Lipids in Neural Function: Modulation of Behavior by Oral Administration of Endocannabinoids Found in Foods

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Introduction

Although Δ9(-)-tetrahydrocannabinol (THC) is not naturally found in the body, receptors for this compound have been described in brain since 1988 [1]. These receptors were later named CB1 and a receptor subtype (CB2) has been found in immune tissues [2]. An endogenous ligand for these receptors has more recently been discovered: it is the ethanolamide of arachidonic acid and was named ‘anandamide’ from the Sanskrit word ‘ananda’ which means ‘bliss’. When anandamide was injected into animals, hypomobility, hypothermia, analgesia and catalepsy were observed [3]. These are the classical behavioral effects of THC administration. Since this discovery, other endogenous metabolites which have been shown to be functional agonists of these receptors have been several polyunsaturated fatty acid derivatives, some of which include Δ4,7,10,13,16,19-docosahexaenoyl ethanolamide, Δ8,11,14,17-docosatetraenoyl ethanolamide, Δ8,11,14-di-homo γ-linolenoyl ethanolamide [4], 2-arachidonylglycerol (2-AG) [5, 6] and Δ5,8,11-eicosatrienoyl (Mead acid) ethanolamide [7] (Fig. 1). These compounds are collectively termed endocannabinoids and have been shown to bind to brain CB receptors with an IC50 value equal to or 1.5-fold that of anandamide [8, 9].
Fig. 1. Structures of endogenous ligands of the cannabinoid CB1 receptor and oleamide.
The presence of CB receptors in diverse invertebrates is evidence that the cannabinoid signalling system has been conserved for at least 500 million years [10, 11].

**Tissue Distribution**

The CB1 receptor is widely distributed in several brain regions including the hippocampus, cortex and striatum, the cervical ganglia and peripheral autonomic fibers [12]. CB1 is also present in non-nervous tissues such as prostate, uterus, testes, bladder, small intestine, spleen and lymphocytes. The CB2 receptor is found in immune tissues such as the spleen, thymus and tonsils and in immunocompetent blood cells with highest concentration in B cells, natural killer cells, mast cells and monocytes.

The distribution of endocannabinoids in the brain is not completely consonant with the regional distribution of their binding sites. This suggests that either they may not necessarily be produced near their targets, or that they play functional roles other than CB receptor activation [13]. The highest concentrations of anandamide found so far occur in uterine tissue in animal studies [14]. Down-regulation of anandamide levels is associated with uterine receptivity for implantation whereas up-regulation is associated with uterine refractoriness. Although this hypothesis is still largely unexplored, it is thought that anandamide will prove to play an important role in reproduction.

**Active Molecules**

*Acylethanolamines (NAEs)*

*N-Arachidonyl*ethanolamine, or anandamide, has been isolated from the human hippocampus, striatum and cerebellum – brain areas known to express high levels of CB1 cannabinoid receptors – together with its putative biosynthetic precursor, *N*-arachidonoylphosphatidylethanolamine [13].

*Docosahexaenoyl*ethanolamide and *di-homo-gamma-linolenoyl*ethanolamide bind to rat brain CB1 receptor and show similar potency in IC$_{50}$ values for inhibition of adenylate cyclase. When injected into animals, these two compounds exerted effects on behavior similar to THC and anandamide, indicating that the two ethanolamides are highly potent endocannabinoids [4].

*Palmitoylethanolamide* was the first NAE discovered in the 1950s as the active anti-inflammatory component in fractions of peanut oil, soybean lecithin and egg yolk with demonstrated activity in animal models [15, 16]. More recently, palmitoylethanolamide has been shown to act by inhibiting synthesis of interleukins-4, -6, and -8 [17] and diminishing inflammatory response in a number of experimental models. Although it has low affinity for CB1 or CB2 receptors, data has been recently presented indicating that
Palmitoylethanolamide competes for the binding of a high affinity ligand to membranes from rat basophilic leukemia cells [18], increasing the likelihood that fatty acid ethanolamides represent a family of transmitters, each with a distinct receptor [19]. The significance of this finding was brought out in a study of pain reception in skin where pain responses were reduced 100-fold more potently when both anandamide and palmitoylethanolamide were administered simultaneously [19]. Synergism and ‘entourage’ effects are highly important concepts in the understanding of endocannabinoid action [20].

