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Targeting cholinergic and endocannabinoid system as a therapeutic intervention for core asd associated phenotypes in autism model: a systematic review

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Targeting cholinergic and endocannabinoid system as a therapeutic intervention for core asd associated phenotypes in autism model: a systematic review

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Running Title: Therapeutics on cholinergic/endocannabinoid system

Abstract

Introduction: Autism spectrum disorder (ASD) is a neurodevelopmental disorder that has been linked to the dysregulation in the cholinergic and endocannabinoid (EC) system. This study systematically reviews the present literature on treatment strategies aimed at enhancing the activity of both systems in ASD models.

Method: We performed a systematic evaluation of literatures that investigated the effects of different therapeutic interventions on the components of the cholinergic and EC systems in ASD models, following the guidelines provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist. Four databases were searched: Google Scholar, Web of science, EMBASE and MEDLINE/PubMed, between August 2012 and February 2023. The selected research papers' references were also examined. Twelve papers (five for cholinergic system, six for EC system and one for the two systems) were reviewed in this study of prior relevant treatment strategies that impact both systems. There were 77 studies cited in total.

Results: The majority of research revealed that different therapeutic interventions down-regulated cannabinoid 1 (CB1) receptors, and the systems hydrolyzing enzymes and up-

regulated EC, Alpha7 nicotinic acetylcholine receptor (α7 nAChR), and acetylcholine signaling molecules. The regulation of the components of the cholinergic and EC systems by the therapeutics generally enhanced behaviors in ASD models.

Conclusion: It is possible that there are therapeutic interventions assessed in one of the systems that may be effective in treating the core ASD-associated phenotype. The benefits of the reviewed therapeutic interventions in this study need to be further investigated in randomized, blind, placebo-controlled clinical trials.

Keywords: Endocannabinoid, Cholinergic, Agonist, Antagonist, Autism.

Introduction

The diagnosis of autism spectrum disorder (ASD), a neurodevelopmental disorder that affects social communication and interaction throughout life, is marked by limited and/or repetitive interests and/or behaviors that first appear before the age of three.[1] ASD now includes a number of disorder that were grouped together under the category of pervasive developmental disorders (PDDs) in the first generation of medical classifications. But because it's a spectrum condition, it also has a high degree of heterogeneity in its phenotypic manifestations, which are linked to a wide range of intellectual and language development levels, intra-individual differences in cognitive profiles, and a history of comorbidity with other developmental disorders and psychiatric conditions.[2,3] According to Rogala *et al*. [4] and Yasuda *et al*. [1], autism is a diverse condition with a complex etiology involving many different elements, including genetic, epigenetic, environmental, and immunological components.

The cholinergic and endocannabinoid systems' neurological signals constitute the body's vast regulatory network, which keeps physiology and homeostasis in check. ASD has been linked to dysregulated endocannabinoid and cholinergic systems.[5-7] Many physiological processes and neuroadaptive reactions depend critically on the cholinergic and endocannabinoid systems. This is involved in numerous stages of brain development and encompasses nociception, reward, learning and memory, movement control, and endocrine function.[8,9] Acetylcholine (ACh) and endocannabinoids (ECs) influence synaptic transmission and plasticity in the central nervous system (CNS) by modulating neurotransmission. By activating type 1 cannabinoid receptors (CB1Rs), which are

predominantly found at presynaptic locations, and nicotinic ACh receptors (nAChRs), these neurotransmitters specifically control the release of both excitatory and inhibitory neurotransmitters.[10,11] The idea of a bidirectional crosstalk between the nicotinic cholinergic and endocannabinoid systems has gained support over time from growing preclinical research. In multiple brain areas, nAChRs and CB1Rs exhibit close overlap and are widely expressed in the central nervous system (CNS).[12,13] When nicotine and delta-9-tetrahydrocannabinol (Δ9-THC), the main psychoactive components of tobacco and cannabis, respectively, are given to animals, they cause a number of common pharmacological effects, including hypothermia, induction of anti-nociception, rewarding effects, dependence, and impairment of locomotion.[14]

One common neuromodulatory system is the endocannabinoid system (ECS). This has a significant impact on the development of the CNS, synaptic plasticity, and the body's reaction to internal and external stressors.[15] The ECS is made up of endogenous cannabinoids, or endocannabinoids, cannabinoid receptors, and the enzymes that synthesize and degrade ECs.[15] Although CB1 receptors are the most dominant kind of cannabinoid receptors, some cannabinoids also activate CB2 receptors, transient receptor potential (TRP) channels, and peroxisome proliferator-activated receptors (PPARs). Cannabinoid receptor interactions enable exogenous cannabinoids, such as tetrahydrocannabinol and cannabidiol, to exert their biological effects. The two endogenous cannabinoids that have been investigated the most are 2-arachidonoyl glycerol (2-AG) and arachidonoyl ethanolamide (AEA- anandamide).[15] Many neurological illnesses are attributed to etiologies involving changes in the EC system's functionality.[16] The observation that the EC system is highly involved in the regulation of social and emotional reactivity as well as in the modulation of behaviors that are frequently altered in ASD, such as learning and memory processes, seizure susceptibility, and circadian rhythm regulation, provides indirect evidence for the system's involvement in ASD.[17-18] Different autism models showed a significant decrease in the levels of AEA and 2-AG,[19] while VPA-autistic animals showed abnormal phosphorylation of the cannabinoid type 1 (CB1) receptor in the dorsal striatum, hippocampus, and amygdala.[20]

