Care and Feeding of the Endocannabinoid System: A Systematic Review of Potential Clinical Interventions that Upregulate the Endocannabinoid System

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Abstract

Background: The “classic” endocannabinoid (eCB) system includes the cannabinoid receptors CB1 and CB2, the eCB ligands anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and their metabolic enzymes. An emerging literature documents the “eCB deficiency syndrome” as an etiology in migraine, fibromyalgia, irritable bowel syndrome, psychological disorders, and other conditions. We performed a systematic review of clinical interventions that enhance the eCB system—ways to upregulate cannabinoid receptors, increase ligand synthesis, or inhibit ligand degradation.

Methodology/Principal Findings: We searched PubMed for clinical trials, observational studies, and preclinical research. Data synthesis was qualitative. Exclusion criteria limited the results to 184 in vitro studies, 102 in vivo animal studies, and 36 human studies. Evidence indicates that several classes of pharmaceuticals upregulate the eCB system, including analgesics (acetaminophen, non-steroidal anti-inflammatory drugs, opioids, glucocorticoids), antidepressants, antipsychotics, anxiolytics, and anticonvulsants. Clinical interventions characterized as “complementary and alternative medicine” also upregulate the eCB system: massage and manipulation, acupuncture, dietary supplements, and herbal medicines. Lifestyle modification (diet, weight control, exercise, and the use of psychoactive substances—alcohol, tobacco, coffee, cannabis) also modulate the eCB system.

Conclusions/Significance: Few clinical trials have assessed interventions that upregulate the eCB system. Many preclinical studies point to other potential approaches; human trials are needed to explore these promising interventions.

Introduction

The endocannabinoid (eCB) system consists of receptors, endogenous ligands, and ligand metabolic enzymes. Metaphorically the eCB system represents a microcosm of psychoneuroimmunology or mind-body medicine. Cannabinoid receptor 1 (CB1) is the most abundant G protein-coupled receptor expressed in the brain, with particularly dense expression in (rank order): the substantia nigra, globus pallidus, hippocampus, cerebral cortex, putamen, caudate, cerebellum, and amygdala [1]. CB1 is also expressed in non-neuronal cells, such as adipocytes and hepatocytes, and in musculoskeletal tissues. Cannabinoid receptor 2 (CB2) is principally associated with cells governing immune function, although it may also be expressed in the central nervous [2,3].

The quintessential eCB ligands are N-arachidonylethanolamide (anandamide, AEA) and sn-2-arachidonoylglycerol (2-AG). AEA and 2-AG are released upon demand from cell membrane-embedded phospholipid precursors. The primary biosynthetic enzyme of AEA is N-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD). 2-AG is biosynthesized by two isoforms of diacylglycerol lipase, DAGLα and DAGLβ. AEA and 2-AG work in a homeostatic fashion, thus they are broken down after they activate CB1 or CB2. AEA is catabolized primarily by fatty acid amidase hydrolyase 1 (FAAH1), and 2-AG is catabolized by monoacylglycerol lipase (MAGL), and, to a lesser extent, αβ-hydrolase-6 (ABHD6), cyclooxygenase 2 (COX2), and FAAH1. This “classic eCB system” has expanded with the discovery of secondary receptors, ligands, and ligand metabolic enzymes [4]. For example, AEA, 2-AG, N-arachidonoylglycine (NAGly) and the phytocannabinoids Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) may also serve, to different extents, as ligands at GPR55, GPR18, GPR119, and several transient receptor potential ion channels (e.g., TRPV1, TRPV2, TRPA1, TRPM8). The effects of AEA and 2-AG can be enhanced by “entourage compounds” that inhibit their hydrolysis via substrate competition,
and thereby prolong their action. Entourage compounds include N-palmitylethanolamide (PEA), N-oleylethanolamide (SEA), and cis-9-octadecenoamide (OEA, oleamide).

The eCB system’s salient homeostatic roles have been summarized as, “relax, eat, sleep, forget, and protect” [3]. It modulates embryological development, neural plasticity, neuroprotection, immunity and inflammation, apoptosis and carcinogenesis, pain and emotional memory, and most importantly from the viewpoint of recent drug development: hunger, feeding, and metabolism. Obese individuals seem to display an increased eCB tone, driving CB₁ activation in a chronic, feed-forward dysfunction (reviewed by [6]). An antagonist or inverse agonist of CB₁ called rimonabant (aka, SR141716 in preclinical studies) was approved for the treatment of obesity. It was subsequently withdrawn from the market due to adverse effects [7].

Other diseases are associated with suboptimal functioning of the eCB system. Russo [8] proposed that migraine, fibromyalgia, irritable bowel syndrome, and related conditions represent CEDS, “clinical endocannabinoid deficiency syndromes.” Fride [9] speculated that a dysfunctional eCB system in infants contributes to “failure to thrive” syndrome. Hill and Gorzalka [10] hypothesized that deficient eCB signaling could be involved in the pathogenesis of depressive illnesses. In human studies, eCB system deficiencies have been implicated in uncompensated schizophrenia [11], migraine [12], multiple sclerosis [13], Huntington’s [14,15], uncompensated Parkinson’s [16], irritable bowel syndrome [17], uncompensated anorexia [18], and chronic motion sickness [19].

Correcting CEDS may be accomplished via at least three molecular mechanisms: 1. augmenting eCB ligand biosynthesis; 2. decreasing eCB ligand degradation; 3. augmenting or decreasing receptor density or function. Clinical interventions for CEDS are largely unknown; this provided a rationale for reviewing potential clinical approaches. The paucity of human clinical trials led us to include preclinical studies in a systematic review. A systematic review uses an objective, transparent approach for research synthesis, with the aim of minimizing bias. Systematic reviews include preclinical studies in a systematic review. A systematic review uses an objective, transparent approach for research synthesis, with the aim of minimizing bias. Systematic reviews usually analyze human clinical trials, but the methodology can be applied to preclinical studies [20,21]. We previously conducted a systematic review of in vitro CB₁ ligand binding affinity and receptor distribution [22]. The review has alerted others to interspecies differences in preclinical studies, and other methodological issues (e.g., [23]).

Potential clinical interventions (intervention groups) include pharmaceutical drugs, such as analgesics (acetaminophen, nonsteroidal anti-inflammatory drugs, opiates, glucocorticoids), antidepressants, antipsychotics, anxiolytic agents, and anticonvulsants. We also investigated therapeutic approaches classified as “complementary and alternative medicine” (CAM). The National Center for Complementary and Alternative Medicine (NCCAM) defines CAM as “a group of diverse medical and healthcare systems, practices, and products, that are not currently part of conventional medicine” (http://nccam.nih.gov/health/whatiscam/). The NCCAM categorizes CAM practices into three broad groups: “natural products” (dietary supplements and herbal remedies), “mind and body medicine” (meditation, yoga, and acupuncture), and “body-based practices” (massage, spinal manipulation). For the purposes of this review, we add “lifestyle modifications,” including diet, weight control, exercise, and commonly-used psychoactive substances—alcohol, tobacco, coffee, and cannabis.

Methods

Data Sources and Search Parameters

This review followed the guidelines proposed by PRISMA, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [24], see Checklist S1. PubMed (www.ncbi.nlm.nih.gov/pubmed/) was searched through March 2013 using three MeSH keywords: endocannabinoids, cannabinoids, cannabinoid receptors. Each keyword was entered in a boolean combination with each of the intervention groups listed in the previous paragraph. Titles and abstracts of identified articles in all languages were screened for inclusion and exclusion criteria. We included randomized clinical trials, observational studies, and preclinical research on model organisms and in vitro studies. We excluded redundant articles that used identical methods and reported parallel results, or review articles that presented duplicate information.

Because this review focuses upon clinical interventions affecting the eCB system, we deemed as irrelevant (and excluded) articles that described the reverse scenario, such as eCB ligands modulating opioid receptors, THC enhancing tobacco or alcohol abuse, etc. Retrieved articles were scanned for supporting citations, and antecedent sources were retrieved and screened for inclusion and exclusion criteria. In addition, we checked reference lists of relevant narrative reviews.

Data Selection, Abstraction, and Synthesis

All three authors selected studies for inclusion and exclusion; the first author abstracted all data, the second and third authors arbitrated uncertainties and disagreements. We undertook a qualitative synthesis across studies because there was substantial heterogeneity with respect to research methodologies amongst the
identified articles—ranging from randomized clinical trials, observational studies, and preclinical research on model organisms and in vitro studies. The substantial heterogeneity amongst these methodologies precluded a single metric of quality assessment.

Many studies utilized in vitro measures of receptor density and signal transduction, as differences in means before- and after-interventions. Briefly: assays for CB1/CB2 receptor density include autoradiography with tritiated ligands (usually [3H]CP55,940 or [3H]SR141716), Western blot or immunostaining with antibodies to CB1/CB2 proteins, and Northern blot with radio-labeled or fluorescent ribonuclease inhibition for CB1/CB2 mRNA. Signal transduction studies measure cannabinoid-stimulated inhibition of adenylyl cyclase, cannabinoid-stimulated [35S]GTPyS binding, or electrophysiological assays of ex vivo brain slices. Electrophysiological studies include depolarization-induced suppression of excitation (DSE, via glutamatergic synapses), depolarization-induced suppression of inhibition (DSI, via GABAergic synapses), long-term depression of excitatory synaptic transmission (LTDE, via glutamatergic synapses), or long-term depression of inhibitory synaptic transmission (LTDI, via GABAergic synapses).

