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Review Article

Modulation of Excitatory and Inhibitory Systems in Autism Spectrum Disorder: The Role of Cannabinoids

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Abstract

Autism Spectrum Disorder includes a group of developmental disabilities characterized by patterns of delay and deviance in the development of social, communicative, cognitive skills and the presence of repetitive and stereotyped behaviors as well as restricted interests. Although the etiopathogenesis of autism has not yet been elucidated, past literature has highlighted an imbalance between glutamatergic and Gamma-Aminobutyric Acid (GABA)-ergic neurotransmission. A cortical deficiency of GABA in young people with ASD has been reported. Endocannabinoids act in numerous synapses of the central nervous system, maintaining adequate synaptic homeostasis, preventing excess stimulation at the level of excitatory or inhibitory synapses. The endocannabinoid system appears to play an important role in some clinical presentations of autism, such as socialization. Indeed, Autism Spectrum Disorder seems to be characterized by a hypo-functionality of the endocannabinoid system.

The present work aims to describe the current state of the art regarding the possible role of cannabinoids in the modulation of the excitatory and inhibitory systems in individuals with ASD.

A literature search was conducted for relevant studies on PubMed database, concerning the randomized clinical trials on the modulating effect of excitatory and inhibitory cannabinoid systems in autism.

Three eligible articles were found according to the purpose of the present study. The results of the three articles considered highlighted a cannabinoid-related increase in glutamate in subcortical regions and a decrease in cortical regions, both in subjects with and without Autism. CBD increased gamma-aminobutyric acid transmission in the subcortical regions of neurotypical subjects, while it decreased it in the same areas of the ASD group. Furthermore, Cannabinoid modulated low-frequency activity used as a measure of brain activity and functional connectivity in the brains of adults with autism spectrum disorder.

Data from the three fMRI studies demonstrated that CBD influences cortical and subcortical connectivity in an adult sample. This effect was notable only in the ASD group but not in the controls. However, further studies are needed to confirm the results obtained so far.

Keywords: Autism Spectrum Disorder; Endocannabinoid System; Cannabinoids

Introduction

Autism Spectrum Disorder (ASD) includes a group of developmental disabilities characterized by patterns of delay and deviance in the development of social, communicative, cognitive skills and the presence of repetitive and stereotyped behaviors as well as restricted interests [1]. In addition to core symptoms, people with ASD often have numerous medical and psychiatric comorbidities that worsen the quality of life of patients and their caregivers [2]. Clinical features are accompanied by an atypical sensory experience present in up to approximately 95% of people with ASD [3].

The clinical presentation can vary considerably over the years, depending on the characteristics of the person, the surrounding environment and above all the habilitation/rehabilitation interventions undertaken [4].

Although the etiopathogenesis of autism has not yet been elucidated, the data in the literature agree that the causes of autism are multifactorial, including genetic, epigenetic, inflammatory, immunological and environmental factors [5-10].

More than one study has reported dysfunctional alterations in E/I neurotransmission in cortical neurons, using glutamatergic and dysfunctional GABAergic neurotransmission, and abnormal levels of GABA concentrations in brain tissue or plasma (for extensive reviews see [11-15]).

Different mechanisms could be responsible for E/I imbalance, such as alterations in genes encoding glutamatergic receptors or synaptic proteins [16,17]. Particularly, the role of neurexins, neuroligins and SHANK proteins, implicated in the formation and maintenance of excitatory and inhibitory synapses, has

been widely established in the pathogenesis of neurodevelopmental disorders [18-22].

Some recent studies have shown abnormalities of the GABAergic and glutamatergic pathways in the prefrontal cortex and Basal Ganglia (BG) regions, now proven to be crucial regions of the core signs and symptoms of ASD [23-26].

Past literature has proven that Endocannabinoid System (or Endogenous Cannabinoid System, ECS) functions as retrograde signal molecules in synapses, especially in glutamatergic and GABAergic synapses, preventing excess excitation or inhibition, respectively [27,28]. Moreover, the ECS can enhance GABAergic transmission and reduce glutamate transmission in different brain regions [29,30].

The ECS is a complex biological system implicated within the pharmacological effects of cannabis [31], neuronal plasticity [32,33], postnatal development [34], pain sensation [35], emotionality [36], appetite [37], learning and memory [38]. It is also concerned with the homeostasis of the organism [39] through the modulation of more than one system such as the cardiovascular (CVS), central nervous (CNS), peripheral nervous (SNP), endocrine, reproductive, immune and digestive systems [40,41].

The ECS includes three major components: endogenous cannabinoids (or endocannabinoids, eCBs), metabotropic receptors and enzymes answerable for the synthesis and degradation of eCBs [42].

