Original article

The endocannabinoidome as a substrate for noneuphoric phytocannabinoid action and gut microbiome dysfunction in neuropsychiatric disorders

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The endocannabinoid (eCB) system encompasses the eCBs anandamide and 2-arachidonoylglycerol, their anabolic/catabolic enzymes, and the cannabinoid CB_1 and CB_2 receptors. Its expansion to include several eCB-like lipid mediators, their metabolic enzymes, and their molecular targets, forms the endocannabinoidome (eCBome). This complex signaling system is deeply involved in the onset, progress, and symptoms of major neuropsychiatric disorders and provides a substrate for future therapeutic drugs against these diseases. Such drugs may include not only THC, the major psychotropic component of cannabis, but also other, noneuphoric plant cannabinoids. These compounds, unlike THC, possess a wide therapeutic window, possibly due to their capability of hitting several eCBome and non-eCBome receptors. This is particularly true for cannabidiol, which is one of the most studied cannabinoids and shows promise for the treatment of a wide range of mental and mood disorders. The eCBome plays a role also in the microbiota-gut-brain axis, which is emerging as an important actor in the control of affective and cognitive functions and in their pathological alterations.

Introduction: from THC to the endocannabinoid system and the endocannabinoidome

For several decades, and starting some 25 years from its discovery, the only plant cannabinoid (phytocannabinoid) with an established mechanism for its pharmacological actions has been Δ^9-tetrahydrocannabinol (THC). To THC are ascribed the most important euphoric and psychotropic effects of recreational preparations (eg, marijuana, hashish) obtained from those varieties of *Cannabis sativa* that are rich in this compound.[1] These effects on the central nervous system are now known to be due to THC capability of activating endogenous G-protein-coupled receptors (GPCRs) that are among the most abundant such proteins in the mammalian brain: the type 1 cannabinoid (CB_1) receptors. THC also activates another GPCR, the type-2 cannabinoid (CB_2) receptor, through which it produces instead anti-inflammatory and immune-modulatory actions.[2] The discovery of CB_1 and CB_2 receptors led to the finding of their endogenous agonists, later named endocannabi-

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noids (eCBs): *N*-arachidonoyl-ethanolamine (anandamide [AEA]) and 2-arachidonoylglycerol (2-AG). The chemical signaling system composed of CB_1 and CB_2 receptors, the two eCBs, and at least five anabolic and catabolic enzymes regulating eCB concentrations in tissues became known as "the endocannabinoid system" eCBS, *Figure 1*.[3] This system has been shown, both in preclinical/animal and human studies, to be altered in several neuropsychiatric conditions, including the following: anxiety,[4] defective extinction of aversive memories (and ensuing posttraumatic stress disorder [PTSD] in man),[5] depression,[6] eating disorders,[7] psychosis and schizophrenia,[8] autism spectrum disorders (ASD),[9] and attention-deficit/hyperactivity disorder (ADHD).[10] The likely function of such alterations is linked to the well-recognized general prohomeostatic role of the eCBS.[11] Accordingly, inhibitors of eCB inactivation by enzymes such as fatty acid amide hydrolase (FAAH, for anandamide) and monoacylglycerol lipase (MAGL, for 2-AG, *Figure 1*) often ameliorate some symptoms of these disorders, in a way usually not accompanied by the typical and unwanted side effects of THC and other direct CB_1 agonists.[4-11]

Recently, the potential therapeutic importance of phytocannabinoids other than THC, which are mostly devoid of euphoric activity and addictive potential, was also recognized. Among these compounds, cannabidiol (CBD), its propyl homolog, cannabidivarin (CBDV), and THC propyl homologue Δ^9-tetrahydrocannabivarin (THCV) (*Figure 2*), are the ones that have been most tested in preclinical and clinical studies. Indeed, CBD is now a major component of two approved therapeutic drugs.[12,13] These phytocannabinoids, unlike THC, seem to act via several molecular targets and there is both preclinical and clinical evidence for their possible use to treat several neuropsychiatric disorders (see below).

