SPECIAL ARTICLE

The endocannabinoid system in social anxiety disorder: from pathophysiology to novel therapeutics

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Social anxiety disorder (SAD) is a highly prevalent psychiatric disorder that presents with an early age of onset, chronic disease course, and increased risk of psychiatric comorbidity. Current treatment options for SAD are associated with low response rates, suboptimal efficacy, and possible risk of adverse effects. Investigation of new neurobiological mechanisms may aid in the identification of more specific therapeutic targets for the treatment of this disorder. Emerging evidence suggests that the endogenous cannabinoid system, also referred to as the endocannabinoid system (ECS), could play a potential role in the pathophysiology of SAD. This review discusses the known pathophysiological mechanisms of SAD, the potential role of the ECS in this disorder, current drugs targeting the ECS, and the potential of these novel compounds to enhance the therapeutic armamentarium for SAD. Further investigational efforts, specifically in human populations, are warranted to improve our knowledge of the ECS in SAD.

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Introduction

Social anxiety disorder (SAD) is characterized by an excessive fear or anxiety of social situations wherein affected individuals worry they may behave in a manner that could lead to embarrassment, humiliation, or rejection by others.[1] These fears may be restricted to performance-like situations, such as public speaking, but may also include more widespread social interactions, such as initiating a conversation or socializing at a gathering. As such, affected individuals typically avoid anxiety-provoking social stimuli.[1] This enduring anxiety and avoidance often results in clinically significant psychosocial impairment, which interferes with the person's daily routines, social engagements, relationships, occupation, and/or academic functioning.[2,3]

SAD is a highly prevalent, predominately youth-onset disorder which may affect 12.1% of individuals in the general population at least once in their lifetime.[3,4] This disorder has a higher prevalence among females, often follows a chronic course, and has increased comorbidity with other psychiatric disorders.[3] Common coexisting health conditions include major depressive disorder, generalized anxiety disorder, agoraphobia, substance use disorders, and increased rates of suicidal ideation, as well as physical health concerns such as cardiovascular disease.[3,5] Individuals with SAD report higher unemployment rates, lower income, and greater financial dependency compared to those without SAD.[3,6] Despite the significant socioeconomic costs and reduced quality of life associated with the disorder, only 35% of respondents with lifelong SAD reported seeking treatment specifically for social anxiety.[6]

In terms of treatment, cognitive behavioral therapy (CBT) serves as a first-line psychotherapy approach for anxiety disorders.[2] A 2018 meta-analysis reported a pre-post CBT effect size of d = 1.37, compared to effect sizes of d = 1.33 for other psychotherapies and d = 0.88 for psychological or pill placebo.[7] Pharmacological treatment with selective serotonin reuptake inhibitors (SSRIs) and serotonin-noradrenaline reuptake inhibitors (SNRIs) has also been demonstrated to yield clinical improvement in SAD patients.[8,9] A 2019 meta-analysis examining 23 randomized-controlled SAD studies reported a standardized mean difference of 0.66 and 0.67 with respect to the measured treatment benefit of SSRIs and SNRIs compared to placebo.[9] A moderate response has been described for treatment with benzodiazepines, although these drugs have been associated with multiple adverse effects, including physiological dependence, withdrawal

symptoms, and impaired cognition when used over longer periods of time.[8,10] Anticonvulsants (pregabalin and gabapentin), monoamine oxidase inhibitors (MAOIs), reversible inhibitors of monoamine oxidase A, and tricyclic antidepressants (TCAs) have also demonstrated efficacy in the treatment of SAD.[8] MAOIs and TCAs carry a higher risk of severe adverse effects and interactions (for MAOIs, e.g., hypertension, serotonin syndrome, potentially dangerous interactions with other antidepressants or food requiring specific dietary restrictions; for TCAs, e.g., anticholinergic effects, cardiac adverse effects) compared to newer antidepressants such as SSRIs and SNRIs.[8]

Overall, current therapeutic options demonstrate suboptimal response rates, limited efficacy, and include risk for potentially severe side effects with certain medications, such as benzodiazepines or MAOIs. The relative paucity of effective treatments for this disorder may be partly due to our limited understanding of the pathophysiology of SAD, despite research efforts implicating several biological systems. Investigation of new neurobiological mechanisms may facilitate the discovery of more specific therapeutic targets for SAD and could aid in the future identification of biomarkers. One notable target includes the endogenous cannabinoid system (ECS), which emerging evidence suggests may mediate aspects of anxiety and social behavior.[11,12]

This article aims to provide a narrative summary of the pathophysiological mechanisms involved in SAD, give an overview of the potential pathophysiological role of the ECS in SAD, and discuss future research directions and potential therapeutic agents for SAD targeting the ECS.