Other naturally occurring NAEs do not bind to CB1 and CB2 receptors with high affinity [21–25] and their biological significance is not clear. These compounds, however, can inhibit the breakdown of the more biologically potent NAEs, such as anandamide, by acting as alternative substrates for fatty acid amide hydrolase, the enzyme which degrades anandamide.

**Simple Amides**

Oleamide (cis-9-octadecenoamide) is a major primary amide found in mammals. It was first identified from the cerebral spinal fluid of a sleep-deprived cat, and when injected into a rat, induced rapid physiological sleep [26]. It can be synthesized from oleic acid and ammonia in brain microsomes [27]. Oleamide does not bind to cannabinoid receptors at physiological concentrations [28], but similarly to linoleoylethanolamide, oleoylethanolamide and 2-arachidonoyl glycerol (see below) it inhibits the breakdown of anandamide by acting as an alternative substrate for fatty acid amide hydrolase [29, 30]. In addition, oleamide has been observed to enhance binding of anandamide to CB1 receptors [31] and potentiate anandamide antiproliferative action on human breast cancer cells [32].

**Sn-2 Arachidonoylglycerol (2-AG)**

2-AG resembles anandamide in its molecular conformation and may have an even greater affinity for CB1 than anandamide itself. It may play an even more important role than anandamide biologically since it was shown to be present at approximately 200- [6] to 800-fold [33] greater than the concentration of anandamide in some regions of the rodent brain. It has also been found in spleen, pancreas and intestinal cells of rats. 2-AG is a CB1 receptor agonist in neuroblastoma concentrations [34] and inhibits the breakdown of anandamide [24]. The two other sn-2-acylglycerol compounds, sn-2-linoleylglycerol and sn-2-palmitoylglycerol, have been shown to potentiate both the activity of 2-AG with respect to its binding to CB receptors, and its capacity to inhibit adenylyl cyclase, as well as in its effects on behavior [20].

Free arachidonic acid, 2-palmitoylglycerol, 2-oleylglycerol, 2-linoleoylglycerol and 2-docosahexaenoylelglycerol are inactive or weakly active on CB1 receptors in neuroblastoma cells [34].
Synthesis and Degradation

NAEs are phospholipase D metabolites of membrane-bound N-acyl phosphatidylethanolamines (NAPE) which are formed from calcium-dependent transacylase reactions utilizing the sn-1 position of phosphatidylethanolamine, phosphatidylcholine or cardiolipin as the acyl group donor [35]. This pathway has been shown to apply also to arachidonoylethanolamide, i.e. anandamide [36]. Typically, saturated or monounsaturated fatty acids predominate on the sn-1 position, thus a selective pool of sn-1 polyunsaturated phospholipid molecules must be used in the transacylase reaction [23–25].

Degradation occurs by the action of fatty acid amide amidohydrolase (FAAH) [30], an enzyme with broad substrate specificity found in many tissues including small intestine, liver, eye and brain [37–39]. The lack of specificity of this enzyme may be important in physiological regulation since the co-release of varying substrates that compete for its hydrolytic activity may potentiate the actions of others. This interaction has recently been demonstrated for oleamide [31]. Although FAAH is capable of synthesis and degradation in vitro [40], degradation is probably more important under physiological conditions.

NAEs are also substrates for lipoxygenases and form new eicosanoid products, some of which have been shown to be bioactive [9, 41, 42].

Functions

Cannabis (marijuana) has been used medicinally for more than 4000 years for the treatment of disorders which include migraine, anorexia, asthma, muscle spasms, seizures, glaucoma, neuralgia, pain, diarrhea, and nausea. When anandamide is injected into rodents, the classical behavioral effects induced by marijuana or its active ingredient, THC – hypomobility, hypothermia, analgesia and catalepsy, are reproduced [3, 43, 44]. In general the effects occur with rapid onset and short duration [45].

