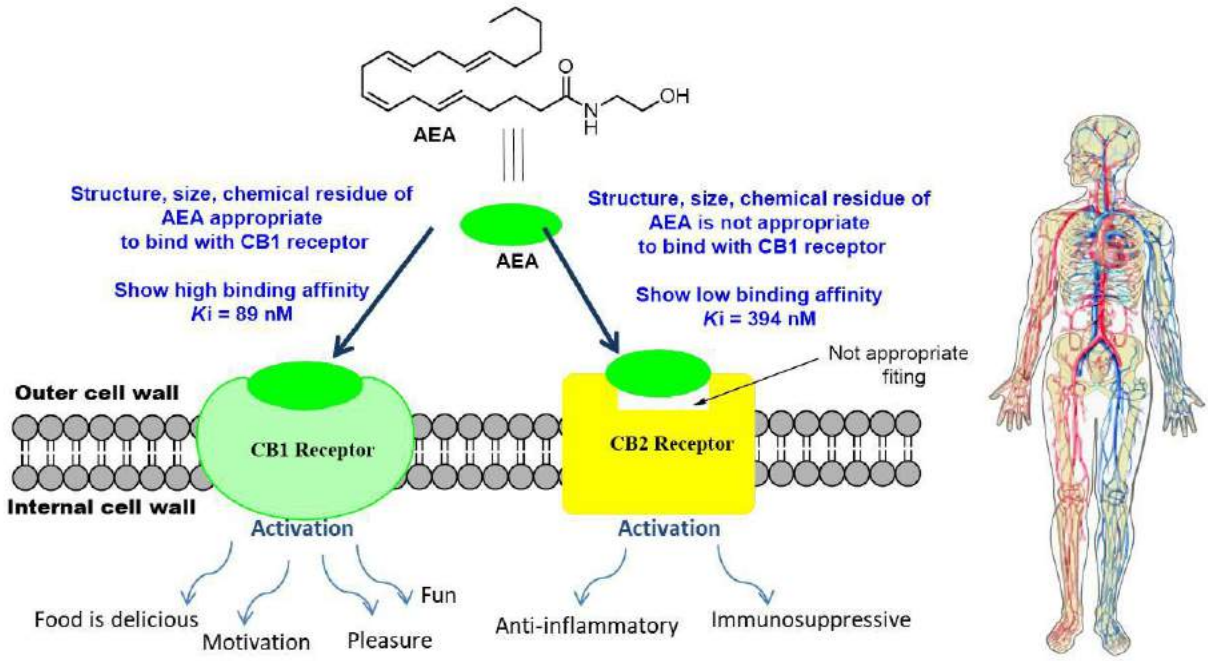

Figure 2: AEA binding affinity CB1 vs CB2 receptor

The word "Anandamide" originated from Sanskrit words "ananda" and "mide", meaning "the bliss chemical." Our brain naturally produces small amounts of AEA, with more being produced in other tissues under certain physiological stimuli such as sexual arousal or increased levels of exercise (sometimes called "runners high") and then passing to the brain to exert beneficial effects on mood, such as reduced anxiety, stress, and providing a general feeling of well-being [14,15].We can also obtain smaller measures of

AEA from food products, such as those containing cocoa beans as in chocolate, which has been claimed to increase systemic levels of AEA.

Among many others, chocolate contains two compounds: N-oleolethanolamine (OEA), and N-linoleoylethanolamine (18:3 NAE), a known anti-inflammatory molecule – analogous to AEA (owing to their structure similarity, and belonging to a family of fatty acids derivatives (Fig. 1). These two compounds can impair the breakdown of endogenous AEA. They were found to inhibit the AEA hydrolysis in rat brain microsomes by Tomaso and his team [16]. The hydrolytic cleavage of AEA yields arachidonic acid and ethanolamine as products. In the presence of OEA and 18:3 NAE, the effect of astrocytes on AEA is strongly reduced, leading to lower hydrolysis rate [17]. Chocolate thus has a potential to increase levels of AEA and prolong the action of this natural brain lipid, largely through the action of NAE and OEA as Tomaso revealed, rather than through the presence of any significant amounts of AEA in cacao/chocolate.