Publication bias was addressed by asking investigators to contribute unpublished studies. Clinical interventions (intervention groups) with five or more studies are provided with an interpretative summary at the end of the section (e.g., the sections on NSAIDs, glucocorticoids, opiates, etc.).

Results and Discussion

The search algorithm identified 6,353 potentially relevant articles. The majority of these were irrelevant. For example, combining the three MeSH keywords with “alcohol” generated 2450 hits, many of which concerned the relationship between alcohol and cannabis in motor vehicle accidents or suicides. Only 322 articles met the predefined selection criteria for relevance. See Figure 1 for a flowchart of articles included in this review. Few randomized clinical trials have been conducted on our topic; most of the articles concerned preclinical research. Fewer studies measured the effects of clinical interventions on CB1 expression in humans. This is because the measurement of CB1 expression requires positron emission tomography (PET) or brain biopsies. Although cannabinoid radioligands for PET scans are available, few PET studies on clinical interventions have been completed. Ethical issues circumcribe brain biopsies in living humans. A few studies measured postmortem CB1 expression.

The use of PubMed as a stand-alone search engine may have generated bias regarding CAM practices. McPartland and Pruitt [23] used PubMed to compile a review of clinical trials regarding the CAM herbal medicine Serenoa repens; PubMed yielded only 35% of articles that they subsequently obtained by screening retrieved articles for supporting citations. Expanding our search by screening retrieved articles for supporting citations improved the yield, as it did in the Serenoa review.

The quality of in vitro studies such as [3H]CP55,940 binding at CB1 was generally high, for example, PMSF was used when appropriate. Methods used in two electrophysiology studies were controversial, and the studies were removed after urging by our manuscript reviewers. The quality of some rodent models of behavior was also questionable. Rather than judge their translational validity—a contentious issue [26]—we have named the specific behavioral assays in each study, allowing the reader to pass judgment.

Pharmaceutical drugs

Non-steroidal anti-inflammatory agents (NSAIDs). NSAIDs inhibit two cyclooxygenase (COX) enzymes, COX1 and COX2, and thereby block the conversion of arachidonic acid (AA) into inflammatory prostaglandins. Ibuprofen, ketorolac, and flurbiprofen also block the hydrolysis of AEA into arachidonic acid and ethanolamine [27]. See Figure 2. A binding site for some NSAIDs on FAAH has also been identified [28]. NSAID inhibition of COX2 blocks the metabolism of AEA and 2-AG into prostaglandin ethanolamides (PG-EAs) and prostaglandin glycerol esters (PG-GEs), respectively [29]. PG-EAs and PG-GEs increase the frequency of miniature inhibitory postsynaptic currents (mIPSCs) in primary cultured mouse hippocampal neurons, an effect opposite to that of their parent molecules [30].

Prostaglandin E2 glycerol ester (PGE2-GE), a COX2 metabolite of 2-AG, induced mechanical allodynia and thermal hyperalgesia in rat paws [31]. PGE2-GE contributed to pain perception and dorsal horn nociceptive neuron hyperactivity, thus providing a rationale for the combined use of COX2 and FAAH inhibitors against inflammatory pain [32].

Electrophysiology studies of rat hippocampal cells showed that meloxicam and nimesulide prolonged and increased DSI; that is to say, the COX2 inhibitors potentiated synaptic 2-AG release and CB2 signaling [33]. Consistent with this, intrathecally applied indomethacin enhanced eCB-mediated antinociception in mice that was blocked by the CB2 antagonist AM251 [34]. Intrathecally applied flurbiprofen produced a similar eCB-dependent antinociception in the rat formalin test [35].

Combining NSAIDs with cannabinoids (either CB1s or exogenous cannabinoids) produces additive or synergistic effects. A sub-effective dose of WIN55,212-2 became fully antinociceptive following administration of indomethacin in rats [36]. A local injection of ibuprofen plus AEA in the rat formalin test produced synergistic antinociceptive effects involving both CB1 and CB2 [37]. The FAAH inhibitor URB937, when coadministered to mice with indomethacin, produced a synergistic reduction in pain-related behaviors [38]. Furthermore, URB937 reduced the number and severity of gastric lesions produced by indomethacin. One contrary study showed that THC’s decrease in intraocular pressure was partially blocked by indomethacin in rabbits [39].

In a small human study, the administration of indomethacin antagonized marijuana effects [40]. Yet a high dose of ibuprofen may cause sedation, possibly a cannabimimetic effect [41]. Clinical anecdotes of NSAIDs eliciting cannabimimetic effects have been reported; the individuals are usually familiar with the effects of cannabis, and usually females [42].

In summary, preclinical studies indicate that some NSAIDs inhibit FAAH and enhance the activity of eCBs, phytocannabinoids, and synthetic cannabinoids. Combinational effects may be particularly relevant at peripheral sites, such as the peripheral terminals of nociceptors.

Acetaminophen. Acetaminophen (paracetamol), following deacetylation to its metabolite N-acetyl-p-aminophenol, is conjugated with AA to form N-arachidonoylphenolamine (NAP, aka AM404). It is likely that deacetylation takes place mainly in the liver, and conjugation occurs in the central nervous system. NAP blocks the breakdown of AEA by FAAH, inhibits COX1 and COX2, and acts as a TRPV1 agonist [43]. The analgesic activity of acetaminophen in rats is blocked by CB1 or CB2 antagonists [44,45]. Acetic acid activity is also lost in CB1/CB2 knockout mice [46]. A sub-effective dose of the synthetic cannabinoid WIN55,212-2 became effective following intracisternal adminis-
tration of acetaminophen in rats [36]. A sub-effective dose of AEA in mice became anxiolytic in the Vogel conflict test and the social interaction test when co-administered with acetaminophen; the effect was blocked by the CB1 antagonist AM251 [47].

Small amounts of acetaminophen are also metabolized via the cytochrome P-450 pathway into N-acetyl-p-benzoquinone imine (NAPQI). Intrathecal administration of NAPQI activates TRPA1 and imparts antinoceptive in the mouse hot-plate test, and a similar action is found for Δ2-tetrahydrocannabinol. These effects are lost in Trpa1(-/-) mice [48].

In summary, preclinical studies indicate that acetaminophen enhances the activity of eCBs and synthetic cannabinoids in rodents. Why acetaminophen fails to elicit cannabimimetic effects in humans is unknown. Acetaminophen-cannabinoid drug interactions may be species-specific; Gould et al. [49] demonstrated strain-specific differences in mice. They suggested that other indirect actions of acetaminophen, including 5-HT receptor agonism, may outweigh any CB1 mediated effects in some mouse strains.

**Glucocorticoids.** The distribution of glucocorticoid receptors (GRs) and CB1 overlap substantially in the central nervous system and other tissues, as do GRs and CB2 in immune cells. Dual activation of GRs and CBs may participate in glucocorticoid-mediated anti-inflammatory activity, immune suppression, insulin resistance, and acute psychoactive effects. In a rat model of spinal nerve injury (sciatic nerve constriction with suture loops), the GR resistance, and acute psychoactive effects. In a rat model of spinal nerve injury (sciatic nerve constriction with suture loops), the GR

In summary, preclinical studies indicate that acetaminophen activates TRPA1 system. Chronic corticosterone administration decreased CB1 antagonism, may outweigh any CB1 strain-specific differences in mice. They suggested that other strains.

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**Opiates.** Naloxone, a μ-opioid receptor antagonist, inhibited THC-induced Fos immunoreactivity in several regions of the rat central nervous system, including the ventral tegmental area, hypothalamus, caudate-putamen, and periaqueductal grey. Conversely, naloxone and THC had an additive effect on Fos immunoreactivity in the amygdala, stria terminals, insular cortex, and paraventricular nucleus of the thalamus [63].

Short-term co-administration of morphine with THC caused an upregulation of CB1 protein in the spinal column of rats, far greater than THC or morphine given alone [64]. A rodent study of chronic but voluntary intake of opiates (rats self-administering heroin) enhanced [3H]CP55,940 binding in the amygdala and ventral tegmental area, plus a marked increase in cannabinoid-stimulated [35]GTPγS binding in the nucleus accumbens, caudate putamen, and amygdala [65]. Superperfusion of ex vivo rat nucleus accumbens slices with 4-aminopyridine and NMDA released glutamate and GABA, respectively, and either morphine or the CB1 agonist HU210 predictably inhibited these responses. Combining HU210 and morphine caused a synergistic inhibition of GABA release, but a non-additive response in glutamate release [66].

Chronic morphine exposure in rats caused a reduction in hippocampal and cerebellar CB1 density measured with [3H]CP55,940, and a strong reduction in CP55,940-stimulated [35]GTPγS binding; 2-AG contents were also reduced [67]. Another rat study showed that chronic morphine exposure caused variable, regionally-specific modulations in [3H]CP55,940 binding and CB1 mRNA levels; CB1 upregulated in some regions and downregulated in other regions [68]. In human CB1-transfected HEK293 cells, morphine induced a desensitization of the μ-opioid receptor and heterologous desensitization of CB1, demonstrated by a reduction in WIN55212-2-induced [35]S]GTPγS binding; [69]. μ-opioid receptor knockout mice showed a dramatic reduction in WIN55212-2-stimulated [35]S]GTPγS binding [70]. In human SH-SY5Y neuroblastoma cells, sequential activation of CB1 and δ-opioid receptor produced synergistic elevations of intracellular Ca²⁺, a response that each receptor alone did not trigger in an efficacious way [71].