In brain, anandamide is synthesized enzymatically in those areas important in memory and higher thought processes, and in areas that control movement as well as nociception. More recently it has been suggested that endocannabinoids are involved in brain development, probably through the activation of second messenger-coupled cannabinoid receptors [46].

Thus, anandamide and its related lipid family are involved in regulating many crucial physiological functions:

**Memory:** There are a number of animal studies which suggest that anandamide and potentially other CB1 receptor agonists induce forgetfulness, a critical ‘sorting’ process necessary in the processing and retention of important memories. Evidence from rodent trials has shown that anandamide impairs...
working memory [47] and memory consolidation [48] and Derkinderen et al. [49] have postulated a role of anandamide in making and breaking short neural connections.

Intracerebroventricular administration of anandamide strikingly increased sleep – both slow wave and rapid eye movement (REM) types at the expense of wakefulness, and deterioration of memory consolidation was confirmed [50]. These observations suggested that the brain cannabinoind system participates in the modulation both of vigilance states and mnemonic processes. In concert with these observations, further rodent studies have shown that the CB1 receptor antagonist SR141716A improved memory [47, 51] and facilitated short-term olfactory memory in the social recognition test [51]. These results strongly support the concept of a role for endocannabinoids in forgetting and lead to the tantalizing suggestion that CB1 receptor blockers might aid the laying down of effective memory.

Sedative: The presence of CB1 receptors and anandamide and 2-AG in the hypothalamus implies that endocannabinoids may play a role in the tuning not only of sleep/wake cycles but also of other hypothalamic functions such as thermoregulation and food intake. Indeed, the physiological responses of hypothermia and hypomobility are found with anandamide or THC injection [45, 52]. One of the mechanisms by which this occurs has been shown to be through interference with dopaminergic and GABAergic transmission [53]. Ultrasonic vocalizations in rat pups separated momentarily from their mothers is a recognized sign of anxiety. This behavior was decreased by administration of the CB receptor agonist CP 55,940 and increased by the CB receptor antagonist SR141716A [54].

When the CB1 receptor antagonist SR141716A was administered, the time spent awake by rats [55] was prolonged. Since oleamide has been recognized as the sleep inducing-agent, there may be hitherto unrecognized links between this bioactive compound and the CB1 receptor system [31]. The control of appetite is partially directed by the central cannabinoind system. Williams and Kirkham [56] observed overeating in mice given any dose of anandamide from 0.5 to 10 mg/kg s.c., an effect that was attenuated by the previous administration, in a dose-dependent manner, of the CB receptor antagonist SR141716A.

Gut Motility: In rodents, gut motility has been shown to be decreased by anandamide [22]. In mice, anandamide injection inhibited the passage of a charcoal meal, an effect which was reversed by the administration of the CB receptor antagonist SR141716A [57].

Hypotension: Anandamide influences vascular tension through vaso-relaxant and neuromodulatory effects as shown by studies in renal arterial segments [58]. Administration of anandamide was shown to decrease systemic blood pressure in rats [59], and in anesthetized guinea pigs where the effect was prolonged if an inhibitor of anandamide transport was administered [60].
The hypotension and bradycardia which has been observed with anandamide administration seems likely to be mediated by action on presynaptic CB1 receptors in sympathetic nerves [61, 62]. More recently, anandamide has been shown to activate vanilloid receptors on perivascular sensory nerves and to induce vasodilation [63].

The hypotensive effect of anandamide is not limited to the systemic vasculature – it also plays a role in ocular pressure [64–66] leading to the development of new strategies for treatment of eye disease such as glaucoma.

**Pain:** THC has well-known antinociceptive properties. Consistent with anandamides being endocannabinoids, peripheral administration of anandamide has been shown to inhibit the induction of hyperalgesia by capsaicin [67]. Calignano et al. [19] have shown highly effective decrease in skin pain (100-fold) with the administration of palmitoylethanolamide and anandamide.

## Endocannabinoids in Foods and Oral Administration

In 1996, a study was published which claimed that anandamide had been discovered in chocolate [68], a rather surprising finding as it is known that higher order plants do not synthesize arachidonic acid. Nevertheless, the press seized on this finding as the explanation for the desirability of chocolate and the feelings of well-being associated with its consumption. Whether the quantitative aspects of this finding were realistic were also discussed. Some calculations indicating that 25 pounds of chocolate would have to be injected in order to feel marijuana-like effects were reported in the press!