Known pathophysiological mechanisms of SAD

Behavioral inhibition and neurocircuitry in SAD

Behavioral inhibition (BI), described as a childhood tendency to withdraw from unfamiliar situations, people and environments,[13] has been associated with the development of SAD across several prospective studies.[14-17] A 2020 meta-analysis found that behaviorally inhibited children had significantly increased odds of developing SAD (odds ratio = 5.84), indicating that childhood BI may be a risk factor/predictor of the disorder.[18,19] One hypothesis suggests that BI is associated with either a lower threshold for or a stronger response to the detection of novel, salient, or threatening information, thereby implicating disruptions in certain neural circuitry.[20] This is supported by studies which found that individuals with a childhood history of BI exhibit a greater striatal response to reward[21-23] and punishment[24] cues, as well as heightened activity in regions including the prefrontal cortex (PFC)[24,25] and anterior cingulate cortex (ACC).[26] Greater amygdala reactivity to novel stimuli has also been reported in adolescents and adults who exhibited BI during childhood.[27-29]

These findings fall in line with those of functional imaging studies in persons with SAD. Results from such studies consistently demonstrate abnormal activity in regions including the amygdala, insula, PFC, and ACC, dubbed the "corticolimbic circuit."[30] Notably, heightened amygdalar and insular activities have been observed in response to performing stressful social tasks[31-34] or viewing negative facial expressions,[33-36] which in some studies has also correlated positively with the severity of SAD symptoms.[37-39] Although less consistent in direction, abnormal activity has also been reported in the PFC and ACC of subjects with SAD compared to controls.[32-34,40] Collectively, these findings suggest that the neurobiology of childhood BI may be associated with corticolimbic disruptions contributing to the development of SAD.

Neurotransmitter systems in SAD

The monoamine hypothesis and pharmacological approaches suggest that the neurobiologies of depression and anxiety share imbalances in the monoaminergic neurotransmission system.[41] In this regard, neuro-molecular positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies in SAD have largely focused on imaging serotonergic and dopaminergic neurotransmission, based on the reported efficacy of antidepressants.[41,42]

Two PET studies examining presynaptic serotoninergic activity found elevated rates of serotonin synthesis in the hippocampus, basal ganglia, amygdala, and ACC of individuals with SAD.[43,44] Additionally, three studies employing two different tracers ([C-11]DASB PET[44,45] and [I-123]-β-CIT SPECT[46]) reported higher serotonin receptor binding potential (a measure of receptor expression) within the raphe nuclei,[44] caudate nucleus,[44] insular cortex,[44] nucleus accumbens,[45] and thalamus.[44,46] Only one study investigated postsynaptic serotonergic function in SAD, in which PET imaging of [carbonyl-C-11]WAY-100635 demonstrated significantly reduced serotonin 1A receptor binding potential in the amygdala, insula, and ACC of patients with SAD.[47]

Imaging of dopaminergic systems has produced less consistent results. Three studies employing [I-123]-β-CIT SPECT reported either an increase,[46] decrease,[48] or no difference[49] in dopamine transporter binding in the striatum. As suggested by Schneier et al.,[49] these discrepancies could possibly be due to small sample sizes (n=12) or differences in SPECT assessment methods (receptor blocking compound, identification of volumes of interest or reference regions). Another SPECT study reported significantly reduced dopamine D2/D3 receptor binding potential in the striatum of patients with SAD and comorbid obsessive-compulsive disorder compared to healthy controls.[50] However, the same group found no difference in striatal dopamine D2/D3 receptor binding in a subsequent PET study, which employed a more reliable dopamine measurement technique and used a larger SAD sample size (n=17).[49] The latter study also reported a decrease in striatal dopamine D2/D3 receptor binding following an amphetamine challenge. Some of the observed differences may be due to variations in age range, symptom severity, treatments, and disease duration of the study cohorts, as well as varying strengths and limitations of the different imaging techniques and tracers employed in the aforementioned studies. While some of these findings suggest that serotonergic and dopaminergic

activity may play a role in SAD, the limited and inconsistent results warrant further investigation to better understand the role of these neurotransmitter systems in SAD pathophysiology.