Elevated AEA levels produce a feeling of well-being, relieve pain and produce a sense of transitional euphoria [18]. As noted above, AEA also influences cells that affect mood, memory, appetite, pain perception, and (overall) may in fact provide medicinal benefits similar to THC. But, to have a generous/notable effect on endogenous AEA levels, several kilos of

chocolate would be needed. [19]. Vincenzo et al [20] reported little or no AEA present in cacao, and noted it was unlikely the amounts present resulted in any observable psychotropic effects. Experiments indicate that AEA may be as important as the more well-known neurotransmitters: dopamine and serotonin. AEA may act as a component in the control of cognition and expression of emotions. AEA, when injected directly into the forebrain of rats, enhances their pleasurable responses to sucrose rewards, and increases their food intake as well [21].

In a normal physiological state, AEA is present in very low levels and has a very short half-life due to the action of the enzyme fatty acid amide hydrolase (FAAH), which breaks it down into two inactive constituents, free arachidonic acid and ethanolamine (Fig. 3). As above, the presence of OEA and NAE in chocolate was found to inhibit this natural breakdown of AEA. Therefore, AEA levels may be enhanced in this manner for a greater period of time allowing for extended beneficial effects.

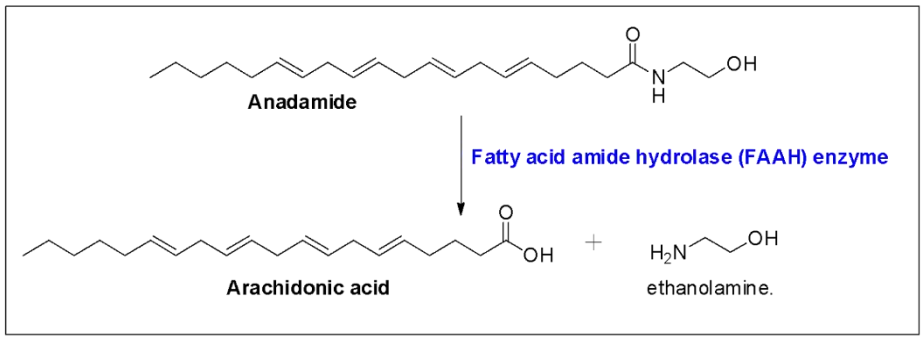

Figure 3. FAAH enzyme assisted conversion of Anandamide to Arachidonic acid.

To attain enhanced levels of AEA, we propose a complementary coupled strategy using exogenous natural FAAH inhibitors, CB2 receptor antagonists, and nanotechnology to take an unabridged advantage of endogenous AEA. The constant urge for amelioration in drug therapeutic efficacy, controlled release, site-specific targeting, shelf life stability, bioavailability, biodegradability and other positive attributes, propel the research of nano-based advanced drug delivery modules. These applications are equally applied to food science technology.

Nanotechnology is a rapidly advancing field that is expected to have a revolutionary impact on many industries, including food and medicine. A myriad of nano-modules has been constructed using various polymers, lipids, inorganic materials or their combinations with the desired physicochemical properties and biological functions for the treatment of various diseases. Nano formulations are capable of enhancing drug efficacy by increasing the retention time and reducing normal tissue toxicity. Many drugs have limitations such as poor solubility, poor bioavailability, lower stability and

toxicity [22]. 95% of all new potential therapeutics have poor pharmacokinetics and biopharmaceutical properties. Nano based delivery systems are taken up by cells more efficiently than the larger micro-molecules and hence can be easily transported and act as efficient therapeutic agents.

Nanoemulsions (NE) are colloidal dispersions that can be fabricated from oil, water and surfactants. It may be oil in water (o/w) or water in oil (w/o), depending on whether water is dispersed as droplets or vice versa. It is defined as a stable colloidal dispersion consisting of two liquids in which one liquid disperses as a droplet (r <100nm) in the other liquid. They are considered to be efficient carriers due to its small size and large surface area. It controls the particle size and properties of the substances. Moreover, the small sizes of particles enhance long-term stability to particle aggregation and gravitational separation, high optical clarity make it suitable for incorporation into products that need to be optically clear and increased bioavailability of certain lipophilic bioactive components.