However, the possible presence of anandamide or other potentially bioactive fatty acid amides was intriguing, and we decided to investigate the possible presence of these compounds in various foodstuffs. In addition, at that time nothing was known about the availability to the brain of NAEs ingested through diet, all previous work having been done in animals with administration by injection. If fatty acid amide biology was to have relevance to food and nutrition, it was therefore crucial to demonstrate that oral administration of these compounds had psychotropic effects. The present study had two aims: first to survey several different foodstuffs for fatty acid amide content, and second to see if administration orally to animals, rather than by injection, of pure compounds was effective in altering classical behavioral responses.

## Methods

**Foods:** The following materials were selected for measurement: coffee cherries and beans, cocoa beans unfermented, fermented unroasted and
fermented roasted, cocoa powder, dark chocolate, peanuts, hazelnuts, walnuts, soybeans, oatmeal, millet, olives, and milks—bovine milk and colostrum and early and mature human milk.

**Analysis:** Materials were extracted with CHCl₃/MeOH 2:1 (v:v) containing [²H₈]anandamide, [²H₄]-C₁₁₆:0, -C₁₁₈:1n-9, -C₁₁₈:2n-3 and -C₁₁₈:3n-3 NAEs, and [²H₈]-2-AG as internal standards. In order to purify and characterize the NAEs and 2-AG, the organic phase was brought to dryness and submitted to a series of chromatographic steps including SiO₂ open-bed chromatography and normal-phase high-pressure liquid chromatography (NP-HPLC). For NP-HPLC, column fractions containing NAEs and 2-AG were eluted with increasing concentrations of 2-propanol in n-hexane as previously described [69]. Quantitation of NAEs and 2-AG was by gas chromatography-electron impact mass spectrometry (GC-EIMS) of the corresponding trimethyl-silyl-ether derivatives is described below. HPLC fractions with the same retention time as synthetic NAEs and oleamide (23–29 min) and 2-AG and 1(3)-AG (16–22 min) were derivatized with 20 ml N-methyl-N-trimethylsilyl-trifluoroacetamide containing 1% trimethyl-chlorosylan for 2 h at room temperature, and analyzed by GC-EIMS in the selected ion-monitoring mode as previously described. Ions were selected at m/z = 427, 419, 412 and 404 for anandamide (corresponding to the molecular ions and the −15 mass units fragment ions of [²H₈]₈- and nondeuterated anandamide, respectively), and at m/z = 530, 522, 515 and 507 for 2-AG (corresponding to the molecular ions and the −15 mass units fragment ions of [²H₈]₈- and nondeuterated 2-AG, respectively). An analogous procedure was followed for the other NAEs. The sensitivity of the measurement was 1 pmol. When the endogenous amounts were of sufficient quantity, GC-EIMS was also run in the total ion current mode, thus yielding whole EIMS spectra. Amounts of NAEs and 2-AG were quantitated by comparison with the corresponding and co-eluting deuterated standards by calculating the ratios between the areas of the peaks obtained at the m/z of the -15 mass units fragment ions. For oleamide quantitation, standard curves were constructed with 0.1–5.0 nmol of the synthetic compound, and run immediately after underivatized aliquots of HPLC fractions containing the ‘unknown’ samples. Both oleamide standards and ‘unknowns’ were analyzed in the total ion current mode.

**Behavioral Tests:** Sabra female mice received food and water *ad libitum* and were maintained at constant ambient temperature (20–22°C) on a 12 h light/dark cycle. Compounds were dissolved in olive oil and gradually administered *per os* (100 µl/10 g mouse) with animal intubation needles (20 g, Perfectum; Popper & Sons, New Hyde Park, N.Y., USA) inserted 3–3.5 cm into the esophagus. Pups were tested 15 min after gavaging, as preliminary information indicates that the peak effect of anandamide starts 10 min after *per os* administration [3]. The mice were subjected to a series of consecutive observations based on a standard procedure employed to evaluate
cannabinoid-induced effects in mice [70] with similar time intervals as previously described [3]. The tests described below are described in detail by Sulcova et al. [71]:

**Ambulation:** Horizontal ambulatory motor activity was measured in an open field of $20 \times 30$ cm, divided into 12 squares of equal size, over a period of 8 min, for the numbers of squares crossed.