The hypothalamic-pituitary-adrenal axis in SAD

It has been well established that the hypothalamic-pituitary-adrenal (HPA) axis plays a major role in stress regulation.[51,52] Activation of the HPA axis leads to increased corticotropin-releasing-hormone (CRH) signaling in the limbic forebrain, which, in turn, stimulates the downstream release of cortisol.[52] While acute stress leads to adaptive activation of the HPA axis with a transient increase in cortisol,[53] chronic stress can result in prolonged activation of the HPA axis via dysregulation of glucocorticoid-mediated feedback inhibition.[54] These disruptive processes may affect the coping mechanisms established by CRH systems, leading to chronic symptoms of fear and anxiety.[51,54]

Previous studies have demonstrated that individuals with SAD had significantly elevated social stressor-induced cortisol levels compared to controls.[55-57] However, these findings are not consistent: other studies employing similar stress paradigms found no significant difference in cortisol levels.[58-60] These discrepancies may be due to variable length of stressor exposure, perception of risk posed by the stressor, comorbidities, or age, which are known to influence cortisol response.[57,61] Interestingly, emerging evidence suggests that the ECS may contribute to the regulation of HPA axis activity.[52] As such, improved understanding of ECS processes may provide insight into the possible aberrations in the HPA axis stress response involved in the pathophysiology of SAD.

The endocannabinoid system

The ECS is a lipid-based signaling system of the central and peripheral nervous system (Figure 1).[62] The ECS is primarily composed of two G protein-coupled cannabinoid receptors, endogenous cannabinoid ligands, and enzymes responsible for ligand synthesis and degradation. Cannabinoid type 1 (CB1) receptors[63] are the predominant cannabinoid receptors in the central nervous system (CNS) and mainly located on terminals of central and peripheral neurons.[64,65] Activation of these receptors inhibits neurotransmitter release, primarily from GABAergic and glutamatergic neurons as well as certain monoaminergic sites.[62] In comparison, cannabinoid type 2 (CB2) receptors[66] are primarily distributed in peripheral tissues and immune system cells, where they modulate cell migration and cytokine release.[65]

The two main endocannabinoids, *N*-arachidonoylethanolamine (anandamide; AEA)[68] and 2-arachidonoylglycerol (2-AG),[69] are lipid ligands that are synthesized and released on demand from the post-synaptic cell, leading to a retrograde suppression of neurotransmitter release.[62,67] Other lipid ligands of cannabinoid receptors include 2-arachidonylglyceryl ether, *N*-arachidonoyl dopamine, *N*-oleoyl dopamine, *O*-arachidonoylethanolamine, and oleamide.[70] Signaling is terminated via metabolization of AEA and 2-AG through their catabolic enzymes, fatty acid amide hydrolase (FAAH)[71] and monoacylglycerol lipase (MAGL),[72] respectively.

These endocannabinoids, collectively with their receptors, biosynthetic proteins, and degradative enzymes, are referred to as the ECS (Figure 1).

Role of the endocannabinoid system in the regulation of stress and anxiety

Over the last two decades, the ECS has emerged and been recognized as a potential regulator of stress and anxiety. Cannabis consumption studies investigating the exogenous cannabinoid delta-9-tetrahydrocannabinol (THC) found that, in addition to its addictive potential,[73] THC consumption produces biphasic physiological effects depending on dose.[74-76] Notably, low-dose THC consumption has been associated with anxiolytic effects, whereas high-dose consumption has been linked to anxiogenic effects.[77,78] Given that the psychoactive effects of THC are mediated by CB1 receptor activation,[63,79] it is possible that ECS signaling may serve to buffer against anxiety and stress symptoms.

Animal studies have converged to show that increasing ECS activity via pharmacological stimulation of CB1 receptors (via CB1 agonism or via elevating AEA/2-AG through inhibition of their degradative enzymes) decreases behavioral measures of rodent anxiety.[80] Conversely, decreasing ECS activity via antagonism or deletion of the CB1 receptor gene elicits an anxiogenic response.[80] These anxiolytic effects seem to depend on CB1 activation in key structures implicated in the fear response, particularly those comprising the corticolimbic circuit.[81-85] In this regard, studies have shown that increased amygdala neuron excitability in rodents exposed to stress can be corrected by FAAH inhibition.[86,87] Moreover, rodent models of anxiety, modeled via exposure to stress paradigms, consistently demonstrate elevated FAAH activity and reduced AEA levels in limbic areas, further supporting the anxiolytic potential of the ECS via FAAH inhibition.[86,88-91] While the status of 2-AG and MAGL in anxiety is less studied, pharmacological and genetic investigations suggest that enhanced 2-AG signaling may play an important role in reducing anxiety-like behaviors and promoting adaptation under conditions of repeated stress exposure (reviewed by Bedse et al.[92]). Several studies have also shown that systemic MAGL inhibition reduces anxiety-like behaviors under basal and highly aversive conditions,[93-97] as well as acute and chronic stress-induced anxiety-like behaviors.[92,93,98,99]