To protect and enhance the properties of lipophilic compounds such as AEA in a safe and effective manner, the lipid-based nano-systems offer an attractive subject of interest owing to the inherent traits of lipids, including cost-effectiveness, biocompatibility, and particle size versatility. Further,

when lipid-based nano-modules like nanoemulsions are fabricated, the riveting properties at nanoscale, which include high surface area, increased solubilisation potential, sufficient drug loading, higher encapsulation, improved bioavailability, resilience in surface functionalization, and protection of bioactive components under stress conditions (e.g. oxidation, pH and enzyme degradation) and more, also contribute to efficacious targeting and delivery of the compound.

AEA is a highly lipophilic, and it is usually supplied as an oil preparation. Since the oral bioavailability of AEA is known to be very low in humans, it is commonly administered via the sublingual route. AEA is metabolized after administration, therefore, the oral bioavailability of AEA is affected by both the poor solubility, i.e., low absorption, and the large first-pass effect [23]. Drug exemplification in nanocarriers may assist with overcoming the impediments related with cannabinoids and similar lipophilic compounds [24].

Our proposition makes use of a combination of natural plant extracted bioactive agents as inhibitors of FAAH, and CB2 antagonists in a nanoemulsion as a method to increase AEA by limiting its breakdown to a level sufficient to reduce inflammation and and reduce anhedonia pursuant to its known action on CB1 receptors.

Unsaturated fatty acid amide hydrolase (FAAH) is a membrane bound protein which plays a pivotal role in ceasing/breaking the signalling of AEA and other fatty acid amides like N-oleoylethanolamine and N-palmitoylethanolamine in the central nervous system. In order to regulate the therapeutic potential of AEA, FAAH inhibitors are being used to limit the expression of FAAH on AEA [25]. They enhance the activity of AEA indirectly by hindering its metabolism and could serve as potential therapeutic agents for the treatment of diseases where the endocannabinoid activation is beneficial for enhancing mood and reducing inflammation. Neurochemical studies on FAAH-knockout mice by Khanna et al. [26] demonstrated a 10-15 fold increase in the AEA concentration in various locations of the mice in the absence of FAAH. Interestingly, the increased levels of AEAs and its analogous compounds correlated well the analgesic effects, therefore demonstrating the role of FAAH in AEA catabolism in-vivo and further proving its hampering effect on the pain pathways.

To date, a large number of chemical scaffolds have been designed, synthesized and investigated for in-vitro FAAH inhibition activity based on extensive structure-activity relationship studies, directed at ameliorating FAAH inhibitory potency and selectivity. However, only very few have reached clinical trials owing to their enormous side-effects including the

tragic disaster of a Phase I clinical trial of BIA, 2016, in France which killed one participant and left 5 irreversibly brain damaged [27]. We therefore make use of natural, safe and potent FAAH inhibitors with minimum side-effects.

Earlier research showed the naturally occurring isoflavone compounds genistein and daidzein inhibit the hydrolysis of AEA by FAAH in very low concentrations, and flavonoids, particularly, Kaempferol, 7-hydroxyflavone and 3,7-dihydroxyflavone were found to inhibit FAAH by activating PPARγ [28]. It was found that they all inhibit FAAH in both intact cells and cell-free preparations at low micromolar concentrations. Subsequent studies showed that this natural potential is also exhibited by Diadzein and soy isoflavone. In another study by Thors et al. [29] Biochanin A was rated a promising inhibitor of FAAH. Another, natural and effective FAAH inhibitor is the extract of the Peruvian plant, Lepidium meyenii (Maca). Maca extract contains numerous macamides shown to have neuroprotective activity. Four of these maccamides (N-benzylstearamide, N-benzyloleamide, N-benzyloctadeca-9Z, 12Z-dienamide, and N-benzyloctadeca-9Z,12Z,15Z-trienamide) exhibited inhibitory effect on FAAH [30].