**Rearing:** Vertical rearing was assessed as the number of rears in an open field.

**Ring test immobility:** Immediately after the open field test, catalepsy was assessed as the number of seconds spent motionless on a 5.5-cm diameter, for up to 4 minutes.

**Body temperature:** Rectal body temperature was measured immediately before food administration and again after the ring test with a telethermometer (Yellow Springs Instruments Co., Yellow Springs, Ohio, USA).

**Analgesia:** Finally, analgesia on a hot plate was measured as the latency until the first hindpaw lick or jump (rarely observed) from the hot plate maintained at $54^\circ C$ with a maximum response time of 45 s. It should be noted that Ankier [72] did not observe any histopathological damage when mice were kept for up to 60 s on a $59^\circ C$ hot plate.

**Intestinal motility:** Inhibition of intestinal mobility has been observed after THC administration [73]. The number of fecal pellets that accumulated during the open field testing was recorded as a measure of intestinal motility.

All procedures met NIH Ethical Standards and Hebrew University Faculty of Medicine/Hadassah Medical Association Institutional Animal Care and Use Committee Standards (Approval No. OPRR-A 5011–01).

**Results**

**Anandamide**

Results (Table 1) show that $N$-arachidonylethanolamine is very low or not present in cocoa beans, unfermented or fermented, unroasted or roasted. Anandamide is also not present in cocoa powder nor in dark chocolate. Thus, di Tomaso et al. [68] reported finding of anandamide in chocolate may be due to an artifact, or to microbial contamination and anandamide is not likely to account for chocolate’s purported pleasurable properties.

In the present analyses, anandamide was also not found in caturra coffee cherries, green beans, peanuts, hazelnuts, walnuts, soybeans, oatmeal, millet or olives.

On the other hand, human breast milk was found to contain small amounts of anandamide (21 ng/g total extracted lipid), and the possible biological significance of this is not known.
Table 1. n-Acylethanolamines, oleamide and arachidonylglycerol in food products and human milk (±SD)