In line with the preclinical literature, human research has shown that treatment with the CB1 inverse agonist/ receptor blocker rimonabant increases symptoms of anxiety and depression in some individuals.[100] Due to serious psychiatric adverse effects, including suicidal ideation, rimonabant was withdrawn worldwide, in an example of the potency and potential risks associated with manipulation of the ECS. Moreover, biochemical studies in humans have found that experimental exposure to the Trier Social Stress Test (TSST) increases serum
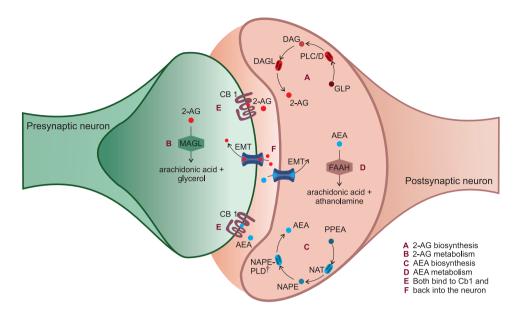
Figure 1 Illustration of endocannabinoid biosynthesis, binding, and metabolism. A) The biosynthesis of 2-AG is mediated by sequential hydrolysis, whereby a GPL is hydrolyzed by PLC or PLD to form DAG. DAG is then rapidly hydrolyzed by DAGL to form 2-AG. B) 2-AG is primarily broken down by MAGL to form arachidonic acid and glycerol. C) AEA is synthesized from phospholipid precursors PPEA which are initially converted to NAPE by NAT. NAPE is subsequently converted to AEA by NAPE-PLD activity. D) AEA is primarily metabolized by FAAH to form arachidonic acid and ethanolamine. E) Both 2-AG and AEA bind to CB1 on the presynaptic neuron. F) After receptor activation, AEA and 2-AG are transported back into the neuron by EMT. † NAPE may be converted to AEA through alternative pathways.[67] 2-AG = 2-arachidonoylglycerol; AEA = anandamide; CB1 = cannabinoid receptor 1; DAG = diacylglycerol; DAGL = DAG lipase; EMT = endocannabinoid membrane transporters; FAAH = fatty acid amide hydrolase; GPL = glycerophospholipid; MAGL = monoacylglycerol lipase; NAPE = N-arachidonoylphosphatidylethanolamine; NAPE-PLD = NAPE phospholipase D; NAT = N-acyltransferase; PLC = phospholipase C; PLD = phospholipase D; PPEA = phosphatidylethanolamine.

concentrations of AEA, 2-AG and the other N-acylethanolamines immediately after the stress period, both in healthy participants (compared to unstressed controls)[101] and in those diagnosed with major depression (compared to TSST-exposed controls).[102] In the former study, baseline anxiety ratings also correlated negatively with baseline AEA concentrations.[101] Similarly, among individuals with PTSD, those with lower peripheral AEA levels experienced more intrusive symptoms.[103] Clinical studies examining the FAAH C385A genetic polymorphism (rs324420) found that carriers of the A allele – which is associated with lower FAAH and higher AEA levels[104-106] – have a blunted amygdalar response to threat, greater ventral striatum response to reward, decreased correlation of amygdala reactivity and trait anxiety, enhanced fronto-amygdalar connectivity, and reduced stress reactivity.[107-109] Green et al.[110] failed to replicate the aforementioned relationship between the FAAH C385A polymorphism and amygdalar functional connectivity, possibly due to a small sample size. However, they did find that FAAH levels measured in vivo in the human brain are negatively correlated with fronto-amygdalar functional connectivity, suggesting that higher brain levels of AEA could increase coupling strength in fronto-amygdalar networks and affect stress response.[110] Another recent fMRI study in healthy males showed that neural activation of the anterior cingulate cortex and anterior insular cortex during extinction learning correlated positively with AEA baseline levels.[111] Moreover, task-related changes in AEA were observed during fear extinction, suggesting that AEA may play a putative role in fear extinction learning.[111] Finally, in the remarkable case report of a patient presenting with pain insensitivity and low fear and anxiety, the C385A polymorphism together with a microdeletion linked to decreased FAAH expression was detected as a possible causal factor. In addition, blood levels of AEA and other fatty-acid amides which are degraded by FAAH were unusually elevated in this individual.[112]