Meanwhile, an *expanded* eCBS has been discovered that encompasses several non-eCB long-chain fatty acid amides and esters, which include: (i) the congeners of anandamide (the *N*-acylethanolamines, NAEs) and 2-AG (the 2-acylglycerols, 2-AcGs); (ii) the *N*-acyl-aminoacids; (iii) acylated neurotransmitters such as the *N*-acyl-dopamines and *N*-acyl-serotonins; and (iv) the primary fatty acid amides. These lipid mediators often share with anandamide and 2-AG biosynthetic (only in the case of the congeners) or inactivating enzymes, but not necessarily their receptors,

which include orphan GPCRs, ligand-activated ion channels, and peroxisome proliferator-activated nuclear receptors (PPARs). These small molecules, therefore, should not be considered eCBs *sensu stricto*, but instead *eCB-like mediators*. This expanded eCBS, including more than 100 lipid mediators, 20 enzymes, and 20 receptors, is known as the *endocannabinoidome* (eCBome, *Figure 1*).[14]

It is now emerging that noneuphoric phytocannabinoids owe their pharmacological activities to their capability of interacting with the eCBome, rather than simply the eCBS, as well as with other signaling molecules. In this chapter, I will discuss to what extent the understanding of the mechanism of action of these peculiar natural plant products, and particularly CBD, CBDV, and THCV, relies on the eCBome. Additionally, I will review data indicating how their proposed therapeutic effects in animal models of neuropsychiatric conditions are due to the interaction with this complex signaling system. Finally, I will describe the potential role of the emerging eCBome-gut microbiome cross-talk in neuropsychiatric disorders.[15,16]

The eCBome explains part of the pharmacology of phytocannabinoids

The most studied non-THC cannabinoid from the pharmacological point of view is CBD. A recent review of the molecular mode of action of this compound[17] pointed out that at least 11 receptors, over 20 different families of enzymes/protein complexes, at least 8 different ion channels, and 10 membrane transporters have been suggested to be modulated by CBD in vitro. Of these proteins, several belong to the eCBome (in as much as they interact also with eCBs and eCB-like mediators), including: CB_1 receptors (negative allosteric modulation); orphan GPCRs such as GPR55 (antagonism); transient receptor potential (TRP) of vanilloid type-1 (TRPV1) channels (activation/desensitization), as well as TRP channels of vanilloid type-2 (TRPV2; activation/desensitization), ankyrin type-1 (TRPA1; activation/desensitization), and melastatin type-8 (TRPM8; inhibition); T-type voltage-activated Ca^{2+} channels ($Ca_{v3.2}$, inhibition); PPAR-γ (activation); and FAAH (inhibition).[17] CBDV, which differs from CBD for only two methylene groups, shares some of these targets, ie, TRPV1, TRPA1, and TRPM8,[18] although studies on most of the other proteins have not yet been carried out. THCV, apart from being a neutral antagonist at CB_1 and