In behavioral studies, heroin reinstated “drug-seeking” behavior for WIN55,212-2 in rats [72]. Morphine did the same for THC in monkeys [73]. The rewarding effects of THC, measured by conditioned place-preference, were reversed by naloxone in rats [74]. In rats trained to discriminate THC, morphine administration markedly potentiated the THC discriminative stimulus [75]. Morphine or codeine potentiated THC-induced antinociception and analgesia in mice and rats [76–79]; inactive doses of the drugs in combination produce potent, synergistic analgesia [80]. Synergistic analgesia was confirmed in an isobolographic analysis [64]. Historically this is the first isobolographic analysis of a cannabinoid since the days Walter Siegfried Loewe, who invented the isobologram to test drug combinations for synergy [81]. Loewe demonstrated synergy generated by cannabis extracts combined
interaction or exploration, and preference for outer or inner zones potentiated THC-induced hypothermia in rats [91], but did not pressant drugs. Treatment with fluoxetine, the archetypal SSRI, inhibitors (MAOIs) are the most commonly prescribed antide-

Chronic fluoxetine also increased WIN55212-2-stimulated cortex, without altering AEA or 2-AG levels in rat brains [93]. The CUS protocol altered CB receptors. Acute administration of chlorpromazine enhanced the enhancement of encephalin-endorphin into the ventral tegmental area potentiated the THC preservation of CB and WIN55,212-stimulated CB density in the hippocampus and hypothalamus, without significantly altering AEA or 2-AG levels in rat brains [98]. The CUS protocol altered CB density in rat brains, and these changes were attenuated by concurrent treatment with imipramine [99]. Desipramine-induced weight gain was reduced by cotreatment with SR141716A, suggesting an eCB pathway [100]. Treatment with the MAOI tranylcypromine increased CB1 binding density in the prefrontal cortex and hippocampus, and increased 2-AG but decreased AEA levels in the prefrontal cortex [93]. Repeated electroconvulsive shock treatment (EST) for depression produced complex and regionally specific effects. Generally EST downregulated CB1 binding density and AEA levels in the cortex, but enhanced cannabinoid-stimulated [35S]GTPyS binding in the amygdala [101].

In summary, the effects of antidepressant drugs or treatments upon the eCB system are not definitive, but likely result in CB1 upregulation, at least in some brain regions. Preclinical studies suggest agonist trafficking may be responsible for variable responses.

**Antipsychotic drugs.** First-generation antipsychotic drugs, such as haloperidol and chlorpromazine (thiorazine), are dopamine D2 receptor inverse agonist. Second-generation “atypical” antipsychotics (e.g., risperidone, olanzapine, clozapine, and aripipra-

with other drugs [82,83], as well as synergy generated amongst the individual components within cannabis itself [84,85].

Normal men subjected to a thermal pain stimulus did not experience analgesia from a low dose of nabilone (a synthetic THC analogue), or a low dose of morphine. But co-administration of the drugs produced an analgesic effect [86].

Endorphins (endogenous opioids) enhance the effects of cannabinoids: Administering a low dose of THC to rats produced an anxiolytic response in the light-dark box test, which was abolished by beta-funaltrexamine, a μ-opioid receptor antagonist [87]. In rats trained to discriminate THC, microinjection of β-endorphin into the ventral tegmental area potentiated the THC discriminative stimulus [75]. Ekephalins (endogenous opioids) also enhance the effects of THC: the inhibition of encephalin-degrading enzymes augmented THC-induced antinociception in mice, an effect blocked by either rimonabant or naloxone [88]. Naltrexone, a μ- and κ-opioid receptor antagonist, significantly increased many of the “positive” subjective effects of oral THC [89] and smoked cannabis [90] in marijuana smokers. These results suggest that endogenous opioids contribute to the effects of cannabis.

In summary, preclinical studies and clinical trials indicate that acute opiate administration enhances the activity of eCBs, phytocannabinoids, and synthetic cannabinoids. Acute opiates may also upregulate CB1 expression. Chronic opiate administration, however, may have deleterious effects on the eCB system.

**Antidepressant drugs.** Serotonin selective uptake inhibitors (SSRIs), tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) are the most commonly prescribed antidepressant drugs. Treatment with fluoxetine, the archetypal SSRI, potentiated THC-induced hypothermia in rats [91], but did not change THC-induced behavioral effects—freezing behavior, social interaction or exploration, and preference for outer or inner zones [92]. Fluoxetine increased CB1 binding density in the prefrontal cortex, without altering AEA or 2-AG levels in rat brains [93]. Chronic fluoxetine also increased WIN55,212-stimulated [35S]GTPyS binding in the rat prefrontal cortex [94]. Conversely, treatment with citalopram reduced HU210-stimulated [35S]GTPyS binding in the rat hypothalamus and hippocampus [95].

<table>
<thead>
<tr>
<th>Supplemented PUFA</th>
<th>assay; result compared to unsupplemented controls</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHA+AA</td>
<td>in vivo piglets, whole brain homogenates; ↑ AEA, −2-AG</td>
<td>[137]</td>
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<tr>
<td>AA</td>
<td>in vivo mice, whole brain homogenates; ↑ AEA</td>
<td>[137]</td>
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<tr>
<td>DHA</td>
<td>in vivo mice, whole brain homogenates; ↓ 2-AG</td>
<td>[325]</td>
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<tr>
<td>AA</td>
<td>in vivo mice, whole brain homogenates; ↑ 2-AG</td>
<td>[325]</td>
</tr>
<tr>
<td>DHA</td>
<td>in vitro mouse 3T3-3442A adipocytes; ↓ 2-AG, ↓ AEA</td>
<td>[326]</td>
</tr>
<tr>
<td>AA</td>
<td>in vitro mouse 3T3-3442A adipocytes; ↓ 2-AG</td>
<td>[326]</td>
</tr>
<tr>
<td>DHA+EPA</td>
<td>in vivo rats, whole brain homogenates; −AEA, −2-AG</td>
<td>[327]</td>
</tr>
<tr>
<td>or AA</td>
<td>in vivo rats, jejunal homogenates; ↑ AEA, ↑ 2-AG</td>
<td>[142]</td>
</tr>
<tr>
<td>DHA+EPA</td>
<td>in vivo Zucker rats, visceral adipose tissue; ↓ 2-AG, ↓ AEA</td>
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<tr>
<td>DHA+EPA</td>
<td>in vivo rats; serum: ↓ AEA, ↓ 2-AG; brain: ↓ AEA, −2-AG</td>
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<tr>
<td>DHA+EPA</td>
<td>in vivo obese mice; serum: ↓ 2-AG, −AEA</td>
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<tr>
<td>DHA+EPA</td>
<td>in vivo mice; liver: ↓ AEA, −2-AG; brain: ↓ AEA</td>
<td>[131]</td>
</tr>
</tbody>
</table>

1 ↑, increase; ↓, decrease; −, no change; doi:10.1371/journal.pone.0089566.t001

Table 1. Effects of PUFA supplementation upon eCB levels.
increased binding of \([^{3}H]CP55,940\) in the substantia nigra>globus pallidus>striatum. Subchronic haloperidol also potentiated CP55,940-stimulated \([^{35}S]GTP\_S\) binding in the substantia nigra [103]. Sundram et al. [104] confirmed haloperidol’s effects on \([^{3}H]CP55,940\) binding, and obtained similar results with chlorpromazine and olanzapine. In monkeys trained to discriminate THC, haloperidol sensitized the THC discriminative stimulus [105]. Risperidone increased \([^{3}H]CP55,940\) binding in rat brain without altering CB\(_{1}\) mRNA levels [106]. Four weeks of aripiprazole upregulated CB\(_{1}\) in rat frontal cortex [107]. Clozapine decreased \([^{3}H]CP55,940\) binding in rat brain [104], and attenuated THC-induced disruption of spatial working memory in the rat radial maze task [108].

Several researchers have proposed that CB\(_{1}\) upregulation during antipsychotic drug treatment may explain appetite enhancement, weight gain, and CB\(_{1}\) supersensitivity. D’Souza et al. [109] conducted a double-blind study on the effects of adding haloperidol to THC. Compared to THC alone, the combination of drugs significantly worsened verbal recall, distractibility, and vigilance scores. The drug combination did not affect other testing parameters, such as euphoric effects and motor outcomes. Another double-blind study showed that haloperidol reversed THC-induced increases in the Positive and Negative Syndrome Scale (used for measuring symptom severity in schizophrenia), but did not affect the THC-induced “high” in healthy male volunteers [110]. A double-blind study in healthy male volunteers showed that olanzapine reduced the effects of THC as measured on the positive and negative syndrome scale, and the visual analogue scale for psychedelic effects, but the reduction fell short of statistical significance, \(p = 0.67\) [111].

In summary, antipsychotic drugs likely upregulate CB\(_{1}\) expression in parts of the rodent brain. In human clinical studies, antipsychotic drugs do not affect THC-induced “high” or “euphoria,” but dampen dysphoria and worsen verbal recall and distractibility.

**Anxiolytics, sedatives, and anesthetics.** Diazepam is used for treating anxiety, insomnia, muscle spasms, and seizure disorders. Combining diazepam with WIN55212-2 produced a supra-additive anxiolytic effect in the rat elevated plus maze test; combining diazepam with the FAAH inhibitor URB597 also led to a supra-additive effect; coadministration of diazepam with the CB\(_{1}\) receptor antagonist AM251 attenuated diazepam’s anxiolytic effect [112]. These findings might be explained by the observation that both chronic and, particularly, acute administration of diazepam to mice is accompanied by strong elevation of brain eCB levels [113].