The role of the ECS specifically in social anxiety is supported by various preclinical findings which demonstrate effects of ECS modulation, via either CB1 receptor activation or FAAH inhibition, on social interaction and social anxiety.[113-118] In comparison, clinical studies investigating this system in SAD are considerably limited. A recent clinical trial investigating the therapeutic effects of a FAAH inhibitor in SAD was negative. However, the authors observed a small to modest anxiolytic effect in patients with severe SAD and suggested that, based on the correlation between low trough concentrations of the inhibitor (i.e., the lowest concentration of the drug in the bloodstream) and low plasma AEA, future trials with a higher dose of the inhibitor may be warranted.[119] In addition, a recently published double-blind, placebo-controlled experimental study in healthy adults found that administration of the FAAH inhibitor PF-04457845 produced a 10-fold increase in peripheral AEA levels and

decreased broad-spectrum fear-related phenotypes.[120] Furthermore, a 2021 double-blind, placebo-controlled clinical trial in healthy males employing the FAAH inhibitor JNJ-42165279 found that the drug attenuated activation in the amygdala, anterior cingulate, and bilateral insula during a face emotion processing task – effects which are consistent with those of previously observed anxiolytic agents.[121] Moreover, higher levels of plasma AEA were associated with greater attenuation in these brain regions.[121] While the latter two clinical studies were not conducted in a population with SAD, they suggest that FAAH inhibitors may have some potential in the treatment of fear-related disorders such as SAD.

Over the past two decades, cannabidiol (CBD), the primary non-psychotomimetic cannabinoid constituent of cannabis,[122] has emerged as a drug with multiple potential therapeutic benefits including neuroprotective, anti-inflammatory, antioxidant, antipsychotic, and antianxiety effects (reviewed by McPartland et al.[123]). Although evidence is emerging that the anxiolytic effects of CBD may be mediated through serotonergic 5HT1a receptors[124,125] and vanilloid receptor type 1 (TRPV1)[126] receptors, some of these effects may also be exerted through modulation of the ECS. Specifically, mild agonistic effects on CB1 receptors and antagonistic effects on exogenous CB1 receptor agonists (THC) have been described.[123,127,128] While the agonistic effects are still limited and controversial, emerging evidence suggests that CBD may act as a negative allosteric modulator of CB1 receptors, resulting in the aforementioned antagonistic effects on cannabinoid agonist activity.[129] Furthermore, there have been inconsistent findings on the effects of CBD on FAAH activity. Some animal studies found an inhibitory effect of CBD on FAAH activity in brain tissue of healthy mice,[129,130] rats,[131] and cell membranes from mouse neuroblastoma,[132] whereas in vivo treatment of mouse glioma tissue with CBD increased FAAH activity.[133] In addition, two studies reported a decrease in FAAH protein expression following CBD administration in chronically stressed mice[134] and LPS-treated mice.[135] Further investigation with more standardized methodologies may help elucidate the actions of CBD on FAAH.

Moreover, several preclinical studies[136-139] investigating effects of CBD in different animal models of anxiety have repeatedly demonstrated anxiolytic effects, as comprehensively reviewed by Blessing and colleagues.[128] Among these, some animal studies have found that CBD produces anxiolytic effects following an inverted U-shaped dose-response curve.[138,140,141] Interestingly, this bell-shaped response is not exclusive to the anxiolytic effects of CBD, as it has also been observed in animal models of depression,[142] compulsive behavior,[143] schizophrenia,[144] cognitive impairment,[145] and other ailments.[140] These findings suggest that the optimal dose of CBD may depend on condition, indicating the need to test different doses in animals and humans in order to elucidate its therapeutic potential for the treatment of SAD and other anxiety disorders.

In terms of clinical findings, one study in human cells suggests that CBD increases AEA levels through binding to fatty acid-binding proteins and thereby prevents AEA uptake and catabolism by FAAH,[146] rather than inhibiting FAAH directly. In addition, results from a clinical trial in schizophrenia indicate higher AEA levels in individuals receiving treatment with CBD.[127] While direct measurement of brain FAAH activity has been made possible by the novel PET radiotracer [C-11]CURB,[147] there have been to our knowledge no studies directly investigating brain FAAH activity before and after treatment with CBD. Finally, three randomized-controlled clinical trials in SAD have shown that CBD may improve SAD symptoms (Table 1).[148-150] Although these results appear to be promising, it should be noted that these trials were conducted in small samples (10 to 37 participants) and employed inconsistent assessment tools across studies. Such limitations warrant further investigation to produce robust findings and better understand the biological mechanisms of CBD action in SAD.