CB$_2$ receptors and, at higher concentrations, a partial CB$_2$ agonist,[19] is also a TRPV1 agonist.[18] Importantly, none of the above interactions, with the only important exception of PPAR-γ activation, are observed with the acid homologs of CBD, CBDV, and THCV (*Figure 1*).[18] These homo- logs are the natural components of the cannabis plant and converted to the corresponding neutral cannabinoids only after desiccation and/or heating of the plant flowers. They often also activate another eCBome target, ie, PPAR-α, as in the case of CBD-acid, and cannabigerolic acid.[20]
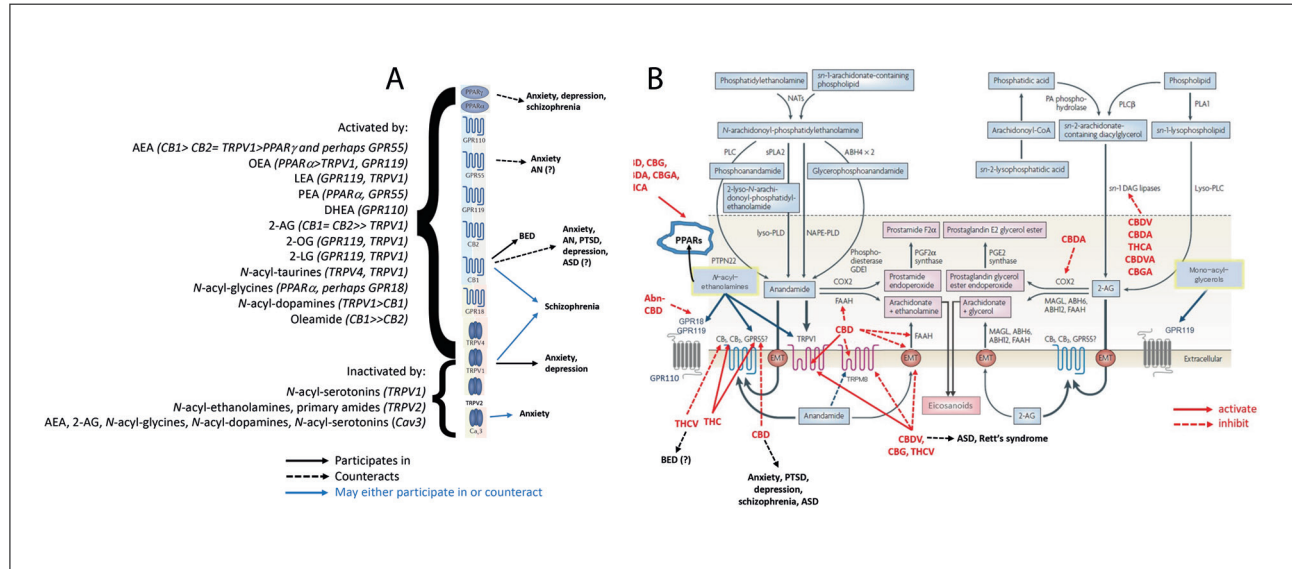
Figure 1. The endocannabinoidome and its interactions with plant cannabinoids. (A) Endocannabinoidome mediators and receptors and their suggested involvement in neuropsychiatric disorders. The endocannabinoids anandamide (AEA) and 2-ar- achidonoylglycerol (2-AG) are often accompanied by their congeners, the *N*-acylethanolamines (NAEs), such as *N*-palmitoyl-, *N*-oleoyl-, *N*-linoleoyl- and *N*-docosahexaenoyl-ethanolamine (PEA, OEA, LEA, and DHEA) and the 2-AcGs, such as 2-oleoyl- and 2-linoleoyl-glycerol (2-OG, 2-LG). These congeners modulate targets other than cannabinoid receptor type 1 (CB$_1$) and cannabinoid receptor type 2 (CB$_2$), such as transient receptor channel type 1, type 2 and type 4 (TRPV1, TRPV2, TRPV4), peroxi- some proliferator-activated nuclear receptor (PPAR)α, and γ, T-type Ca^{2+} (Ca$_{v3}$) channels, and orphan G-protein coupled recep- tors (GPR), such as GPR18, GPR55, GPR110, and GPR119. Other long-chain fatty acid amides, such as primary amides, *N*-acylated amino acids (lipoamino acids), and some *N*-acyl-neurotransmitters (*N*-acyl-dopamines and *N*-acyl-serotonins) have also been identified as elements of the expanded endocannabinoid system with promiscuous targets. Endocannabinoidome targets have been implicated in the etiology of neuropsychiatric disorders, as indicated. (B) The endocannabinoids anandamide and 2-AG, their congeners, and the various long-chain fatty acid amides often share receptors and anabolic/catabolic enzymes, although these may have different substrate selectivity. Fatty acid amide hydrolase (FAAH) breaks down all long-chain *N*-acyl- ethanolamines, *N*-acyl-taurines, and *N*-acyl-glycines; FAAH-2 (so far found only in human tissues) has a preference for OEA and LEA; *N*-acylethanolamine acid amidohydrolase (NAAA) recognizes saturated N-acylethanolamines such as PEA; monoa- cylglycerol lipase (MAGL) is specific for long-chain 2-AGs, especially those that are unsaturated, and so do α, β-hydrolases 6 and 12 (ABHD6, ABHD12), which also have non-endocannabinoidome ester substrates. In addition, some oxidizing enzymes of the arachidonate cascade, such as cyclooxygenase-2 (COX2), recognize the polyunsaturated fatty acid-containing endocan- nabinoid congeners. Several metabolic products of these congeners have their own receptors. Plant cannabinoids modulate receptors and enzymes of the endocannabinoidome and have been proposed as treatments for neuropsychiatric disorders, as shown. ABH4, α, β-hydrolase-4; Abn-CBD, abnormal cannabidiol; ADHD, attention-deficit/hyperactivity disorder; AN, anorexia nervosa; ASD, autism spectrum disorder; BED, binge eating disorder; CBD, cannabidiol; CBDA, cannabidiolic acid; CBDV, can- nabidivarin; CBDVA, cannabidivarinic acid; CBG, cannabigerol; CBGA, cannabigerolic acid; CoA, coenzyme A; EMT, endocanna- binoid membrane transporter; GDE1, glycerophosphodiester phosphodiesterase 1; lyso-PLC, lyso phospholipase C; NAPE-PLD, N-acyl-phosphatidylethanolamine-specific phospholipase D; NATs, *N*-acyl-transferases; OA, oleamide; PA, phosphatidic acid; PG, prostaglandin; PLA, phospholipase A; PLC, phospholipase C; PLD, phospholipase D; PTPN22, tyrosine-protein phosphatase nonreceptor type 22; PTSD, posttraumatic stress disorder; *sn*-1-DAG lipase, *sn*-1-specific diacylglycerol lipase; sPLA2, secretory phospholipase A2; THC, Δ^9-tetrahydrocannabinol; THCA, Δ^9-tetrahydrocannabivarinic acid; THCV, Δ^9-tetrahydrocannabivarin; TRPM8, transient receptor potential melastatin type-8
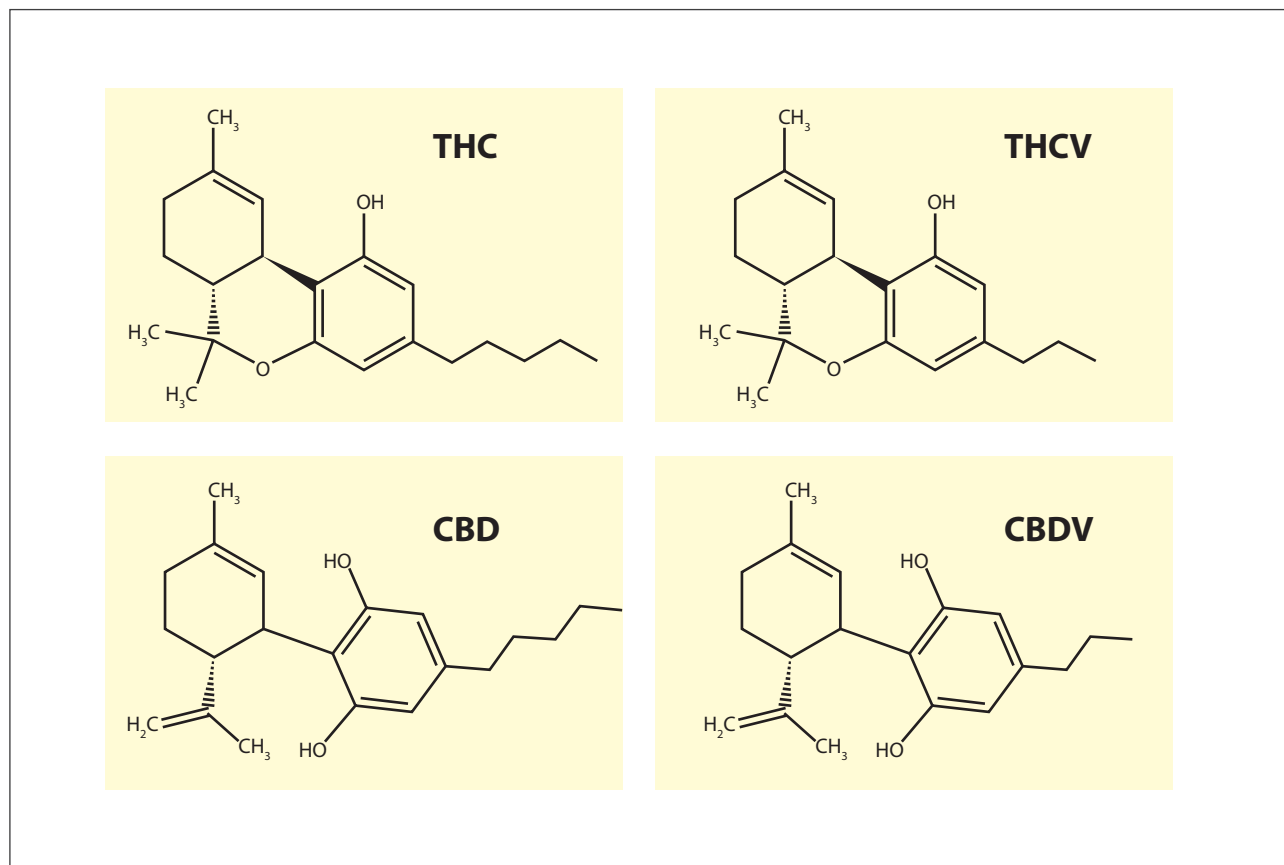
Figure 2. Chemical structures of Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabivarin (THCV), cannabidiol (CBD), and cannabidivarin, the four most clinically studied plant cannabinoids in the context of neuropsychiatric disorders.