The anxiolytic and sedative effects of alprazolam were also attenuated by AM251 in mouse behavioral assays (light-dark box test, neurological severity score, and step-down inhibitory avoidance test) [114]. Surprisingly, however, the administration of alprazolam reduced WIN55212-2-stimulated \([^{35}S]GTP\_S\) binding in mouse amygdala and hippocampus [114]. CB\(_{1}\)^−/− knockout mice showed impaired anxiolytic responses to both buspirone and bromazepam in light/dark box, elevated plus maze, and social interaction tests [115]. N-arachidonoyl-serotonin (AA-3-H), a dual FAAH/TRPV1 blocker, imparted anxiolytic effects in the mouse elevated plus maze assay [116].

A sub-effective dose of THC given to mice caused catlepsy in the horizontal bar test after sub-effective doses of either flurazepam or baclofen were added [117]. The beta-adrenergic blocking agent propranolol causes mild sedation, but pretreatment with propranolol blocked cannabis-induced cardiovascular effects and learning impairment in a small clinical trial [118].

General anesthesia (midazolam, sufentanil, isoflurane, and sufentanil) resulted in decreased serum AEA in patients stressed by the anticipation of cardiac surgery [119]. The dissociative anesthetic phencyclidine (PCP) impairs rota rod performance and open-field behavior in rats, effects shared by THC; combining the two caused supra-additive results [120]. Low-grade Mexican marijuana was adulterated with PCP and marketed as “superweed” in the 1970s [121]. Nitrous oxide and THC both increase pain threshold in the tail-flick and hot-plate test, and their combination caused supra-additive effects [122].

**Anticonvulsants.** Combining diazepam with WIN55212-2 produced a supra-additive anticonvulsant effect in rats; combining diazepam with the FAAH inhibitor URB597 also led to a synergistic effect; coadministration of diazepam with the CB\(_{1}\) receptor antagonist AM251 attenuated the anticonvulsant effect of diazepam [123]. Chronic administration of valproate in rats increased CB\(_{1}\) binding of the PET scan tracer \([^{18}F]MK-9470\); this was not seen with levetiracetam [124]. Tiagabine, an anticonvulsant GABA reuptake inhibitor, augmented THC-induced catalepsy [117] but not antinoceptive or hypothermia [125]. In a human study, tiagabine augmented THC discrimination and enhanced THC effects in other outcomes [126].

Pregabalin is a Ca\(_{v}^{2+}\) channel antagonist used for treating epilepsy and neuropathic pain. Isobolographic analysis demonstrated that combining WIN 55,212-2 with pregabalin exerted synergistic antinociceptive effects in the mouse hot-plate test [127]. Vagus nerve stimulation (VNS) is used as an add-on treatment to patients with drug-resistant epilepsy. Implantation of a vagus nerve stimulator in rats significantly decreased AEA and 2-AG in mesenteric adipose tissue, but increased PEA [128]. Chemical VNS by administration of the peptide hormone cholecystokinin

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**Table 2. Effects of short- and long-term caloric restriction upon the brain eCB system in animal studies.**

<table>
<thead>
<tr>
<th>species, exercise</th>
<th>measure</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>rats administered leptin</td>
<td>leptin (appetite-reducing hormone) decreases hypothalamic AEA and 2-AG levels</td>
<td>[6]</td>
</tr>
<tr>
<td>rats fasted</td>
<td>fasting for 24 h increased AEA and 2-AG in limbic forebrain and 2-AG in hypothalamus;</td>
<td>[328]</td>
</tr>
<tr>
<td>mice fasted</td>
<td>time-dependent effects: short-term fasting (24 h) increased hypothalamic 2-AG; long-term fasting (12 d) decreased hypothalamic 2-AG</td>
<td>[329]</td>
</tr>
<tr>
<td>goldfish fasted</td>
<td>food restriction decreased CB(_{1}), mRNA in the forebrain and increased AEA levels in the telencephalon, two effects reversed by refeeding</td>
<td>[330, 331]</td>
</tr>
<tr>
<td>rats after gastric bypass</td>
<td>weight loss after Roux-en-Y gastric bypass surgery decreased AEA and with no change in 2-AG levels in skeletal muscle</td>
<td>[332]</td>
</tr>
<tr>
<td>Zucker obese rats fasted</td>
<td>fasting decreased CB(_{1}), mRNA in brainstem but not in hypothalamic nuclei</td>
<td>[333]</td>
</tr>
</tbody>
</table>

doi:10.1371/journal.pone.0089566.t002
Complementary and alternative medicine

Dietary supplements: PUFAs. Polysaturated fatty acids (PUFAs) play fundamental roles in many cellular and multicellular processes, including inflammation, immunity, and neurotransmission. They must be obtained through diet, and a proper balance between omega-6 (ω-6) PUFAs and ω-3 PUFAs is essential. The typical Western diet contains a surplus of ω-6s and a deficiency of ω-3s [130].

Arachidonic acid (AA) is the archetypical ω-6, with 20 carbons and four double bonds (20:4ω-6). Some of its metabolites cause chronic diseases seen in Western populations: prostaglandins cause pain and swelling, and leukotrienes cause bronchoconstriction and asthma. The inflammatory metabolites of AA are countered by dietary ω-3s. The two best-known ω-3s are eicosapentaenoic acid (EPA, 20:5ω-3) and docosahexaenoic acid (DHA, 22:6ω-3).

cCBs are derived from AA (see Figure 2). Several preclinical studies showed that dietary supplementation with AA increased serum levels of AEA and 2-AG, summarized in Table 1. Although we clearly need AA to biosynthesize eCBs, ω-3 PUFAs and ω-3s predictably increased the concentration of EPA and/or DHA in tissues, cells, and plasma, and decreased the relative concentration of AA in tissues, cells, and plasma [132,133]. ω-3 supplementation also decreased AEA and 2-AG in tissues, cells, and plasma (Table 1). However, the effects of ω-3 supplementation are nuanced and complex:

Piscitelli et al. [134] fed mice a high-fat diet (cholesterol and saturated fatty acids) with little AA. This diet caused a decrease in AEA and 2-AG in the liver. Supplementing that diet with DHA and EPA increased AEA and 2-AG in the liver. In contrast, the high-fat diet increased AEA and 2-AG in muscle tissue, and supplementation with krill oil decreased AEA and 2-AG. Similar trends were seen in heart, kidneys, and white adipose tissue.

Adequate levels of dietary ω-3s are required for proper cCB signaling. Mice supplemented with ω-3s, compared to mice on a control diet, expressed greater levels of CB1 and CB2 mRNA. Mice supplemented with ω-3s also expressed greater levels of cCB synthetic enzymes—NAPE-PLD, DAGLα, and DAGβ [132].

In apparent contrast with the above findings, Lafourcade et al. [135] showed that ω-3 deficiency abolished cCB-mediated neuronal functions. They reasoned that lifelong ω-3 deficiency causes chronically elevated cCB levels within brain synapses, which leads to CB2 desensitization. They tested a rodent model of depression-like behavior (the forced-swim test), and the ω-3-deficient mice performed like CB2−/− knockout mice. The administration of WIN55212-2 did not change their behavior, whereas in ω-3-rich mice, WIN55212-2 imparted typical cannabinoidic effects. Larrieu et al. [136] demonstrated depressive-like symptoms in ω-3-deficient mice compared to mice fed an ω-3 enriched diet. They used the forced-swim test as well as the more valid open-field and social-investigation tests. Mice deficient in ω-3 showed impairment in the CB1 signaling pathway—ERK1/2 phosphorylation in the hippocampus was reduced after treatment with WIN55212-2, and the antianxiety effects of WIN55212-2 were absent in ω-3-deficient mice.

ω-3 PUFAs may impact the eCB system via a second mechanism: cCB biosynthetic enzymes readily accept ω-3s as substrates. An ω-3-rich diet markedly elevated the N-acyl-ethanolamide metabolite of DHA, called DHEA, the N-acyl-ethanolamide metabolite of EPA, called EPEA, and the sn-2-glycerol-ester metabolite of EPA, called 2-EPG [133,137]. FAAH catalyzed DHEA [138,139], DHEA and EPEA act as cCBs: DHEA and EPEA showed high binding affinity for CB1 (Kd = 124 and 55 nM respectively) and acted as partial agonists [139]. Their affinity nearly equals that ofAEA—a meta-analysis of affinity studies using the same binding assay (mouse brain, [140]CP55940 displacement, presence of PMSF) produced a modal kᵰ value of 61 nM for AEA [122]. DHEA, aka synaptamide, stimulates neurite growth and synaptogenesis in developing hippocampal neurons [140].

In natural fish oil, DHA and EPA are esterified in triacylglycerides (TAG), whereas in many fish oil capsules, DHA and EPA are esterified in EE (ethyl-ester) or TAG (rTAG). Krill oil contains DHA and EPA esterified in phospholipids, primarily phosphatidylcholines, which may improve their bioavailability; furthermore krill oil contains less AA than fish oil [141]. Batetta et al. [142] supplemented the diet of obese Zucker rats with fish oil or krill oil, which contained nearly identical amounts of EPA and DHA. The visceral adipose tissue of krill oil-supplemented rats contained less AEA and 2-AG than fish oil-supplemented rats. In the liver only AEA levels were significantly less. The effects of these dietary sources of DHA and EPA on brain cCB levels were much less pronounced, with krill oil producing only a small decrease of 2-AG levels [143]. The same research group reported similar results in an obese cohort mostly composed by women: krill oil but not fish oil significantly decreased serum 2-AG levels; no significant changes were seen in normo-weight subjects [144]. In a yet unpublished study, one of us observed that in obese men, dietary krill oil reduced plasma AEA levels and concomitantly counteracted hypertriglyceridemia (Di Marzo, unpublished data).