Interaction of the endocannabinoid system with other systems and biological findings

As discussed, previous research has largely focused on establishing monoaminergic impairment affecting the limbic system[2,152] and dysfunction of the HPA axis[153] as pathophysiological mechanisms of SAD. Accordingly, treatments developed and/or tested for SAD have mainly targeted serotonin (5-HT) and norepinephrine (NE) neurotransmission, specifically via drugs blocking their reuptake.[8] However, these drugs are ineffective in more than one-third of SAD patients.[2]

Recently, the ECS has been identified as a modulator of the aforementioned systems and incited interest in the development of new pharmacological treatments for mood and anxiety disorders. In particular, two preclinical studies found that synthetic CB1 receptor agonism was associated with increased NE efflux and anxiety-like behavior in healthy rodents.[154,155] The latter study also showed that local administration of a CB1 receptor antagonist (SR 141716A) followed by local administration of a CB1 receptor agonist (WIN55,212-2) produces a paradoxical inhibition of NE efflux.[155] Together, these findings suggest that enhanced NE activity may be mediated, in part, by direct CB1 receptor agonism, localized at noradrenergic axon terminals. However, the paradoxical decrease in NE observed in the second study may also indicate that NE efflux is modulated indirectly via interneurons or other chemically distinct afferents.[154] Similarly, direct or indirect stimulation of CB1 receptors has been linked to enhanced 5-HT neuronal activity.[156-158] However, these studies found that increased 5-HT neurotransmission following CB1 receptor agonism,[156] FAAH inhibition,[157] or FAAH knockout[158] was associated with enhanced stress-coping behaviors and antidepressant effects in otherwise healthy rodents.

Accumulating evidence also suggests that the ECS may act as a homeostatic regulator of HPA axis activity under basal and stress-related conditions.[159] In this regard, studies have found that chronic stress exposure or repeated corticosterone treatment activate the HPA axis response, which has been associated with changes

Table 1 Randomized, double-blind, placebo-controlled clinical trials of drugs targeting the ECS in SAD

Reference	Sample size	Pharmacological treatment	Treatment duration (# days, treatment/day)	Symptom scale	Principal finding
Crippa et al.[148]	SAD = 10	400 mg CBD (n=5) Placebo (n=5)	1 day, 1/day	VAMS	CBD decreased subjective anxiety scores compared to placebo ($p < 0.001$).
Bergamaschi et al.[149]	SAD = 24 HC = 12[†]	600 mg CBD[‡] (n=12) Placebo[‡] (n=12)	1 day, 1/day	VAMS, NSSS	CBD pre-treatment reduced subjective anxiety scores compared to placebo (VAMS, $p < 0.001$; NSSS, $p < 0.004$).
Masataka[150]	SAD = 37	300 mg CBD (n=17) Placebo (n=20)	4 weeks, 1/day	LSAS, FNEQ	CBD decreased post-intervention social anxiety scores compared to placebo (FNEQ, $p = 0.0002$; LSAS, $p = 0.0018$).
Schmidt et al.[119]	SAD = 149	25 mg JNJ (n=74) Placebo (n=75)	12 weeks, 1/day	LSAS, HAM-A, HDRS	No significant difference in anxiety scores between subjects treated with JNJ and placebo.
NCT03549819[151]	SAD among other anxiety disorders Not yet recruiting	200 mg CBD (n=TBD) Placebo (n=TBD)	8 weeks, 1/day[§]	HAM-A	Clinical trial registered[∥]

CBD = cannabidiol; ECS = endocannabinoid system; FNEQ = Fear of Negative Evaluation Questionnaire; HAM-A = Hamilton Anxiety Rating Scale; HC = healthy control; HDRS = Hamilton Depression Rating Scale; JNJ = JNJ-42165279 (FAAH inhibitor); LSAS = Liebowitz Social Anxiety Scale; MCT = medium-chain triglyceride; NSSS = Negative Self-Statement Scale; SAD = social anxiety disorder; TBD = to be determined; THC = delta-9-tetrahydrocannabinol; VAMS = Visual Analogue Mood Scale.
[†] Healthy controls underwent the same study procedures without receiving pharmacological treatment.
[‡] Treatment administered 1.5 hours prior to participation in a simulated public speaking test.
[§] Titrated as tolerated up to a maximum 2 capsules, twice daily (200-800 mg total dose).
[∥] Clinical trial is registered but has not yet started.