CBDV-acid and THCV-acid were also shown to weakly inhibit 2-AG biosynthesis by diacylglycerol lipase-α in vitro, an effect also exerted by CBDV,[18] thus potentially behaving as indirect antagonists of cannabinoid receptors. Conversely, most neutral cannabinoids, such as CBD, weakly inhibit the putative eCB membrane transporter (EMT), which was suggested to mediate eCB cellular reuptake or transport through the plasma membrane but is yet to be molecularly characterized.[18] CBD-acid weakly inhibits cyclooxygenase-2 (COX-2), the first, rate-limiting-step enzyme in the oxidation of both anandamide and 2-AG to the corresponding prostaglandin-like metabolites and eCBome mediators, known as prostamides and prostaglandin-glycerol esters, respectively. These latter compounds are inactive at cannabinoid and prostanoid receptors and activate instead other, as yet not fully iden-

tified, molecular targets. By inhibiting eCB inactivation through either canonical (eg, EMT- and FAAH-mediated) pathways or COX-2, phytocannabinoids may indirectly: i) activate receptors that are stimulated by eCBs, ie, CB_1 and CB_2 receptors, and also TRPV1 channels and possibly GPR55; or ii) inhibit receptors that are instead inactivated by eCBs, such as TRPM8 and $Ca_{v3.2}$ channels,[20,21] or activated by prostamides and prostaglandin-glycerol esters.