In summary, dietary ω-3s seem to act as homeostatic regulators of the eCB system. In obese rodents fed a high-AA diet, ω-3s significantly decrease cCBs, especially 2-AG, particularly in tissues that become dysregulated, such as adipose and liver tissues. Plasma cCB levels are reduced by krill oil also in obese humans. Little change in cCB levels are seen in normo-weight individuals not fed a high-ω-6 diet, and dietary ω-3s are required for proper cCB signaling.

Dietary supplements: Probiotics. "Probiotics" are endosymbiotic microorganisms that confer a health benefit upon their human hosts. Probiotics occur in fermented foods, such as yogurt and kimchi. The best known organisms are Lactobacillus acidophilus and Bifidobacterium species. "Prebiotics" such as oligofructose are carbohydrates that serve as substrates for probiotic organisms. Human intestinal epithelial cells incubated with L. acidophilus produced more CB2 mRNA [145]. Feeding L. acidophilus to mice and rats increased the expression of CB2 mRNA in colonic epithelial cells. Lastly, mice fed L. acidophilus showed less pain behavior following colonic distension with butyrate than control mice, an effect reversed by the CB2 antagonist AM630 [145].

Probiotics and prebiotics also modulate CB1 expression. Acute probiotic treatment with Enterococcus faecium upregulated CB1 mRNA in Sola salt [146]. Pathologically obese ob/ob mice expressed elevated levels of colon CB1 mRNA [147]. When fed prebiotics such as oligofructose, they expressed less CB1 mRNA, produced less AEA (due to increased FAAH mRNA expression in adipose tissue), and gained less fat mass.

Other dietary considerations. A natural phosphate derivative of vitamin E, α-tocopheryl phosphate (α-TP), is a common supplement...
constituent in plant and animal tissues. Although α-TP does not bind to CB₁, it modulates synaptic transmission in rodent hippocampus slices, an effect blocked by the CB₁ antagonist AM251 [148].

Human breast milk contains small amounts of AEA and high levels of 2-AG, but the biological significance of this is not known [149]. The oral administration of AEA (300 mg/kg), OEA (200 mg/kg) and especially 2-AG (400 mg/kg) in rats produces calming properties [150]. Mouse breast milk also contains eCBs, and when newborn mice are fed the CB₁ antagonist SR141716A, they stop suckling and die [151].

Pesticides such as chlorpyrifos and diazinon alter normal eCB system function [152,153]. We hypothesize that eating organic foods lacking pesticide residues may promote eCB homeostasis. Piperonyl butoxide, which is a synergist added to insecticides such as pyrethrum, is an efficacious but low-potency antagonist of CB₁ and CB₂ [154]. Phthalates are plasticizers added to water bottles, tin cans, food packaging, and even the enteric coating of pharmaceutical pills. Phthalates may act as endocrine disruptors and carcinogens. They also block CB₁ as allosteric antagonists [153].

### Herbal remedies.
Some plants besides Cannabis produce vaguely cannabimimetic effects. Copaïc incense, extracted from *Pistium species* (same plant family as *Bombax*) contains a pentacyclic triterpene with high affinity for CB₁ and CB₂ [156]. Absinthium contains thujone, a constituent of wormwood, *Artemisia absinthium*. Thujone has weak affinity for CB₁ [157]. Pristimerin, an alkaloid found in khat, *Catha edulis*, acts as a potent inhibitor of MAGL (IC₅₀ = 93 nM) and causes an elevation of 2-AG levels in rat cortical neurons [158]. Salvinorin A in *Salvia divinorum* produces cannabimimetic activity in NG108-15 cells expressing CB₁, but the study was retracted [160].

**Table 3.** Effects of exercise upon the eCB system in rodent studies.

<table>
<thead>
<tr>
<th>species, exercise</th>
<th>measure</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>rats, forced swimming for 1 h/d x 6 months</td>
<td>decreased CB₁ antibody expression in adipocytes</td>
<td>[334]</td>
</tr>
<tr>
<td>rats, voluntary wheelrunning, 24 h</td>
<td>running reversed chronic stress-induced deficits in GABAergic synapses to CB₁ stimulation by eCBs and HU210</td>
<td>[208]</td>
</tr>
<tr>
<td>mice, voluntary wheel running, 42 d</td>
<td>running rescued the sensitivity of striatal GABA synapses to CB₁ stimulation downregulated by FAAE induction</td>
<td>[335]</td>
</tr>
<tr>
<td>mice, voluntary wheel running for 15 d</td>
<td>sensitivity of striatal GABA synapses to CB₁ stimulation increased</td>
<td>[336]</td>
</tr>
<tr>
<td>rats, forced treadmill running for 40 d</td>
<td>reduced CB₁ expression in the striatum and hippocampus</td>
<td>[337]</td>
</tr>
<tr>
<td>rats, voluntary wheel running for 8 d</td>
<td>increased CB₁ expression in the hippocampus, increased CB₁-mediated GTP-cs binding, and increased AEA content in hippocampus</td>
<td>[338]</td>
</tr>
<tr>
<td>mice, voluntary wheel running for 10 d</td>
<td>increased CB₁ expression in the hippocampus</td>
<td>[321]</td>
</tr>
<tr>
<td>rats, forced treadmill running for 40 d</td>
<td>no change in gene expression of CB₁, CB₂, or FAAH in liver</td>
<td>[339]</td>
</tr>
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</table>

doi:10.1371/journal.pone.0089566.t003

Compounds with phytocannabinoid-like moieties have been extracted from legumes [167,168], *Helichrysum* [169], *Rhododendron* sp. [170], liverworts [171,172], and fungi [173–175]. Falcariol is a skin irritant found in several plants that causes contact dermatitis. It covalently binds with the CB₁ receptor, causing potent inverse agonistic and pro-inflammatory effects in human skin [176].

Higher plants (angiosperms and gymnosperms) produce PUFAs with acyl tails limited to 18 carbons in length [177]. Hence reports of PUFAs in plants with longer acyl tails, such as AA, AEA, and 2-AG are controversial. Di Tomaso et al. [178] detected AEA in chocolate and cocoa powder derived from *Theobroma cacao*. A subsequent study showed very little, if any, AEA in cocoa powder [149]. Nakane et al. [179] reportedly extracted sciadonic acid (20:3o-6) from seeds of a pine tree, *Sciadopitys verticillata*. This analog of 2-AG exhibited cannabimimetic activity in NG108-15 cells expressing CB₁.

Unlike higher plants, non-vascular plants such as club mosses, mosses, and algae express Δ⁶-elongase enzymes, so they are capable of producing PUFAs with longer acyl tails [177]. Some plants besides *Echinacea* species bind to CB₂, but this was retracted [160]. Semiplenamide A, an AEA-like PUFA isolated from a blue-green alga, *Lyngbya majuscula*, has micromolar affinity for CB₁ and also blocks the AEA transporter, thereby inhibiting AEA breakdown [180]. Grenadamine, a PUFA in *Lyngbya maiquesula*, has micromolar affinities for CB₁ [181]. Soderstrom et al. [182] extracted but did not identify an eCB-like compound from *L. maiquesula*. Soderstrom also extracted a dozen eCB-like PUFAs from unidentified green algae (Chlorophyta), the brown alga *Laminaria angustata*, and the sponge *Mycale micranthsea*.

Some plant ligands bind to CB₂ and modulate the immune system, but have no affinity for CB₁ and do not elicit psychoactivity. Alkaloids from *Echinacea* species bind to CB₂ with nanomolar affinity, and act as CB₂ agonists with immunomodulatory effects [183]. Several constituents from *E. purpurea* root and herb produce synergistic, pleiotropic effects—they bind to CB₂ as well as inhibit AEA uptake [184]. Other constituents from *Echinacea purpurea* act as weak CB₁ antagonists [185].

The principal terpenoid in black pepper, (E)-β-caryophyllene (BCP), binds to CB₂ with nanomolar affinity and acts as an agonist. Its anti-inflammatory effects are reduced in CB₂ knockout

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mice [186]. The protective effects of BCP on colitis in mice are reversed by the CB1 antagonist AM630 [187]. The protective effects of BCP on cisplatin-induced nephrotoxicity in mice are absent in CB2 knockout mice [188]. Lastly, the antiinflammatory effect of BCP in mice is prevented by pretreatment with AM630 [189].

Rutamarin in Ruta graveolens has micromolar affinity for CB2 [190]. An unidentified constituent in noni fruit, Monneda cymbula, shows weak affinity for CB2 [191]. The aromatic resin extracted from mastic, Pistacia lentiscus, contains an essential oil (EO) rich with monoterpenoids and sesquiterpenoids. Rats fed mastic EO showed higher plasma levels of DHA, EPA, PEA, and OEA than control rats, with no change in AEA or 2-AG [192].