Given the large density of cholinergic synapses found in the neocortex, limbic system, thalamus, and striatum, it is likely that cholinergic transmission plays a key role in memory, learning, attention, and other higher-order brain functions.[21] Numerous research directions point to additional functions of cholinergic systems in the general homeostasis and plasticity of the brain. As a result, current research on cognitive and social deficiencies heavily relies on the brain's cholinergic system.[21] The neurotransmitter molecule, ACh, cholinergic receptors (AChRs), choline acetyltransferase (ChAT), and acetylcholinesterase (AChE) are all components of the cholinergic system. These molecules have dual roles in the brain, acting as neurotransmitters and neuromodulators. They are crucial for arousal, motivation, memory, attention, and homeostasis maintenance. In response to neuronal inputs, the majority of innate and adaptive brain cells release or express these molecules on their surfaces. ASD-related core behavioral deficits may result from dysregulation of this neural system communication. A number of preclinical ASD animal models seem to have dysregulated cholinergic systems. [6,7] Meyza and Blanchard [22] describe the BTBR mouse model for ASD, which is an inbred mouse strain that has an Itpr3 gene deletion. Mice with BTBR exhibit abnormal nicotinic cholinergic neurotransmission, repetitive behaviors, and social communication problems.[23] In BTBR mice, nicotine treatment reduced these distinctive behaviors associated with ASD.[23] Similar results were observed when donepezil, an acetylcholinesterase inhibitor, was given to BTBR mice in another study.[24]

Targeting the cholinergic and endocannabinoid systems, a number of agonists, antagonists, and inhibitors have been developed to help with the fundamental behavioral deficits associated with ASD. This review will address the various therapeutic interventions for dysregulated cholinergic and endocannabinoid systems in ASD, offering a comprehensive and current overview of the systematic literature on potential therapies that could improve the activities of the cholinergic and endocannabinoid systems in ASD. Which molecules influence the defective behaviors in animal models of ASD, and which agonists and antagonists affect the components of the cholinergic and endocannabinoid systems? Improved methods for regulating these systems in ASD may result from a greater understanding of the varied roles played by pharmacological compounds and other behavioral therapy approaches.

Method

We completed our systematic literature review in December 2023 using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) approach. This was the guideline for the approval of the study by the review and research ethics committee in the department of anatomy, college of medicine, University of Nigeria, Enugu Campus.

In order to aid in the review, the following questions were asked: 1) How safe and effective were the treatments, and how well did they enhance the components of the cholinergic and endocannabinoid systems? 2) What impact did the therapies have on the behavioral deficits linked to the system's activities in the ASD models? 3) Which key method were used to assess the improvement in behavior?

Google Scholar, Web of science, EMBASE, and MEDLINE/PubMed were the four databases that were searched. The search strategy for the databases was developed based on terms found in the title or abstract, using descriptors related to the endocannabinoid and cholinergic systems (the system receptors, signaling molecules, and enzymes) as well as descriptors related to autism (autistic, autism, Asperger, transgenic autism, BTBR mouse model, sodium valproate autism model, and pervasive developmental disorder). Articles in any language were considered in the analysis of eligibility; there were no language restrictions during the selection process. The search operators "AND" and "OR" were used, in addition to enclosing descriptors in quotation marks. Terms linked to cholinergic (components), ECS (components), ASD, autistic animal model and autism were clustered together using the "OR" operator. These two groups of linked sentences were then combined using the "AND" operator.

Papers published between August 2012 and February 2023 that satisfied the inclusion requirements were added. Book chapters, abstracts, studies on animals, and studies on other illnesses or alterations associated with symptoms and indicators similar to those shown in the autism model were all disregarded as irrelevant to the subject. Articles

discussing enhancers, agonists, or antagonists of the cholinergic and endocannabinoid system components of ASD models were considered in this study.