Role of eCBome mediators and their molecular targets in neuropsychiatric disorders

The ever-accumulating evidence for the involvement of eCBome receptors and mediators in the etiology of the most frequent neuropsychiatric disorders (*Figure 1a*), will be discussed in this section.

Anxiety and fear conditioning

Stemming from the anecdotal use of marijuana and other THC-rich preparations to reduce anxiety, several studies have shown how CB$_1$ receptor activation, if exerted in a site- and time-dependent manner, can produce anxiolytic effects in animal models.[5,22] In fact, CB$_1$ receptors are mostly expressed on presynaptic terminals of both GABAergic and glutamatergic neurons and, once activated by retro-actively acting eCBs released from postsynaptic neurons, inhibit either GABA or glutamate release. Therefore, only the population of receptors expressed on glutamatergic neurons needs to be activated in order to reduce the release of "anxiogenic" glutamate, but not of "anxiolytic" GABA. Although relatively simplistic, since complex circuitries (particularly in the amygdala) also involving glutamatergic signaling take part in the control of anxiety and fear conditioning, this concept explains why: i) glutamatergic CB$_1$ receptors are necessary for appropriate fear extinction, whereas rescue of CB$_1$ receptors on forebrain GABAergic neurons of CB$_1$ receptor–knockout mice is sufficient to restore an anxiety-like behaviour[22,23]; and ii) systemically administered CB$_1$ receptor agonists, such as after marijuana smoking, can be either anxiogenic or anxiolytic, depending on context.[22] Conversely, inhibition of eCB inactivation by FAAH or MAGL, by preserving the site- and time-specific prohomeostatic action of eCBs, is being proposed as a safer means to reduce anxiety. Indeed, several animal and human studies suggest that this condition is often accompanied by defective eCB signaling, due, for example, to excessive expression of FAAH,[24] whereas resilience to anxiety is found in individuals carrying a FAAH polymorphism that makes the enzyme less stable.[25] Inhibitors of FAAH and MAGL (as well as the putative EMT) produce anxiolytic actions mostly due to CB$_1$ receptor activation, although evidence exists for GPR55 also negatively controlling this condition.[26] Conversely, TRPV1 activation by anandamide, or other NAE substrates of FAAH (*Figure 1*), can exacerbate anxiety, to the point that dual FAAH/TRPV1 blockers produce more efficacious anxiolytic actions than selective inhibitors of these two proteins.[27] Ca$_{v3.2}$ channels, which are inhibited by eCBs and several eCB-like mediators, instead can play both anxiogenic and anxiolytic roles depending on context.[28] Finally, amygdalar PPAR-γ and PPAR-α were suggested to counteract emotional stress and anxiety[29] and reduce fear learning.[30] However, no role has been identified yet for GPR55, Ca$_{v3.2}$ channels and PPARs in eCB and eCB-like mediator actions on anxiety and fear.

Depression

Also in the case of major depressive disorder there is evidence supporting a role for direct or indirect CB$_1$ receptor activation as a protective mechanism.[6] This role is probably played also in humans, given the well-established negative effects on mood and the induction of depression and suicidal ideation by CB$_1$ receptor antagonists in some obese patients,[31] and is again likely effected in a site-specific manner. In fact, knockdown of CB$_1$ receptors in afferents from basolateral amygdala cholecystokinin neurons to nucleus accumbens dopamine-D$_2$ receptor–expressing neurons elevated synaptic activity of the latter and promoted stress susceptibility.[32] Conversely, selective inhibition of this circuit or administration of synthetic cannabinoids in the nucleus accumbens was sufficient to produce antidepressant-like effects.[32] Another mechanism, unmasked by using a FAAH inhibitor, consists in the potentiation of serotonergic signaling in the raphe nucleus via CB$_1$ receptors in the ventromedial prefrontal cortex.[33] Indeed, both FAAH and MAGL inhibitors reduce depression-like signs in animal models via indirect activation of CB$_1$ receptors.[34] By interfering with stress coping, TRPV1 activation instead may exacerbate these signs, and dual inhibitors of FAAH and this channel were shown to produce efficacious effects.[35,36] Finally, PPAR-γ agonists reduce depression-like signs in rodents,[37] probably due in part to antineuroinflammatory or neurogenesis-stimulatory actions that have been proposed to be important in the treatment of major depressive disorder.