Shellfish are not herbal remedies, but they have been used medicinally. AEA and/or 2-AG have been isolated from the species, assay | result compared to controls¹ | reference |
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>human neuroblastoma cell line</td>
<td>↑ [3H]AEA</td>
<td>[340]</td>
</tr>
<tr>
<td>rat cerebellar granule neurons</td>
<td>↑ [3H]2-AG</td>
<td>[341]</td>
</tr>
<tr>
<td>rat, oral administration</td>
<td>↑ AEA limbic forebrain, ↓ AEA+2-AG midbrain</td>
<td>[258]</td>
</tr>
<tr>
<td>rat cerebellar granule neurons</td>
<td>↑ [3H]AEA via ↓ AEA transport and =FAAH</td>
<td>[342]</td>
</tr>
<tr>
<td>mouse, ethanol vapor inhalation</td>
<td>↑ AEA cortex via ↓ FAAH</td>
<td>[252]</td>
</tr>
</tbody>
</table>

¹↑, increase; ↓, decrease; =, no change; assay; result compared to unsupplemented controls.
doi:10.1371/journal.pone.0089566.t004

Mind and body medicine: chronic stress. Chronic or repeated stress results in a chronic elevation of endogenous corticosterone via the hypothalamic-pituitary-adrenocortical (HPA) axis. Chronic stress (repeated restraint) reduced AEA levels in the hypothalamus and hippocampus, but not in the striatum or the frontal cortex; 2-AG levels increased after the last, but not the first, session in the hypothalamus, hippocampus, and frontal cortex [207]. Fear expression after the sessions was prolonged in mice receiving rimonabant and in CB1−/− knockouts. Conditional knockouts lacking CB1 in two defined neuronal subpopulations—glutamatergic neurons and GABAergic neurons—indicated that the former CB1 subpopulation was responsible for the fear responses.

Electrophysiological studies confirm the effects of chronic stress upon the eCB system: Chronic social defeat stress in mice (exposure to aggression) impaired GABAergic synapse sensitivity to eCBs (probably 2-AG) mobilized by group I metabotropic glutamate receptor stimulation [208]. The CUS protocol attenuated eCB-mediated DSE, LTD, and depression of field excitatory postsynaptic potentials [96]. Chronic restraint stress attenuated eCB-mediated DSI in rat hippocampus [209]. These chronic stressors also desensitized CB1 to exogenous cannabinoids: they reduced electrophysiological responses to HU 210 in mouse striatum [205], and to WIN55,212-2 in mouse striatum [96]. Chronic immobilization stress in rats impaired retrograde eCB signaling at GABAergic synapses, and a functional downregulation of CB1 in the paraventricular nucleus of the hypothalamus [210].

Acute restraint challenge in rats induces corticosterone release in the paraventricular nucleus of the hypothalamus (PVN). This is inhibited by dexamethasone, a response blocked by the CB1 antagonist AM251—suggesting that fast feedback requires local release of eCBs. Indeed, PVN content of 2-AG is elevated by the restraint challenge [200].

Acute footshock stress increased 2-AG and AEA levels in the periaqueductal gray and contributed to stress-induced analgesia (SIA) in male rats. SIA enhancement by a MAGL inhibitor and not by a FAAH inhibitor indicated that 2-AG was the primary eCB responsible for SIA [211]. SIA was modulated via CB1 receptors in the basolateral nucleus of the amygdala (BLA); microinjection of SR141716A into the BLA suppressed SIA [212]. Glucocorticoid enhancement of memory consolidation in the acute footshock stress is dependent upon CB1 activation in male rats; WIN55,212-2 infused into the amygdala enhances memory in an inhibitory avoidance apparatus, and AM251 impairs the
response [213]. Acute handling stress in male newts increased serum cortisol levels and induced behavioral changes (less sexual behavior); the latter was blocked by a cannabinoid antagonist, AM281, indicating dependence upon CB1 activation [214].

Acute restraint stress in male rats increases hippocampal content of 2-AG and enhanced eCB-dependent modulation of GABA release measured by whole-cell voltage clamp of inhibitory postsynaptic currents (IPSCs) in hippocampal CA1 cells [215]. Responses in female rats are much more complex, because eCB levels fluctuate across the estrous cycle [216]. The eCB system has been implicated in cycle-dependent changes in pressure pain thresholds in human females [217].

In summary, chronic stress impairs the eCB system, via decreased levels of AEA and 2-AG. Changes in CB1 expression are more labile. Stress management may reverse the effects of chronic stress on eCB signaling, although few studies exploring this possibility have been performed to date. Clinical anecdotes suggests that stress-reduction techniques, such as meditation, yoga, and deep breathing exercises impart mild cannabimimetic effects [218].

Rossi et al. [208] found that mice given access to a running wheel recovered their chronic stress-induced synaptic defects. Accordingly, social play in rats increased CB1 phosphorylation (a marker of CB1 activation) in the amygdala and enhanced AEA levels in the amygdala and nucleus accumbens [219]. The effects of exercise on the eCB system are elaborated below. Grooming behavior, which is a stress-reduction behavior in rodents, increased in response to SR141716A administration [220].

Mind and body medicine: acupuncture. Acupuncture reduced stress-related behavior (from maternal separation in rats) and normalized HPA-induced corticosterone release [221]. Electroacupuncture (EA) reduced thermal hyperalgesia and mechanical allodynia induced by an injection of complete Freund’s adjuvant into rat paws. EA increased AEA levels in skin tissue. The antinoceptive effects of EA were attenuated by the CB2 antagonist AM630, but not by the CB1 antagonist AM251 [222]. Moreover, EA upregulated the expression of CB2 receptors in skin tissues [223]. It appears likely that CB2 activation in the skin stimulates the release of β-endorphin, which then acts on peripheral μ-opioid receptors to inhibit nociception [224].

However, CB1 may play a role in the central effects of EA: rats treated with EA showed reduced GABA levels in the ventrolateral periaqueductal gray, an effect reversed by CB1 blockade with AM251 [225]. Enhanced activation of epsilon protein kinase C in rat brain by EA was reversed by CB1 blockade with AM251 and not by CB2 blockade with AM630 [226].

Mind and body medicine: body-based practices. Massage and osteopathic manipulation of asymptomatic participants increased serum AEA 168% over pretreatment levels; mean OEA levels decreased 27%, and no changes occurred in 2-AG. Participants receiving sham manipulation showed no changes [218]. Osteopathic manipulation of participants with low back pain increased serum PEA 1.6-fold over pretreatment levels, with no change in AEA. Participants receiving sham manipulation showed no changes [227].

Lifestyle modifications

Diet and weight change. Dozens of animal studies and human cohort studies have shown that diets rich in fats and sugars alter levels of AEA, 2-AG, their metabolic enzymes, and CB1. The reverse causality is also true—many studies show that CB1 agonists stimulate the consumption of fat and sugar. The rewarding properties of palatable foods are attenuated by CB1 blockade and in CB1−/− knockouts. Stimulation of feeding behavior by CB1 agonists occurs across the phylogenetic scale, from humans to Hydra, although there is no molecular evidence for CB1 orthologs in invertebrates other than the boneless chordates Ciona intestinalis and Branchiostoma floridae. Reviews on this topic are available [7,228,229], which we do not intend to duplicate here.

Upregulation of the eCB system in obese humans seems to be driven by excessive production of eCBs in several peripheral tissues such as visceral adipose tissue, liver, pancreas, and skeletal muscle. Differences arise between central (intra-abdominal) adipocytes versus peripheral (subcutaneous) adipocytes, with additional variations due to gender, age, and genetic polymorphisms in metabolic enzymes. Visceral adiposity particularly correlates with elevated levels of 2-AG in blood plasma [230]. Increases in circulating eCBs likely reflect spillover from adipose tissues and liver parenchyma, where CB1 activation promotes de novo lipogenesis and reduces insulin sensitivity, respectively. In mice with diet-induced obesity, CB1 mRNA and protein levels increased in the hippocampus, compared to lean controls [231]. Furthermore, hippocampal slices from obese mice showed increased CB1 functionality, with no sign of CB1 desensitization. We find it surprising that sustained elevations of eCB ligands do not result in CB1 downregulation. This may be due to the fact that such elevations are not as dramatic as those caused, for example, by chronic MAGL inhibition. The lack of downregulation may contribute to the hedonic aspects of overeating, and influence cognitive processes.

Weight loss by caloric restriction or fasting predictably modulates the eCB system. Animal studies have demonstrated the complexities arising in adipose tissue versus the central nervous system (Table 2). In human studies, weight loss from caloric restriction has produced conflicting results. Engeli et al. [232] measured CB1 and FAAH gene expression, and serum AEA and 2-AG, in obese postmenopausal women. They reported no changes after 5% weight loss from caloric restriction. Bennetzen et al. [233] analyzed a younger population of obese men and women; a 10–12% weight loss resulted in elevated 2-AG levels in gluteal adipose tissues, with no change in AEA levels. Weight loss increased CB1 mRNA in abdominal adipose tissues but decreased CB1 mRNA in gluteal adipose tissues.

In centrally obese men, decreased plasma AEA and 2-AG levels accompanied a weight loss intervention consisting of both caloric restriction and exercise. Only 2-AG levels correlated with decreased visceral adipose tissue, plasma triglycerides and insulin resistance, and improved HDL-cholesterol levels [234]. However, the influence of caloric restriction and exercise separately was not analyzed in this study. You et al. [235] measured CB1 and FAAH mRNA in subcutaneous abdominal and gluteal adipose tissue in overweight or obese postmenopausal women. Caloric restriction resulted in 11% weight loss, which led to a reduction in gluteal CB1 and FAAH gene expression but no significant changes in abdominal adipose tissue. You and associates also tested the effects of exercise, see below. A 12-week hospital-based weight loss program (moderate caloric restriction along with counseling by dieticians and physical activity teachers) resulted in a mean weight loss of 9.5% and a significant reduction in salivary AEA levels, while salivary 2-AG, OEA and PEA did not significantly change [236].