<table>
<thead>
<tr>
<th>Material</th>
<th>Ethanolamides</th>
<th>Oleamide</th>
<th>AA MAG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/g starting material</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c16:0</td>
<td>c18:1n-9</td>
<td>c18:2n-6</td>
</tr>
<tr>
<td>Fatty acyl moiety</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Coffee</strong></td>
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<tr>
<td>Caturra coffee cherries with skin</td>
<td>54 ± 35</td>
<td>228 ± 75</td>
<td></td>
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<tr>
<td>Coffee green beans, Arabica</td>
<td>63 ± 22</td>
<td>17 ± 6</td>
<td></td>
</tr>
<tr>
<td><strong>Cocoa</strong></td>
<td></td>
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<tr>
<td>Cocoa beans, unfermented, unroasted, unhulled</td>
<td>10 ± 4</td>
<td>148.8 ± 87</td>
<td>108 ± 35</td>
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<tr>
<td>Cocoa beans, fermented, unroasted, unhulled</td>
<td>121 ± 53</td>
<td></td>
<td></td>
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<tr>
<td>Cocoa roast, fermented, roasted, hulled</td>
<td>20 ± 18</td>
<td>214 ± 144</td>
<td>41 ± 9</td>
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<tr>
<td>Cocoa powder</td>
<td>1,464 ± 401</td>
<td>2,172 ± 695</td>
<td>5,844 ± 1,515</td>
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<td>Dark chocolate, 70% cocoa</td>
<td>4 ± 3.9</td>
<td>435 ± 147</td>
<td>224 ± 125</td>
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<td><strong>Nuts, soya, grains, olives</strong></td>
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<td>Peanuts, with salt</td>
<td>77 ± 27</td>
<td>273 ± 31</td>
<td>260 ± 98</td>
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<td>Hazelnuts</td>
<td>58 ± 22</td>
<td>1,055 ± 399</td>
<td>247 ± 102</td>
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<td>Walnuts</td>
<td>13 ± 4</td>
<td>21 ± 4</td>
<td>76 ± 27</td>
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<tr>
<td>Soybeans, white, whole, dehulled, dried</td>
<td>126 ± 43</td>
<td>302 ± 101</td>
<td>805 ± 249</td>
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<td>Oatmeal large flakes</td>
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<td>890 ± 298</td>
<td>2,750 ± 977</td>
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<td>Millet, Rustica</td>
<td>44 ± 12</td>
<td>9 ± 4</td>
<td>431 ± 133</td>
</tr>
<tr>
<td>Olives, green, with salt, spice water, acidifiant</td>
<td>11 ± 4</td>
<td>95 ± 27</td>
<td>47 ± 20</td>
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<tr>
<td><strong>Milk</strong></td>
<td></td>
<td></td>
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<tr>
<td>Bovine milk, immature, fresh frozen</td>
<td>112 ± 37</td>
<td>111 ± 41</td>
<td>4.2 ± 0.6</td>
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<tr>
<td>Bovine milk, mature, fresh frozen</td>
<td>271 ± 53</td>
<td>65 ± 39</td>
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<tr>
<td>Bovine milk, pasteurized</td>
<td>140 ± 29</td>
<td>452 ± 210</td>
<td>117 ± 31</td>
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<td>Human milk early, pooled, frozen</td>
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<tr>
<td>Human milk, mature, pooled, frozen</td>
<td>156 ± 117</td>
<td>227 ± 7</td>
<td>38 ± 15</td>
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<tr>
<td>Goat milk, Commercial</td>
<td>528 ± 7</td>
<td>57 ± 5</td>
<td>9 ± 4</td>
</tr>
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Lipids in Neural Function

MAG and NAE have calming properties when orally administered

**Fig. 2.** Effects on behaviors of oral administration of anandamide, 2-arachidonylglycerol (2-AG) and oleamide. The positive control was Δ⁹(−)-tetrahydrocannabinol (THC) and the negative control was olive oil.

**Other Fatty Acid Amides and Arachidonylglycerol**

In contrast to the absence of anandamide in plant materials, other NAEs and oleamide were found to variable degrees in the materials studied. Oleoyl- and linoleoylethanolamides were the most important ethanolamides found in the plant materials. Some palmitoylethanolamide was also present and this congener was found in milk as well. All materials contained oleamide, and milk proved to be the richest source of this fatty acid amide of all materials tested. Human milk contained higher 2-AG compared to bovine milk.

**Bioactivity**

Anandamide (300 mg/kg), oleamide (200 mg/kg) and 2-AG (400 mg/kg) had demonstrated effects on three of the four behaviors tested and body temperature, but had no influence on analgesia or intestinal motility (Figs. 2–4). Lower doses were inactive in all tests. Intraperitoneally injected doses of these compounds have been shown to be effective at much lower doses [3, 43].

**Conclusion**

As might be expected, higher order plants do not contain arachidonyl species, in particular amides, ethanolamides or glycerides of arachidonic acid.
Fig. 3. Effects of oral administration of anandamide, arachidonylglycerol (2-AG), oleamide, Δ⁹(-)-tetrahydrocannabinol (THC) and olive oil on body temperature (±SE).

Fig. 4. Effects of oral administration of anandamide, arachidonylglycerol (2-AG), oleamide, Δ⁹(-)-tetrahydrocannabinol (THC) and olive oil on fecal output (±SE).
On the other hand, ethanolamides of other fatty acids, oleic, linoleic and, to a lesser extent, palmitic, are present. Oleamide is the most abundant of the fatty acyl amides in plant and animal food sources.