Eating disorders

One of the best established roles of CB$_1$ receptors is to induce food intake after food deprivation.[38] This, and the widely described "munchies" effect of marijuana smoking, suggested that defective or aberrant eCB signaling at CB$_1$ receptors might underlie disorders such as anorexia nervosa (AN) or binge eating disorder (BED). Accordingly, strong evidence exists in both animal models and, particularly, human studies, for the occurrence of a dysregulation of central or peripheral eCB levels in these conditions.[39-42] For example, the changes in peripheral blood levels of eCBs, particularly 2-AG, and/or eCB-like mediators occurring in anticipation to, or immediately after, the consumption of palatable/favorite foods in healthy human volunteers are disrupted in individuals with AN or BED.[40-42] However, these findings, beyond the proposed use of CB$_1$ receptor antagonists in animal models of BED,[43] and the so-far unsatisfactory clinical results with THC in AN,[44] have not yet brought a breakthrough in the

pharmacological treatment of these disorders. Regarding other eCBome receptors, a malfunctioning polymorphism of GPR55 has been associated with AN,[45] whereas no evidence exists for other eCBome targets being associated with the control of symptoms of eating disorders.

Psychosis and schizophrenia

A plethora of studies have addressed the question of whether the alterations in eCB, namely anandamide, and NAE levels observed in the blood of schizophrenic studies and in the brain of animal models of psychotic behaviors, play a protective or maladaptive role in the etiology of schizophrenia and other psychosis.[46-49] These alterations are, in fact, often corrected by treatment with antipsychotic drugs.[46] Marijuana abuse is associated with earlier onset of psychoses in genetically predisposed individuals, but the role of CB_1 receptor in these conditions is controversial. Some preclinical studies in chemical and developmental animal models of schizophrenia have proposed that CB_1 receptor activation might be deleterious, and CB_1 receptor antagonism beneficial, to most negative, positive, and cognitive behavioral signs of this condition.[49] However, in agreement with the hypothesis arising from human studies, in these models, anandamide might have a beneficial effect,[46] possibly also via non-CB_1 receptors.[49] Indeed, TRPV1 activation/desensitization has proven beneficial in some models,[50] whereas PPAR-γ agonists (such as anandamide at low-medium micromolar concentrations) might be beneficial,[51] also in view of the possible contribution of neuroinflammation to schizophrenia.

ASD and ADHD

Recent data have revealed the potential role of the eCBome in neurodevelopmental pediatric disorders such as ASD, in agreement with the increasingly recognized role of eCBome receptors in social behavior.[9,10] Significantly lower anandamide and NAE, but not 2-AG, levels were found in the serum of children with ASD.[52,53] Although in this study it was not possible to assess the pathophysiological meaning of these alterations, experiments in the valproate-induced model of autism, where brain FAAH levels are increased[54,55]

> One way through which perturbation of the gut microbiota composition can produce effects on mental health is via the release of molecules that influence cognitive or social behaviors

and inhibition of the enzyme attenuates both cognitive and synaptic dysfunction,[56,57] suggest that reduced anandamide and NAE signaling might underlie some of the symptoms of ASD. Accordingly, CB_1 receptor activation is also beneficial in the neurolignin-3^{R451C} mouse model of ASD,[58] which exhibits impaired tonic eCB signaling.[59] FAAH inhibition produces beneficial effects also in models of fragile-X syndrome, a rare pediatric disorder with ASD features.[60] However, brain levels of anandamide and NAE were found to be higher in the hippocampus of valproate-exposed rats immediately after social exposure,[55] suggesting that signaling through these mediators might represent an initial adaptive response in ASD, to be effected possibly via the observed enhanced expression of CB_1 receptor, but not PPAR or GPR55.[54,55]

Alterations in eCB levels in children with ADHD and loss of striatal CB_1 receptors in mice with point mutation in the dopamine transporter, an animal model of this disorder, have been reported.[61,62] However, whether CB_1 receptors, or other eCBome targets, play a protective or maladaptive role in this disorder is not yet clear. Indeed, a dysfunctional polymorphism of FAAH leading to elevated NAE levels was recently found to be associated with ADHD,[63] whereas organophosphate-induced inhibition of MAGL and FAAH caused ADHD-like signs in rats in a manner antagonized by a CB_1 receptor inverse agonist.[64]

The eCBome mediates in part the therapeutic effects of phytocannabinoids in neuropsychiatric disorders