The most studied eCBs are N-arachidonylethanolamine (or anandamide, AEA) [43] and 2-arachidonylglycerol (2-AG) [44]. Cannabis contains more than 500 compounds and the most abundant are represented by Δ-9-tetrahydrocannabinol (THC), cannabidiol (CBD), flavonoids and terpenes [45]. Cannabidiol (CBD) and delta-9-tetrahydrocannabinol (Δ9-THC) are the most intensively studied phytocannabinoids. In addition to eCBs, phytocannabinoids and synthetic derivative compounds represent other substances that act as ligands for ECS receptors.

THC has a similar affinity for both CB1 and CB2 receptors, but most of the psychoactive effects of THC are related to the activation of CB1 receptors [46]. Cannabidiol (CBD) does not have psychotropic properties, as recently confirmed [47]. CBD has a very low affinity for CB1 and CB2 receptors, and several results have suggested that CBD operates as a negative allosteric modulator/inverse agonist in both CB1 and CB2 receptors [48-50].

There are other potentially therapeutic phytocannabinoids, which have been tested in pre-clinical studies, but not yet extensively in vivo and are represented by: Δ8-Tetrahydrocannabinol [51], Cannabinol [52], Cannabigerol [53], Cannabichromene [54], Δ9-Tetrahydrocannabivarin (Δ 9-THCV) [55] and Cannabidivarin (CBDV) [56].

The two main receptors of the ECS are the cannabinoid receptor type 1 (CB1) and the cannabinoid receptor type 2 (CB2). The CB1 is expressed in both the peripheral and the central nervous systems such as in the hippocampus, cortical regions, basal ganglia (BG) and cerebellum. On the other hand, the CB2 receptor is mostly expressed in peripheral cells and tissues of the immune system. Although the presence of CB2 in the brain is very low compared to CB1, CB2 appears to play a crucial role in macrophage/microglia functions [57]. Indeed, CB2 expression drastically increases in activated microglia and CB2 activation decreases the production of pro-inflammatory molecules [58]. Both of these receptors are coupled to the G protein signaling pathway [59].

The ECS appears to play an important role in some clinical presentations of autism as well as in the regulation of emotional responses, behavioral reactivity, reciprocity, social play and social interaction [60]. Indeed, numerous murine studies have shown that the ECS plays a crucial role in the pathophysiological mechanisms underlying ASD [61-63]. Human studies support growing evidence of ECS abnormalities in people with ASD [64-67].

Currently, only risperidone and aripiprazole are approved drugs for the treatment of irritability associated with ASD [68]. Due to the high incidence of adverse effects of conventional psychotropic therapies [69], the use of phytocannabinoids could become an alternative therapeutic strategy in these situations [70].

In the literature, there is preclinical evidence to support the positive effects of the use of cannabinoids in balancing the pathological mechanisms of ASD [71-74] and ASD-like symptoms [75-78].

Cannabinoids are already being used for a wide range of conditions such as multiple sclerosis, Tourette's syndrome, Parkinson's disease, epilepsy, glaucoma, nausea and pain [79], but clinical data on the use of cannabinoids in people with autism are still very limited.

Materials and Methods

The present work aims to describe the current state of the art regarding the possible role of cannabinoids in the modulation of the excitatory and inhibitory systems in individuals with ASD.

A literature search was conducted for relevant studies using PubMed database concerning the randomized clinical trials using functional Magnetic Resonance Imaging (fMRI) or Magnetic Resonance Spectroscopy (MRS) on the modulating effect of cannabinoids on the excitatory and inhibitory systems in people with autism.

Results and Discussion

Three eligible papers [80-82] were found according to the purpose of the present study.

Over the years, technological progress has made it possible to measure the cerebral effects of psychotropic substances. fMRI has already been used in people with ASD, to study the brain effects of riluzole, propranolol and oxytocin during cognitive tasks [23,83,84]. MRS has been already used to determine alteration in E/I dynamics in BG and dorsomedial prefrontal cortex (DMPFC) [24].

All three studies used a randomized double-blind, cross-over design. Two studies [80,81] acquired data following a single oral dose of 600 mg CBD or a matched placebo while one study utilized CBDV [82] (see **Table 1**).

Two studies compared MRS measures of glutamate and GABA [81] and Glx (glutamate + glutamine) and GABA+ (GABA+ macromolecules) [82] levels in the BG and DMPFC in men with and without ASD.

Considering that differentiating the cognitive response to a task from normal brain activation can be challenging in people with ASD [85,86], one study rightly used a resting state design (task-free) to examine the fractional amplitude of low-frequency fluctuations (fALFF) as a measure of spontaneous regional brain activity [87]. Indeed, low-frequency oscillations appear to be important in synchronizing activity between brain regions [88].