What is the potential for these compounds to influence behavior when consumed in a normal diet? Based on the present study, calculations show that unreasonably large amounts of a food would have to be consumed to achieve levels of ingestion of fatty acid amides such as were shown to be effective in the mouse. However, there are several factors to consider. First it is known from in vitro studies that some of the amide species, while having lesser affinity to the receptor themselves, will inhibit the breakdown of other, more active species. Therefore, a mixture of the compounds may have a synergistic effect which is difficult to predict. Second, in the present study, the animal testing was based on clearly evident responses to stimuli: very strong, discernable and pharmacologic. The question is left whether lower levels result in a much attenuated effect, although not discerned and perhaps not discernable with the present methods used. Clearly in the nutrition of normal daily life, this strong an effect would not be desirable.

Finally, as discussed in the introductory paragraphs of this monograph, CB receptors are found in many tissues, including the gut. The potential for influences of these lipid compounds on enterocytes or gastroenteral or systemic immune cells deserves further study.

References


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Discussion

**Dr. Langhans:** What is known about the effects of endocannabinoids on immune cells? You mentioned that there are receptors on immune cells.

**Dr. Crozier-Willi:** These compounds seem to have anti-inflammatory effects when given by injection subcutaneously, so there is a potential therapeutic application in the field of inflammation.

**Dr. Fernstrom:** With a lot of drugs, you can’t just apply a mg/kg dose from one species to another. I wonder if there is a conversion factor required here; 39,000 kg of chocolate equivalents may not be right! The compound may be more potent or more actively metabolized in the human. Have you thought about that?

**Dr. Crozier-Willi:** I gave you that number to illustrate how carefully we did the work, and we found no anandamide in chocolate. Plant species do not make arachidonic acid so it would be extremely unusual to find arachidonyl species in plants. Oleamide has the most potential, and I also think that some products on the market may contain oleamide, which has not yet been recognized but is having a psychotropic effect. For example in the United Kingdom there are drinks that have been traditionally been consumed to help sleep. These are mixed with milk, which may have sedative properties for other reasons. But I would like to see whether these products contain oleamide, which may be contributing to their pacifying effects.

**Dr. Fernstrom:** But I think you may get further faster if you look at the bioavailability issues. Drugs like fenfluramine, for example, are actively concentrated in the brain, and depending on the species this concentration is greater or lesser, so there are numerous examples where you can’t compare dose from one species to another. If you are interested in the active principle you should try to find out as quickly as possible if you think it’s centrally acting, and how much in various species is actually getting from the mouth to the brain, if that’s where you think it is acting, because it may be that only a very tiny amount is required in humans.

**Dr. Crozier-Willi:** That’s a good point.

**Dr. Haschke:** Your calculations on the psychotropic effect in mice indicate a dose of 200 mg/kg. Now even if you have exaggerated somewhat, an intake of 68 mg in a 5-kg infant cannot be very far below the no-effect level. In classical toxicology that would create a problem if you put the substance into food. I’m not sure that we are orders of magnitude away from an effect in another species.

**Dr. Crozier-Willi:** Well, I did carry that calculation, even using the somewhat exaggerated figures that I showed you. For the infant I calculated that there would be a 3,000-fold difference. But what we saw in mice in my opinion is a huge behavioral effect. This was quite clear from the experiment, where the behavioral endpoint was immobility[OG1]. I think we could already be under the influence of much lesser effects and much smaller quantities. So I think you’re quite right – we are finding out things about foods that we didn’t know before, and some of these foods may be having psychotropic effects.

**Dr. Holm:** You used olive oil as a placebo. Can you be sure that olive oil doesn’t have any effects on the variables you tested?

**Dr. Crozier-Willi:** Well, we can be pretty sure, because we found very low levels of cannabinoids in olives and also olive oil is extracted and refined.

**Dr. Holm:** If you continue with investigations on the immune system you must be cautious with olive oil.
Dr. Crozier-Willi: You are referring to the oleic acid. Yes, I agree.

Dr. Kaye: What happens if you eat a lot of arachidonic acid in your diet?

Dr. Crozier-Willi: We tend to eat a fair amount of arachidonic acid if we consume animal products. You mentioned that your anorexic patients do not eat animal fats and are mostly vegetarian, so I think you have made an interesting point which probably bears following up. Arachidonic acid can be synthesized from linoleic acid, but depending on what your patients are eating this could be problematic in itself as there is a lot of competition for the enzymes that form arachidonic acid. Thus, depending on the balance of linoleic and linolenic acid that you take in, there may be problems in synthesizing enough arachidonic acid. If you are not obtaining it through animal products your question is very relevant.