Because of the generally prohomeostatic role of CB_1 and CB_2 receptors in brain function, the possibility of the use of THC, or its synthetic analog nabilone, for the treatment of neuropsychiatric disorders has been discussed for decades. Such use, however, still appears to be limited by the unwanted central side effects and the addictive/tolerance potential of CB_1 receptor agonists and their narrow therapeutic window. To date, non-THC phytocannabinoids that have proven to be efficacious in animal models (and, in some cases, also clinical trials) of disorders such as anxiety,

PTSD, depression, schizophrenia, and ASD have been suggested to do so through several targets, such as indirect activation (as inhibitors of eCB inactivation) of CB_1/CB_2 receptors, antagonism of GPR55, or direct activation/desensitization of TRPV1 channels (*Figure 1b*). Furthermore, non-eCBome targets, such as serotonin 1A ($5-HT_{1a}$) receptors, have been suggested to underlie some actions of CBD.[65]

CBD

CBD, recently approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of rare and otherwise untreatable pediatric epilepsies,[13] has been described to produce beneficial actions in:

i) anxiety, PTSD, and depression in: a) animal models, in a manner attenuated by either $5-HT_{1a}$ or CB_1/CB_2 receptor antagonists depending on the model and the potential role therein of chronic pain and stress, substance abuse, defective neurogenesis, and neuroinflammation[65-67]; and b) humans, particularly in patients with neurological or other neuropsychiatric conditions that are accompanied by anxiety.[68,69] The capability of CBD to activate TRPV1 might somehow reduce its efficacy against anxiety and possibly explain why bell-shaped dose-response curves are often observed in preclinical studies[70];

ii) schizophrenia, where the effects in animal models have been suggested to be due to reversal of overexpression of CB_1 receptors,[71] activation of $5-HT_{1A}$ receptors,[65] or activation/desensitization of TRPV1 channels[72]; whereas the beneficial effect of CBD per se in a clinical trial carried out with a 800 mg/day dose of the compound was found to be accompanied by elevation of anandamide (and NAE) levels (in a manner that, for anandamide, correlated with positive and negative symptom amelioration),[73] in agreement with CBD weak inhibitory action on FAAH and the putative EMT. A subsequent clinical study, carried out with CBD (1 g/day) as an adjunct therapy, also showed beneficial effects on positive and cognitive symptoms.[74] The dose of CBD used in these trials might be important, given the fact that a recent study with 600 mg/day oral CBD reported no significant therapeutic effects[75];

iii) ASD, for which beneficial effects in children with this condition have been reported in three observational studies,

of which, however, only one was performed with purified botanical CBD.[76] Two brain imaging studies, one with functional magnetic resonance imaging (fMRI) and the other with magnetic resonance spectroscopy (MRS), have shown that CBD (600 mg, single oral administration) modulates the glutamate-GABA systems and the low-frequency activity and functional connectivity in the brains of adults with ASD in a manner different from their controls.[77,78] Since studies with CBD in animal models of ASD are still lacking, it is difficult to assess through what mechanism of action this phytocannabinoid exerts these effects, as well as to what extent they are related to clinical efficacy, which, however, still needs to be confirmed in double-blind placebo-controlled trials (see Poleg et al[79] for a recent review).

CBDV

Somehow considered the neglected cousin of CBD, from which it differs by the lack of only two methylene groups in the alkyl chain, CBDV has been tested so far in the valproate model of ASD and in the *Mecp2* mutant mouse model of Rett's syndrome, an ASD-related rare pediatric disorder. Beneficial effects and/or increased survival were observed also at relatively low (eg, 2 mg/kg, intraperitoneal) doses.[80-82] Interestingly, in both the valproate model and *Mecp2* mutant mice, CBDV reversed the alterations of eCBS proteins observed in the brain (such as the increased levels of CB_1 receptors).[80,82] In adults with ASD submitted to an MRS study,[83] CBDV (600 mg, single oral dose) produced, in the left basal ganglia, effects on glutamate-GABA signaling similar to those exerted by the same dose of CBD in the prefrontal cortex.[78]

THCV

The object of a clinical study in overweight/obese patients with dyslipidemia, with mixed results,[84] THCV, which differs from THC by the lack of only two methylene groups in the alkyl chain, has been tested in the phenylcyclidine-treated rat model of schizophrenia. The compound was found to produce antipsychotic effects in a manner partly antagonized by a $5-HT_{1a}$ antagonist and mimicked by a CB_1 antagonist.[85] THCV, at the single oral dose of 10 mg, has also been shown to antagonize the neural effects of both palatable food reward and aversion in human volunteers, suggesting a possible use against BED.[86] Interestingly, 5-day dosing of human volunteers with THCV was found to counteract some of the pharmacological effects of THC in a placebo-controlled, double-blind, crossover pilot trial,

but to potentiate others, thus raising some doubts that it may be used to treat the psychotic effects of marijuana smoking in predisposed subjects.[87]