In summary, increased food intake, adiposity, and elevated levels of AEA and 2-AG apparently spiral in a feed-forward mechanism. Weight loss from caloric restriction breaks the cycle, possibly by reducing CB1 expression and reducing eCB levels.

Exercise. Rodent studies have shown that exercise modulates the eCB system (Table 3). The results of these studies show a critical difference between short-term, voluntary exercises (e.g.,
levels increased incrementally at 55% maximum work output, at exercise, whereas 2-AG concentrations remained stable. AEA male cyclists—serum AEA levels increased significantly during increase [237]. Heyman

The CRM and CRV groups showed a slight increase in CB to a control group that participated solely in caloric restriction. decrease in FAAH mRNA in abdominal adipose tissue, compared combining data from the two groups (CRM

30 minutes of moderately intense treadmill running (70–80% age-

neurobiological rewards are linked to high-intensity exercise in cursorial mammals. The authors showed that humans and dogs share significantly increased exercise-induced eCB signaling, following high-intensity endurance running, whereas eCB signaling did not significantly increase following low-intensity walking, nor did it increase in the non-cursorial ferrets following exercise at any intensity. The same research group showed that serum AEA levels in male and female runners significantly increased after 30 minutes of moderately intense treadmill running (70–80% age-adjusted maximum heart rate), and not after very high or very low intensity exercises [240,241].

In summary, medium- to high-intensity voluntary exercise in cursorial mammals, including humans, increases eCB signaling, via increased serum AEA levels (but not 2-AG), and possibly increased CB1 expression. “Runner’s high” may be an eCB-induced reward for exercise.

Alcohol. Acute administration of a high dose of ethanol in rats decreased AEA levels in brain, serum, and adipose tissue; PEA also decreased in the brain. AEA decrease was associated with inhibition of AEA release and no change in NAPE-PLD or FAAH hydrolysis [242]. However, exposing ex vivo murine hippocampal neuron cultures to lower doses of ethanol increased AEA and 2-AG release [243]. This increase led to reduced presynaptic glutamate release in neuron cultures, which was blocked by SR141716A. There was no change in CB1 density.

Electrophysiological studies of anesthetized rats showed that alcohol enhanced eCB signaling in mesolimbic circuits [244]. This effect was blocked by SR141716A, and by the FAAH inhibitor URB597—indicating AEA involvement. Another study by the same group showed parallel responses in rat amygdala. The downregulation of amygdala CB1 with chronic WIN55212-2 blunted the response to alcohol [245].

Ex vivo exposure of rat striatal slices showed ethanol shifts synaptic plasticity from LTP to eCB-mediated LTDI. Ethanol-enhanced LTDI was blocked by the CB1 antagonist AM251 [246]. The same group showed that ethanol modulated eCB-mediated striatal plasticity in a synapse-specific manner. Ethanol prevented CB1-dependent long-lasting disinhibition (DLL) in the dorsolateral striatum [247]. Furthermore, the study showed that LTDI by an exogenous cannabinoid, WIN55,212-2, was actually prevented by ethanol.

Chronic ethanol treatment decreased CB1 density and decreased cannabinoid-stimulated [35S]GTP[S] activation in various animal models [248–251]. One study of chronic ethanol did not alter CB1 binding of [3H]CP55,940 or CB1 mRNA levels in rat brain homogenates [68]. Short-term chronic exposure (72 hours) of ethanol vapor in mice increased CB1 density in the cortex, hippocampus, striatum and cerebellum, with downregulation of CB1 receptor-stimulated [35S]GTP[S] binding [252]. The effects of chronic ethanol treatment upon eCB levels in various in vitro and animal models are shown in Table 4.

Vinod et al. [251] compared alcohol-prefering (aP) and alcohol-non preferring (NaP) rats, a pair of rat lines selectively bred for opposite alcohol preference. CB1 receptor density, CB1 receptor-stimulated [35S]GTP[S] coupling, and levels of AEA and 2-AG were higher in the brains of alcohol-naive aP compared to NaP
rats. Ethanol consumption in aP rats decreased CB₁ receptor-stimulated \[^{35}S\]GTP\(_S\) binding after 10 days, and moreso after 60 days. 2-AG levels elevated after 10 days, and both 2-AG and AEA levels increased after 60 days; FAAH levels decreased with no change in MAGL. Ethanol withdrawal upregulated \[^{35}S\]GTP\(_S\) binding.

A rat model of binge drinking—serial cycles of ethanol intoxication and withdrawal—increased CB₁ mRNA in the prefrontal cortex [253]. Another study of serial cycles in rats showed a transient decrease in hippocampal CB₁ mRNA and protein levels (two days after cessation of cycles), followed by a long term up-regulation in CB₁ mRNA and protein, 40 days after cessation of cycles. Serial cycles increased 2-AG in the hippocampus, two days and 40 days after cessation of cycles; AEA increased only at 40 days [254].

An electrophysiological study of intermittent ethanol consumption in rats showed depression of CB₁-dependent long-lasting disinhibition (DLL) in excised slices of the dorsolateral striatum [255]. Furthermore, the study showed that LTDI by an exogenous cannabinoid, WIN55,212-2, was prevented by intermittent ethanol consumption.

A human clinical trial assigned 55 adults to one of three groups—drinking either 250 ml of red wine, grape juice, or plain water. Within 10 minutes, the consumption of a moderate amount of alcohol reduces plasma AEA and 2-AG concentrations, whereas an equal volume of grape juice did not affect plasma eCBs. Interestingly, plain water reduced 2-AG concentrations without affecting AEA [256].

Alcoholics who died of natural causes or motor vehicle accidents expressed decreased CB₁ densities in the ventral striatum, decreased CP55,940-stimulated \[^{35}S\]GTP\(_S\) binding, and decreased FAAH activity, compared to controls [257]. Alcoholics who died of suicide in the same study had increased CB₁ densities, decreased CB₁ receptor-stimulated G(i/o) protein activation, and decreased FAAH activity, compared to controls. Lehtonen et al. (2010) measured eCB levels in post-mortem brains of Cloninger type 1 and type 2 alcoholics. Type 1 alcoholics had lower levels of AEA than controls in the nucleus accumbens (NAcc), anterior cingulate cortex, and frontal cortex. PEA, OEA, and 2-AG were unchanged. They also showed dopaminergic deficiencies in the NAcc, suggesting a compensatory mechanism one direction or the other. Type 2 alcoholics produced slightly higher eCB levels than controls, but not significantly.

In summary, acute ethanol may enhance endogenous eCB release and eCB signaling, although it varies by brain area and synapse, and this complexity requires further testing. Two studies suggest ethanol dampens the effects of the eCB system. Chronic ethanol consumption and binge drinking likely desensitize or downregulate CB₁ and impair eCB signaling, except perhaps in areas involved in reward and motivation to self-administer this substance of abuse [258].

**Nicotine.** In a human randomized controlled trial, nicotine augmented THC-induced “high” and heart rate [259]. In rodent behavioral studies, acute nicotine augmented THC discrimination and THC-induced hypothermia, antinociception, locomotor inactivity, anxiolysis, and place aversion [260–264]. Nicotine-potentiated THC discrimination was blocked by rimonabant and URB-597 (a FAAH inhibitor), suggesting nicotine potentiation is mediated by the release of AEA acting at CB₁ [263]. CB₂ is also involved—the CB₂-selective agonist JWH133 induced antinociception in the mouse formalin test, and this effect was potentiated by nicotine [265]. Acute nicotine elicited marked increases in AEA in the amygdala, hypothalamus, and prefrontal cortex but decreased levels in the hippocampus; variations in 2-AG were less pronounced [266].

In a contrary study, intracerebellar microinfusion of nicotine attenuated THC-induced ataxia in mice. Microinfusion of synthetic subtype agonists indicated the involvement of \(\alpha_2\beta_2\) but not \(\alpha_2\) nicotinic receptor subtypes [267]. Buczynski et al. [260] compared volitional self-administration (SA) versus forced nicotine exposure (FA) in the ventral tegmental area using in vivo microdialysis. SA but not FA increased AEA; both SA and FA increased 2-AG; these subclue changes were not seen in corresponding bulk brain tissue analysis of eCBs. Acute nicotine enhanced THC-induced c-Fos expression in various brain regions [264].

**Chronic nicotine increased AEA levels in the limbic forebrain and increased AEA and 2-AG contents in rat brainstem, but decreased AEA and/or 2-AG contents in the hippocampus, the striatum and the cerebral cortex [258]. Chronic nicotine increased CB₁ density in the prelimbic prefrontal cortex, ventral tegmental area, and the hippocampus [269]. Seven days of nicotine exposure increased brain CB₁ densities in adolescent male rats and sensitized them to the locomotor-depressing effects of THC and CP55,940 [270]. These changes were not seen in adult male rats. Chronic nicotine inhibited the development of tolerance to antinociceptive and hypothermic effects of THC [264].

Other plant products that exert cholinergic effects, such as calamus, Acorus calamus, have been admixed with cannabis to decrease cannabis-induced memory deficits, and “calm and center the effects of marijuana” [42]. Consistent with this, the synthetic cholinergic agent rivastigmine reversed memory deficits in rats induced by the synthetic cannabinoid WIN55,212-2 [271].