in AEA levels, FAAH activity, and CB1 receptor expression.[90,159-161] Moreover, Di et al.[162] showed that the paraventricular nucleus (PVN) participates in glucocorticoid-mediated feedback regulation of the HPA axis via endocannabinoid release. Specifically, this model suggests that, during the stress response, activation of hippocampal glucocorticoid receptors (via corticosterone) induces the synthesis of endocannabinoid ligands which bind to CB1 receptors on glutamatergic neurons.[162] This binding activates a signaling cascade that leads to the inhibition of glutamate release onto the hypothalamic PVN, decreasing PVN neuronal activity and further hormone secretion.[162] It is possible, then, that disruption of certain ECS processes may lead to dysregulation of HPA axis activity, which together may contribute to the development of sustained anxiety-related symptoms.

ECS interactions with oxytocin, the neuropeptide that reinforces social bonding and social reward, have also been observed. Specifically, Wei et al.[163] showed that oxytocin drives AEA mobilization in the mouse nucleus accumbens, leading to reinforcement of social reward behavior. Pharmacological blockade of oxytocin receptors attenuated this response, whereas FAAH inhibition or gene deletion offset the behavioral effects of oxytocin receptor blockade.[163] These findings suggest that the ECS may modulate the prosocial effects of oxytocin, which may open potential avenues of research in disorders of social impairment such as SAD.

Genetic studies examining ECS candidate genes relating to anxiety disorders have produced limited findings. Current evidence suggests that a single nucleotide polymorphism in the *FAAH* gene (rs324420; C385A)[104] influences FAAH protein expression,[105,106] which has been found to correlate with trait anxiety.[107] However, there is little to no evidence to support an association between genetic variants of other ECS genes and symptoms of SAD.

Thus, further investigation of ECS interactions with other biological systems and genetic variables may help improve our current knowledge and address the gap with respect to SAD pathophysiology, potentially allowing for the development of more targeted treatment options.

Summary of current drugs targeting the endocannabinoid system

Based on current evidence, it appears that the ECS may play a role in buffering against stress and anxiety. Thus, targeting components of this system could serve as a potentially valid strategy for the treatment of social anxiety symptoms. In this regard, several ECS-targeting drugs have been developed, with some demonstrating promising therapeutic potential.

Logically, CB1 agonists were considered promising, given their ability to enhance ECS activity and reduce anxiety-like behaviors in rodents.[80,164-166] Notable synthetic CB1 agonists include WIN55,212-2, CP-55940, and HU-210. Although these compounds are generally well-tolerated, accompanying risk of other psychotropic adverse effects has been observed.[167] Direct CB1 agonism has also been associated with disturbances in social behavior and social play in some preclinical studies, prompting the exploration of other ECS targets.[113,168-170]

One of the most effective strategies to target anxiety through modulation of the ECS has been inhibition of

the endocannabinoid degradation enzymes FAAH and MAGL. This allows for the indirect activation of CB1 receptors by increased levels of AEA and 2-AG. Accumulating evidence has demonstrated the anxiolytic effects of FAAH inhibitors (URB597, PF-3845, and PF-04457845), most prominently in rodents,[80] as well as the aforementioned human experimental PF-04457845 trial.[120] Interestingly, prosocial effects of these compounds have also been consistently observed in rodent models of social impairment.[117,171-174] Although more limited in study, MAGL inhibitors (URB602, JZL184) have demonstrated similar results in terms of improved anxiety and social behavior.[80,175,176] While a handful of inhibitor studies have reported insignificant results,[177-179] the vast majority suggest that inhibition of endocannabinoid degradation enzymes may serve as a promising novel treatment strategy for anxiety-related disorders.

The opposing effects on anxiety and social behavior exerted through direct versus indirect CBR activation may be due to differences in the mechanism of action (e.g. partial vs full agonistic properties at CBR) and/or affinities of endogenous vs synthetic ligands for CB1R vs CB2R affinity.[180] However, this variability may also be explained by existing differences in the underlying status of the ECS in animals with social impairments, such as ECS receptor expression, ligand levels, or enzyme activity.[173,174,181] These aspects warrant further investigation of changes in ECS components in social impairment models as well as in human studies of disorders associated with social impairment in order to validate the therapeutic potential and potential risks of these ECS-targeting agents in clinical settings.