The eCBome-gut microbiome interaction and how it underlies neuropsychiatric conditions

The gastrointestinal system is very closely connected to the brain. The eCBome, mostly through CB_1 receptors and TRPV1 channels in myenteric and vagal fibers, and PPAR-α and GPR119 receptors in enteroendocrine epithelial cells of the small intestine, plays a major role in this context. These receptors affect myenteric neuron activity, vagal and sympathetic nerve function, and the release of gastrointestinal neuropeptides, which in turn may modulate eCB levels.[88] In the context of the gut-brain axis and its implication in neuropsychiatric disorders, the role of the gut microbiota is also starting to be appreciated. Perturbations, generally described under the definition of "gut dysbiosis," of this "symbiotic additional organ" occur during disorders as different as major depressive disorder, schizophrenia, and ASD, and seem to contribute to exacerbating their symptoms, as shown, for example, through the use of fecal microbiome transplant from patients with these disorders to germ-free mice.[89] One way through which perturbation of the gut microbiota composition can produce effects on mental health is via the release of molecules that influence cognitive or social behaviors. Such molecules may either directly affect myenteric or vagal nerve activity or be released into the blood stream—also thanks to the increased intestinal permeability that is a feature of proinflammatory gut dysbiosis— to act on the brain. Interestingly, commensal microorganism-derived molecules include not only neurotransmitters such as serotonin or GABA, but also eCB-like mediators that are capable of modulating host eCBome receptors.[90] On the other hand, dramatic alterations in gut microbiota amount and/or composition, such as in antibiotic-treated or germ-free mice, was shown to affect, through as yet unidentified mechanisms, the messenger RNA (mRNA) expression of eCBome receptors and enzymes, and the concentrations of eCBome mediators, in the gut.[91] Also this interaction may influence the host gut-brain axis. For example, antibiotic-treated mice contain less *N*-oleoyl and *N*-arachidonoyl-serotonin in the small intestine.[92] Given the antidepressant-like activity of these molecules, exerted by inhibiting FAAH and/or antagonizing TRPV1,[35,36] it was proposed that such alteration was partly responsible for the depression-like signs of these animals.[92] Accordingly, treatment with probiotics reversed both such signs and the reduction in intestinal *N*-oleoyl and *N*-arachidonoyl-serotonin levels.[92]

On the other hand, CB_1 receptors were suggested to: i) mediate part of the enhanced intestinal permeability induced by gut dysbiosis (and hence its systemic proinflammatory effects) during obesity, a condition often accompanied by affective disorders; and ii) modulate the composition of commensal microorganisms toward species that favor dysmetabolism.[93] TRPV1 and PPAR-α instead inhibit intestinal permeability.[94] Alterations of the tissue levels of the eCBome ligands of these receptors may, therefore, mediate the negative or beneficial effects on the "leaky gut," respectively, of dysbiosis or "beneficial" commensal microorganisms, such as *Akkermansia muciniphila*.[93] Accordingly, a non CB_1 receptor–activating NAE, *N*-palmitoylethanolamine, which activates PPAR-α and, indirectly, TRPV1, was recently shown to produce therapeutic effects in models of ASD or vitamin D-deficiency-induced pain, and at the same time reduce systemic or central inflammation associated with disrupted gut microbiota composition.[95,96] Evidence also exists for beneficial gut microbiota modulation by another non-eCB NAE, *N*-oleoylethanolamine,[97,98] as well as by the TRPV1 agonist capsaicin.[99]

In summary, alterations not only in the eCBome but also in its emerging tight bidirectional relationships with the gut microbiome, are starting to be viewed as a potentially important mechanism in the etiology, progress, and symptoms of neuropsychiatric disorders.[15,16]

Conclusions

The expanded eCBS, or eCBome, encompasses hundreds of biomolecules: lipid mediators, their metabolic enzymes, and their molecular targets (*Figure 1*). These molecular entities are deeply involved in the onset, progress, and symptoms of major neuropsychiatric disorders, and, as such, they provide a substrate for future therapeutic drugs against these illnesses, including phytocannabinoids other than THC (*Figure 2*). These latter compounds, unlike the major psychotropic component of marijuana, seem to possess a wide therapeutic window, possibly due to their capability of hitting several eCBome and non-eCBome receptors. Among noneuphoric phytocannabinoids, CBD is certainly the most

studied and promising for the treatment of a wide range of mental and mood disorders, whereas CBDV and THCV have only now started to be investigated. Finally, studies on the eCBome in both animals and humans affected by neuropsychiatric disorders may also cast light on the increasingly recognized role of the gut-brain axis, and in particular of the gut microbiota, in the control of brain affective and cognitive function and dysfunction. ∎

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