**Caffeine.** Co-administering caffeine and cannabis has a long history. Bell [272] claimed that oral administration of hashish with coffee increased the effects of cannabis, and at the same time diminished its duration. He proposed a pharmacokinetic mechanism—coffee promoted more rapid absorption of hashish.

Caffeine and theophylline are antagonists of adenosine receptors. Adenosine receptors are tonically activated by adenosine, their endogenous ligand. Rodent studies indicate that \(\alpha_1\)-subtype adenosine receptors tonically inhibit CB₁ activity [273]. Thus the antagonism of \(\alpha_1\) receptors by caffeine and theophylline enhances eCB system function (e.g., activation of CB₁ by 2-AG). Caffeine potentiated CB₁-mediated activity stimulated by THC and WIN-55,212 in hippocampus slices [273]. Consistent with this, the simultaneous application of WIN-55,212 plus an \(\alpha_1\) agonist produced less than additive stimulation of \[^{35}S\]GTP\(_S\) binding in mouse cerebellar membranes [274].

In whole animals, however, caffeine’s effects are biphasic and vary by dosage and acute versus chronic administration. In humans, the acute administration of caffeine decreases headache pain, but exposure to chronic high doses, \(\geq 300\) mg/day, may exacerbate chronic pain [275]. In rabbits, an acute dose of caffeine antagonized THC-induced changes in cortico-hippocampal electroencephalogram recordings [276]. In mice, chronic caffeine at high doses potentiated CB₁-dependent stimulation by eCBs and HU210 at striatal GABAergic, but not glutamatergic, synapses [277]. A single dose or a subacute dose (one day of caffeine in water) rescued the sensitivity of GABAergic synapses to HU210 in mice exposed to chronic stress.

Chronic caffeine at moderate doses increased THC’s effects on short-term memory in mice [278]. Surprisingly, CB₁ density decreased in the caudate nucleus, measured by \[^{3}H\]SR141716A binding. Cortical and hippocampal tissues also showed a decrease in WIN55,212-stimulated \[^{35}S\]GTP\(_S\) binding, but this attenuation was not seen in THC-stimulated \[^{35}S\]GTP\(_S\) binding. This
highlighted the fact that caffeine-induced changes observed in vitro do not necessarily reflect the effects of caffeine upon integrated brain circuitry in vivo. Lastly, acute antagonism of A1 with DPCPX did not modulate the effects of THC on short-term memory [278], which further supports our hypothesis that chronic and acute blockade of A1 receptors have different functional consequences.

Cannabis. Cannabis and cannabis products are complex polypharmaceuticals, consisting of THC, cannabinol (CBD), dozens of minor cannabinoids, as well as terpenoids, flavonoids, and other compounds. Fundamentally, THC mimics AEA and 2-AG by acting as an agonist at CB1 and CB2 [279]. But rather than simply substituting for AEA and 2-AG, McPartland and Guy [280] proposed that Cannabis and its many constituents work, in part, by “kick-starting” the eCB system. The acute administration of THC increased CB1 density in rodent brains [281,282]. Acute upregulation of CB1 mRNA continued for up to 14 days in some rat brain regions [283]. Acute THC also increased the sensitivity of CB1 to cannabinoids, measured by WIN-55,212-2-stimulated [35S]GTP[S] binding in rat brains [284]. Lastly, acute THC stimulated AEA biosynthesis [285].

Chronic, high dosing of THC causes a predictable desensitization and downregulation of CB1 and CB2, accompanied by drug tolerance. Chronic THC decreased CB1 density in rodent brains, and dampened cannabinoid-stimulated [35S]GTP[S] [282,284,286,287] in different regions of the brain; downregulate and desensitize at unequal rates and magnitudes, with greatest decreases in the hippocampus and little or no change in the nucleus accumbens and basolateral amygdala. Chronic THC elicited few changes in AEA or 2-AG levels in rat brains, except for a significant augmentation of AEA levels in the limbic forebrain [288].

Similar results have been reported in two human studies. Villares [289] collected postmortem brain tissues from known cannabis smokers; [3H]SR141716A binding and CB1 mRNA was downregulated in several brain regions, compared to non-smoking control autopsies. Hirvonen et al. [290] employed PET scan imaging in living subjects. The degree of CB1 downregulation correlated with years of chronic cannabis smoking. CB1 densities returned to normal after four weeks of abstinence. Variable downregulation in different brain regions may explain why frequent users of cannabis develop tolerance to some effects of THC, such as anxiogenesis and cognitive impairment, but not to frequent users of cannabis develop tolerance to some effects of THC. Further, THC was more potent at producing antinociception than rats injected with vehicle. In other words, inhibiting AEA degradation led to an increase in the potency of THC. Further, THC was more potent at producing antinociception, decreasing spontaneous activity, and increasing ring immobility when co-administered with PMSF as compared to vehicle.

Chronic THC antagonized the effects of THC upon the eCB system oscillate between potentiation and suppression, depending on acute versus chronic dosage. The dividing line between “acute” and “chronic” is a gray zone, and likely differs amongst individuals. Suplita et al. [316] summarized the situation: they studied “stress antinociception,” where rodents become less responsive to painful stimuli following exposure to an environmental stressor. Stress antinociception is mediated, in part, by the coordinated release of 2-AG and AEA. Acute administration of THC potentiated eCB-mediated stress antinociception. The converse was also true: animals exposed acutely to foot shock, which elicits eCB-mediated stress antinociception, became sensitized to the effects of THC. Chronic administration of THC predictably dampened stress antinociception. The converse was not true: chronic exposure to foot shock (3 min/day for 15 days) failed to dampen antinociception induced by either WIN-55,212-2 or by further footshocks. The potential synergy between THC and the eCB system is analogous to the potential synergy between AEA and 2-AG: Rodent studies that combined FAAH and MAGL inhibitors indicated that AEA and 2-AG may activate CB1 receptors in different parts of the central nervous system. Each causes unique
behavioral effects, and when both are enhanced, new effects emerge. Long and colleagues [317] showed that AEA and 2-AG independently dampen pain sensation, but together their effects are dramatically enhanced.

Cannabis is more than THC [318,319]. Adding CBD to THC in mice enhanced CB1 expression in hippocampus and hypothalamus [320]. CBD increased hippocampal cell survival and neurogenesis, whereas THC had the opposite effect; the CBD response was absent in CB1−/− knockout mice [321]. CBD inhibited the cellular uptake of AEA and its breakdown by FAAH [322,323]. A separate systematic review regarding the effects of CBD on THC is currently underway (McPartland, unpublished).

Several other non-THC cannabinoids interact with enzymes of the eCB system. For example, cannabidivarin and cannabidiolic acid are moderately potent inhibitors of DAGLα and 2-AG may be counterproductive. Faced with constant expression, these drugs are generally administered in a chronic fashion, and patients (exercise, maintenance of ideal body weight) and CAM interventions (e.g., dietary supplements, stress modification, acupuncture, massage and manipulation). In our opinion these are sensible methods of enhancing the eCB system.

Preclinical studies identified useful prescription drugs, such as SSRIs, anxiolytics, antipsychotics, and anticonvulsants. However, these drugs are generally administered in a chronic fashion, and this comes with a caveat: generating chronic elevations in AEA and 2-AG may be counterproductive. Faced with constant activation by agonists, CB1 and CB2 desensitize and downregulate. A desensitized receptor drives less receptor-mediated signal transduction, and develops cross-tolerance to all agonists—eCBs and phytocannabinoids alike. A downregulated receptor is not functional—either it does not bind ligand or has internalized away from the cell membrane.

The difference between acute and chronic augmentation has been demonstrated in rodent studies: acute blockade of MAGL with JZL184 elevated 2-AG levels and provided analgesia [324]. In the face of chronic blockade with JZL184 this analgesia was lost, because sustained elevation of 2-AG caused CB1 desensitization. This led to a loss in eCB-dependent synaptic plasticity, cross-tolerance to other cannabinoids, and physical dependence.

Other drugs identified in preclinical studies have side effect profiles too severe to warrant their use for upregulating the eCB system (e.g., corticosteroids, opioids, nicotine). Preclinical studies suggest a number of over-the-counter medications, such as analgesics, seem to be acting through eCB-mediated mechanisms. Clinical trials are warranted, although over-the-counter medications lack patent protection, so expensive clinical trials seem unlikely.

Conclusions

Many randomized controlled trials identified in this systematic review have been conducted on lifestyle modifications (e.g., diet, exercise, maintenance of ideal body weight) and CAM interventions (e.g., dietary supplements, stress modification, acupuncture, massage and manipulation). In our opinion these are sensible methods of enhancing the eCB system.

Preclinical studies identified useful prescription drugs, such as SSRIs, anxiolytics, antipsychotics, and anticonvulsants. However, these drugs are generally administered in a chronic fashion, and this comes with a caveat: generating chronic elevations in AEA and 2-AG may be counterproductive. Faced with constant activation by agonists, CB1 and CB2 desensitize and downregulate. A desensitized receptor drives less receptor-mediated signal transduction, and develops cross-tolerance to all agonists—eCBs and phytocannabinoids alike. A downregulated receptor is not functional—either it does not bind ligand or has internalized away from the cell membrane.

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Supporting Information

Checklist S1 Online supporting material. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist. (DOC)

Author Contributions

Conceived and designed the experiments; JM GWG VD. Performed the experiments: JM GWG VD. Analyzed the data: JM GWG VD. Contributed reagents/materials/analysis tools: JM GWG VD. Wrote the paper: JM GWG VD.

References

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