In contrast, CB1 inverse agonists/antagonists have been shown to further exacerbate anxiety-like behaviors. As mentioned above the inverse agonist Rimonabant, found to be effective for weight-loss and smoking cessation, was quickly withdrawn following the emergence of severe psychiatric side effects.[182] In line with this, CB1 inverse agonist, AM251 has been found to reduce social interactions in rodents[183,184] and non-human primates.[115] Interestingly and in contrast to CB1 inverse agonists, two preclinical studies found that administration of a CB1 neutral antagonist, AM4113, did not produce anxiogenic or depressive side effects when administered in rats.[185,186] Similarly, two other rodent studies found that Rimonabant, but not the CB1 neutral antagonist tetrahydrocannabivarin, produced depressive and anxiogenic-like behaviors following administration.[187,188] While inverse agonists induce a pharmacological response opposite to that of agonists, neutral antagonists do not activate the receptor – differences which may partly explain the absence of these behavioral side effects following administration of the latter group of agents.[185]

Overall, the development of ECS-targeting agents has produced some promising results with regards to modulation of anxiety-like symptoms and social behavior, particularly in the field of FAAH and MAGL inhibitors. However, current evidence largely focuses on measures of general anxiety in animal models, and lacks the support of clinical trials. Further investigation of the effect of ECS-targeting drugs, specifically in animal models of social impairment and human studies, may provide greater insight as to how these compounds effect other biological systems, the possibility of risks and adverse effects, as well as their therapeutic potential in SAD.

Future research and potential challenges

This article highlights some of the currently known pathophysiological mechanisms of SAD and the therapeutic potential of the ECS. Targeting specific components of the ECS which have been shown to play a role in anxiety-related symptoms and social behavior may serve as a novel and possibly more targeted treatment for such disorders. However, there are a number of challenges to consider with respect to this new approach.

Firstly, while CB1 activation has been hypothesized to improve social anxiety-like behaviors by enhancing ECS signaling, activation of this receptor has since also demonstrated increased risk of social[113,168-170] and cognitive[189] impairments, as well as disturbances in the reward system.[190,191] As previously discussed, indirect CB1 activation via FAAH or MAGL inhibition has been the most commonly proposed solution to possibly avoid these adverse effects. Additionally, peripheral CB1 agonists and CB1 positive allosteric modulators have also demonstrated the ability to avoid certain CB1-related side effects.[192-194] Despite these alternative solutions, the vast majority of current supporting evidence is a result of animal experimentation. As such, the efficacy, safety, and tolerability of such drugs must be further validated through clinical studies to evaluate not only their therapeutic potential, but also their possible risks.

Secondly, it is important to consider that the ECS is a complex biological system that does not function in isolation, but rather through interactions with an array of other systems. In this regard, the ECS may interact with and modulate other systems including the endovanilloid,[194,195] opioid,[196] noradrenergic,[197] prostanoid,[198] serotonergic,[199] dopaminergic,[200] glutamatergic,[201] and GABAergic[202] systems, among other biological networks.[194] Accounting for these additional biological networks will be crucial in understanding the larger physiological context and environment in which the ECS functions, and, by extension, the biological pathways that pharmacological interventions in the ECS can potentially influence.

A third challenge to consider is the pathophysiological heterogeneity of individuals with SAD. While the pathophysiological mechanisms of SAD are not yet certain, a number of biological systems have been described above which may additionally interact with the ECS. In this regard, it is possible that SAD in some individuals may be due to greater dysregulation of the HPA axis,[51] whereas others may have more severe disruptions in their serotonergic/dopaminergic systems[42] or inflammatory processes.[203,204] Accordingly, it is possible that ECS-targeted treatment may be beneficial to certain

neurobiological subgroups of SAD patients, but potentially ineffective or even possibly harmful to others. As such, future research efforts should also focus on elucidating biological subpopulations in SAD and how ECS-targeted therapies could be individualized to maximize their therapeutic potential.

Conclusion

Overall, the ECS presents as a potential biological pathway in the pathophysiology of SAD and as promising avenue for developing novel therapeutic approaches. The lack of human ECS studies and clinical trials, combined with the complex nature of the ECS and the heterogenous pathophysiology of SAD, highlight significant gaps in our knowledge and possible challenges, though at the same time great potential for future research. Further investigational efforts – specifically in human populations – are warranted to improve our knowledge of the ECS in SAD and help clarify these emerging questions. In turn, such research may allow for the development of more targeted pharmacological treatment interventions for individuals with SAD.

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Disclosure

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