All three studies taken into consideration show how cannabinoids shift both fALFF and FC [80] as well as the levels of excitatory and inhibitory neurotransmitters [81,82] in the living adult human brain both in the ASD group and in the control group.
Two studies [81,82] showed no differences in baseline glutamate and GABA levels in BG and DMPFC as evidenced by some MRS studies [23,24], but not all [89]. However, it should be noted that individual responses in autistic brains varied according to baseline Glx levels.

Changes in fALFF were more prominent in the ASD group and not significant in controls [80]. Furthermore, in ASD the shift in fALFF in the cerebellum was accompanied by diffuse changes in vermal FC with many of its subcortical and cortical targets. As suggested by the authors, CBD appears to 'tune' FC in a region or connection-specific manner. These data are supported by previous studies correlating levels of E/I cerebellar and cerebro-cerebellar FC in adolescents and adults with ASD [90].

The most important FC alterations were attributable to the vermis but not to the fusiform probably, as explained by the authors, due to reduced expression of GABA receptors [91], decreased levels of enzymes responsible for converting glutamate to GABA, alterations of inhibitory Purkinje cells [92] and structural alterations in ASD [93,94], although data in the literature are conflicting [95,96].

Given the impairments [85,97] of functional spindle-shaped abnormalities in face processing [98] and the study evidence, one could hypothesize the use of CBD to improve the recognition of faces in ASD.

Regarding Glx, both people with ASD and neurotypicals responded to CBD in an overlapping manner [81]. On the other hand, CBD decreased GABA+ levels in the BG and (markedly) in the DMPFC voxel of autistic adults, probably due to metabolic or genetic alterations of GABA receptors [92,99-101]. Probably these results are explained by the ability of CBD to suppress the activity of prefrontal glutamatergic neurons through the 5-HT1A receptors [102,103]. Furthermore, 5-HT1a receptor dysfunction in ASD is now known [104]. These data support on the one hand the theory of E/I alteration in ASD and on the other the possible use of CBD as a therapeutic approach.

The cortical, sub-cortical, and thalamic connections of the BG appear to be important for the clinical presentation of the features of the autism diagnosis [105-107].

CBDV induced an increase in BG Glx levels (left BG) in both groups. In contrast, CBDV had no impact on Glx in DMPFC, nor on GABA+ levels in either voxel [82]. Only in the ASD group, the drug-induced shift in Glx levels in BG was significantly negatively correlated with baseline Glx value.

Moreover, based on the basal levels of Glx, a different response was highlighted in subjects with ASD. Indeed, in autistic individuals with lower baseline Glx levels, CBDV increased Glx levels, while individuals with higher baseline Glx values experienced a decrease in Glx. From the data collected, CBDV seems to normalize the altered levels of Glx.

These effects could be explained by the binding of CBDV to TRP receptors coupled to pyramidal excitatory neurons highly represented in BG as demonstrated by preclinical studies [108,109]. Furthermore, CBDV could modulate excitatory neurotransmission through the activation of microglial TRP receptors, a mechanism already known to increase extracellular vesicular shedding and subsequent glutamate release [110].

In our view, these findings appear important to clinicians in further differentiating subgroups within the autistic disorder spectrum in such a way as to make treatments increasingly individualized.

Table 1: Summary of studies.

Author	Study Design	N. of people with ASD	N. of controls	Treatment	Daily Dosage	Main Outcomes
Pretzsch et al. 2019 [80]	RCT Crossover	17	17	CBD	600 mg/die single administration	CBD significantly increased fALFF in the cerebellar vermis with many of its subcortical and cortical targets in ASD group
Pretzsch et al. 2019 [81]	RCT Crossover	17	17	CBD	600 gm/die single administration	CBD modulates glutamate-GABA systems, but prefrontal-GABA systems respond differently in ASD group
Pretzsch et al. 2019 [82]	RCT Crossover	17	17	CBDV	600 gm/die single administration	CBDV modulates the glutamate-GABA system in the BG but not in frontal regions in ASD group

The studies considered, have some limitations such as the low sample size, the acute use of cannabinoids only, having recruited only male subjects, the stringent inclusion criteria which excluded psychiatric comorbidities often frequent in people with ASD. Although the data are very encouraging, future research is needed to confirm the data exposed on a large scale and on more representative samples of the population of people with ASD.

Conclusion

Data from the three studies considered in this review demonstrated that CBD and CBDV influence cortical and subcortical connectivity in an adult sample. This effect was notable only in the ASD group but not in the controls. Indeed, data showed how cannabinoids shift both fALFF and Functional Connectivity as well as the levels of excitatory and inhibitory neurotransmitters in the living adult human brain more consistently in the ASD group. Furthermore, these data, in addition to confirming the hypothesis of alteration between excitatory and inhibitory systems, lay the foundations for the use of drugs acting on the GABA system as a treatment target in ASD. However, further studies are needed to confirm the results obtained so far.

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