Dr. Kaye: Are all chocolates equivalent? Is it possible that different chocolates have different chemical compositions?

Dr. Crozier-Willi: We did find variable levels of these different bioactive ingredients depending on how the cocoa beans had been treated. If they were fermented there were more of these compounds, so there may have been a microbial contribution. If they were roasted there was more also, which reflects water loss but may also have something to do with the processing – if you have oleic acid and amide or protein amide sources, then drying under heat may result in the formation of oleamide, for example. So it’s conceivable that different chocolates and different processing technologies could contribute differing amounts.

Dr. Kaye: Are there different species of cocoa beans that are chemically different?

Dr. Crozier-Willi: I can’t answer that question. This area is too new. There are different species of cocoa beans for sure, but whether or not there are differences in their composition in terms of these compounds I don’t know.

Dr. Rosenberg: I’m interested in the information you gave us about a knock-out mouse. Perhaps that could lead to a better understanding of what this CB receptor is there for. What is the phenotype of the knock-out mouse?

Dr. Crozier-Willi: They have very uncoordinated motor movement. I’ve never worked with that animal so I don’t know about it in detail.

Dr. Rosenberg: Do we only have a knock-out for the CB1 receptor?

Dr. Crozier-Willi: To my knowledge that’s true. However, I don’t think it will be very long before we have the CB2 receptor, as it’s already been characterized.

Dr. Uauy: How about other psychotropic compounds such as opioids? Have you explored other factors that may be in food that could make us addicted or aversive to foods?

Dr. Crozier-Willi: No, we haven’t investigated that at all. When people found the opioid receptor, they started looking also for the endogenous ligands and they found them in terms of endorphins. It wouldn’t surprise me if foods contained endorphins, but we haven’t investigated that. I think it’s quite clear that when we eat, there are psychotropic effects – for example, if we are very hungry with a low glucose and so on, we immediately feel much better if we eat something. Some of those changes reflect variations in the usual metabolites and nutrients, but there may also be a contribution from these psychotrophic compounds in foods.

Dr. Freeman: You talked about the modest levels of ethanolamides in bovine milk. What about if you fractionated the milk? In the ketogenic diet we use heavy cream. I’m struck by some major side effects of this type of diet: we have terrible problems with constipation, but we have major positive effects on hyperactivity and behavior; the children don’t become immobile but they certainly become less mobile, and they are also less sensitive to pain. If you looked at cream, would the ethanolamides be higher?
**Dr. Crozier-Willi:** I didn’t do the calculations for such an exaggerated diet but I wonder if there could be something there. Those symptoms are perfectly consistent with cannabinoid agonism.

**Dr. Woods:** Do animals self-administer these compounds? If so, how do the doses compare with the ones that were gavaged in the mice?

**Dr. Crozier-Willi:** No, THC is not an addicting compound and animals don’t self-administer.

**Dr. Møller:** There is a particular form of depression in women, associated with the consumption of large amounts of chocolate – affected people are in fact chocaholics. Do you think that these amounts of chocolate could be having a beneficial effect to such individuals?

**Dr. Crozier-Willi:** From the results of our study, I would say that there is very little chance that the euphoric or pleasurable effects of chocolate come from anandamides, as we didn’t find any in chocolate. I’m the first one to agree that chocolate does have something wonderful about it, but it’s certainly not the anandamide content. I think there are a lot of psychosocial factors around chocolate consumption that contribute to the desire for chocolate in these women.

**Dr. Fernstrom:** If you push up the amount of arachidonic acid consumed in the diet, can you increase the formation of these compounds? Is there some connection between how much of a precursor is consumed and the level of the final active compound in the brain?

**Dr. Crozier-Willi:** That’s a very good question, but we haven’t seen any published data on it.

**Note Added in Press**

Since the discussion was held, new data has been released showing that in contrast to previously published studies, monkeys which had been used in prior cocaine abuse studies will self-administer THC. Tanda G *et al.* *Nature Neuroscience*, 2000; 3: 1073–4.