As way of example, a number of plant-based dietary ingredients have been reported to enhance the endocannabinoid system, including, capsaicin, the active ingredient in hot peppers, which binds specifically to the vanilloid TRPV1 receptor and binds weakly to cannabinoid CB1 and CB2 receptors. TRPV1 activation results in release of AEA, which binds strongly to CB1 and weakly to CB2 [31].

Arachidonic acid (ARA) is an unsaturated, essential fatty acid which also exhibits CB2 antagonism in greater fashion. ARA is synthesized from dietary linoleic acid or by denaturation and chain elongation of the essential fatty acid, linoleic acid [32, 33}. It is a fundamental constituent of cell structure required for development and growth, and a vital constituent of the biological cell membrane to maintain fluidity and flexibility which is necessary for the functioning of cells in the nervous system, skeletal muscle and the immune system. The fluidity, mobility and selectivity towards membranes is due to the presence of a cis double bond in ARA. It evokes K+ channel opening in neurons which play a critical role in modulating cortical neuronal excitability [34]. ARA in free state modulates the function of the ion channels and several receptors and enzymes *via* activation as well as inhibition [35]. ARA and its metabolites promote and modulate type 2 immune responses that are very important in resistance to assault by

parasites and allergens, directly *via* action on eosinophils and basophils. ARA in cell membranes undergoes reacylation/deacylation cycles, which keep the concentration of free ARA in cells at very low level and limit ARA availability to oxidation [36]. The free ARA levels should be kept low in the cells as uncontrolled accumulation of unesterified ARA has been shown to decisively impair cell survival by inducing apoptosis.

N-oleoyethanolamide (OEA) is a naturally occurring ethanolamide lipid which controls feeding and body weight in vertebrates ranging from mice to pythons and has the ability to activate three or four superfamilies of receptors. OEA is a monounsaturated form of AEA, but unlike anandamide it acts independently of the cannabinoid pathway in which Peroxisome Proliferator-Activated Receptor Alpha (PPAR-α) activity is regulated to stimulate lipolysis. OEA performs as an endogenous ligand and has been found to bind the novel cannabinoid 7-transmembrane receptor GPR119, the transmitter-gated channel TRPV1 and the nuclear receptor PPARα. OEA is not an agonist at cannabinoid receptors, as it does not bind to CB1 or CB2. It has been shown to potentiate anandamide responses by the so-called 'entourage effect'[37]. It was identified as a satiety factor, linking the gastrointestinal system, the pancreas, and, potentially, the brain. Rodriguez de Fonseca *et al.* [87] reported that OEA also plays a major role

in feeding behaviour. Its presence fluctuates in the small intestine with feeding, decreasing when animals are prevented access to food. OEA reduces both food intake (for 12 h) and long term weight gains in rats and mice.

Our proposition embodies a combination of Kaempferol, maca extracts, ARA, and OEA in a nano delivery format to optimize absorption and maximize bioactivity through extended release, in a base of chocolate from cacao beans which naturally contain N-oleolethanolamine (OEA), and N-linoleoylethanolamine (18:3 NAE) as additional FAAH inhibitors. T and potentiators of AEA. The optimal levels and ratios of these compounds are reserved for future research.

Conclusion:

The activation of the CB1 receptors of the endocannabinoid system by the endogenous ligand Anandamide (AEA) results in pain relieving and mood enhancing effects. Anandamide is biologically hydrolyzed by the fatty acid amide hydrolase enzyme (FAAH). The concentration of anandamide in the brain can be increased through FAAH enzyme inhibition and antagonizm of the CB2 receptor. Therefore, a combination of natural plant extracts from the commonly derived flavonoid Kaempferol with Lepidium meyenii (maca)

and Theobroma cacao as FAAH inhibitors, along with Arachidonic acid (ARA) and N-oleoyethanolamide (OEA) from plant sources as CB1 antagonists and AEA potentiators all in a nanoemulsion will act as CB1 agonist as well as strongly inhibit FAAH enzymes to increase AEA to a level sufficient to stimulate mild euphoria and relieve pain through reduction of inflammation pursuant to its known action on CB1 receptors.

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