The first screening was done by reading the abstracts and titles of the papers that were found in the databases. The articles that were deemed appropriate for the proposed topic were then read in full. Following the screening procedure, we examined the papers' references to determine if any additional relevant research met the eligibility requirements. Three authors independently and concurrently carried out the search, and one experienced author vetted the selected articles. The most knowledgeable and experienced author made the final decision about whether or not to include a given study, always making sure to verify the qualifying requirements. A total of 17, 12, 8, 6, and 5 articles were found after searches were conducted in the MEDLINE/PubMed, Google Scholar, Web of science, EMBASE and References of the reviewed papers, respectively. These were reduced to 9, 2, and 1 item, respectively, as they did not meet the inclusion criteria. After additional screening, 12 papers were found to be able to entirely match the inclusion criteria.

Each trial's data extraction method involved completing a standardized information sheet. A third reviewer verified the data that had been gathered after three reviewers had extracted the scientific information. Any disagreements were discussed and decided upon by the reviewers and writers.

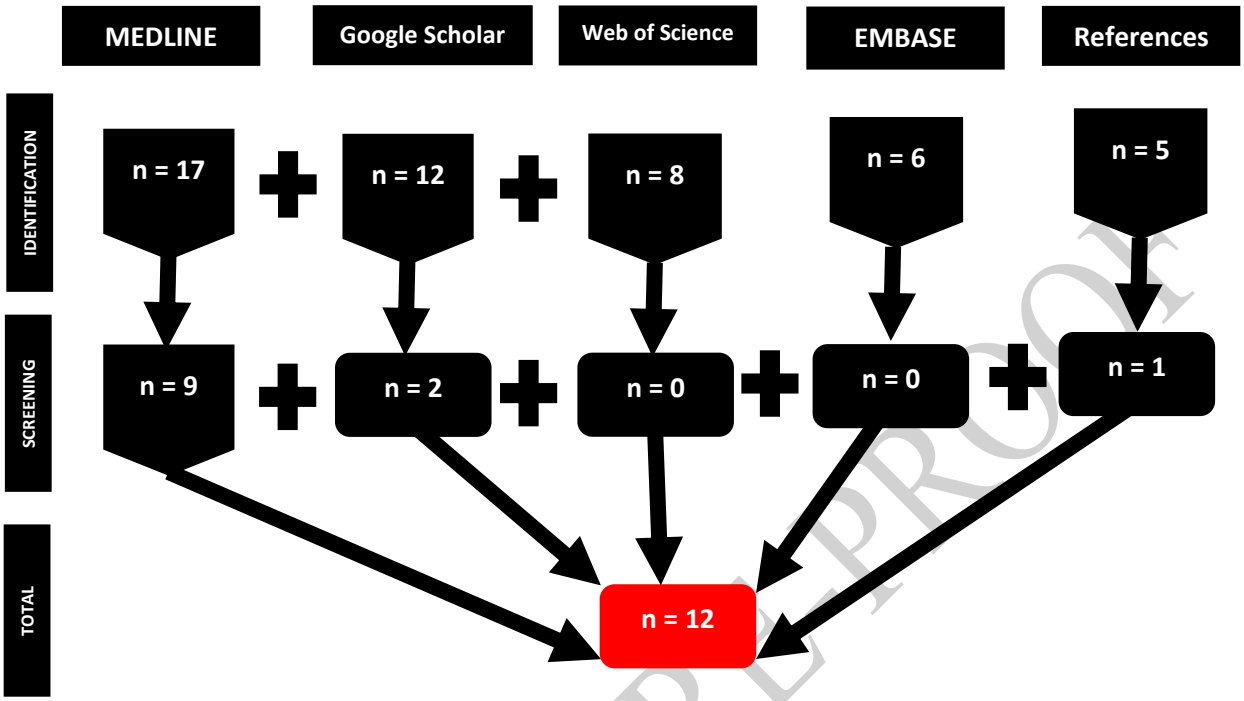

Figure 1 - Study selection flowchart according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for targeting the cholinergic and endocannabinoid systems as a therapeutic intervention for core ASD-associated phenotypes in the ASD model: a systematic review

Result

48 articles were found in the search's initial results. Following the initial screening, 36 studies were disqualified for failing to meet the criteria because they didn't investigate any components of the cholinergic or endocannabinoid systems, and some papers involved primary research on a non-specific ASD animal model, studies looking into the therapeutic potentials of other brain signaling systems, and investigations into the symptoms of other disorders and conditions that share some traits with the ASD animal model. Following that, three articles from Google Scholar, nine articles from

MEDLINE/PubMed, and one article from the references of reviewed papers were obtained while none of the articles from web of science and EMBASE were selected. 12 papers in all were picked for analysis in the end (Figure 1). The systematic review includes 4, 3, 2, 1, 1, and 1 studies that were conducted in the United Arab Emirates, United States of America, China, Egypt, Ireland, and Italy, respectively.

Several therapeutic interventions were identified in this review with a positive impact on the components of the endocannabinoid system and the cholinergic system. E100 was administered to autistic models of VPA-C57BL/6 and BTBR. The E100 which is a known H3 receptor antagonist and AChE inhibitor, down-regulated acetylcholinesterase in the hippocampus of VPA-C57BL/6 and BTBR autistic models and up-regulated anandamide, with no significant difference in the level of 2 arachidonyl glycerol when compared to the untreated VPA-C57BL/6 and BTBR autistic models.[25,26] The impact of E100 on the various components of the endocannabinoid and cholinergic systems reversed the various phenotypes associated with the core ASD symptoms.[25,26] The E100 enhanced sociability and social novelty index, reduced the number of buried marbles, increased time spent and the number of entries in the open arm, and decreased the percentage escalation of shredded nestlets. [25,26] Cannabidivarin, JZLI84, and environmental enrichment which are known to directly or indirectly modulate CB1 receptors were identified to down-regulate CB1 receptors with a positive impact on social activities, cognition, and repetitive behaviors.[27,28,29] JZLI84 down-regulated CB2 receptors in the hippocampus and prefrontal cortex with decreased escape latency and time of platform crosses, increased sociability and novel preference index, decreased number of buried marbles, and decreased grooming time.[28] Acetaminophen, URB59, and PF3845 are

known as either direct or indirect modulators of CB1 receptors together with E100 increased the level of anandamide in specific regions of the brain,[25,26,30,31,32] with acetaminophen and URB59 enhancing social activities.[30,31] MJN110 which is known to inhibit MAGL, increased the level of 2-AG in the prefrontal cortex with a significant decrease in the time spent in the open arm, a significant decrease in the number of entries, and a significant decrease in the time spent in the inner zone.[32] URB59, JZL184, and Cannabidivarin down-regulated most of the enzymatic components of the ECS, enhancing social activities, cognition, and repetitive behaviors.[27,28,31] While URB59, JZL184, and Cannabidivarin downregulated FAAH, and JZL184, Cannabidivarin downregulated MAGL.[27,28,31]

E100 and ST-2223 which are H3 receptor antagonist together with curcumin, which are allosteric modulator of α7-nAChRs, canagliflozin, which are sodium-glucose co-transporter type 2 (SGLT2) inhibitor, and duloxetine which belong to the class of serotonin and norepinephrine reuptake inhibitors (SNRIs), positively impacted various components of the cholinergic system with enhancement of social activities, anxiety, locomotion, and repetitive activities.[25,26,33,34,35,36] Curcumin enhanced α7-nAChR in the hippocampus,[33] while ST-2223 and canagliflozin increased the level of ACh in the autistic animal models.[34,36] The level of AChE was decreased in different autistic models by duloxetine and E100.[25,26,35]

The expression of CB1 receptors in the prefrontal cortex presents no significant difference in the autistic animal models when treated with acetaminophen and JZL184.[28,30] Apparently, JZL184 enhanced cognition, social activities, and repetitive behaviors, while acetaminophen enhanced only social activities.[28,30] 2-AG showed no significant

difference in the forebrain, cerebellum, and prefrontal cortex of autistic animal models when treated with URB59, E100, and PF3845, respectively.[25,26,31,32] While social activities were enhanced in the treatment with URB59 and E100,[25,26,31] decreased repetitive behaviors and anxiety activities were recorded in the treatment of the autistic animal models with E100.[25,26] NAPE-PLD in the VPA-autistic animal model showed no significant difference in the hippocampus and prefrontal cortex when treated with JZL184,[28] while it also showed no significant difference in the hippocampus when treated with cannabidivarin.[27] FAAH and DAGLα in the VPA-autistic animal models showed no significant difference in prefrontal cortex and hippocampus, specifically, when treated with JZL184 and cannabidivarin.[27,28] The treatment of VPA-autistic animal models with JZL184 and cannabidivarin enhanced cognition, social activities, and repetitive behaviors. [27,28]

After acetaminophen (APAP) is broken down into p-aminophenol, it readily passes through the blood-brain barrier and is changed into AM404 by FAAH, which increases the release of anandamide.[37] The CB1R are modulated by anandamide, environmental enrichment, and cannabidivarin.[37-40] The enzyme fatty acid amide hydrolase (FAAH), which increases the release of anandamide and modifies CB1R, is effectively and irreversibly inhibited by PF-3845 and URB59.[41,42] MJN10 and JZL184 inhibit monoacylglycerol lipase (MAGL), which in turn modifies CB1R by increasing the production of 2-arachidonoylglycerol (2-AG). 43-45 ST-2223 inhibits H_3 receptors, which in turn inhibits dopamine receptors by increasing histamine levels.[36] E100 functions as both an AChE inhibitor and an H_3R antagonist.[25,26] Curcumin modulates α7-nAChR allosterically,[46,47] canagliflozin inhibits sodium-glucose co-transporter type 2 (SGLT2);[48] and duloxetine prevents serotonin and norepinephrine (SNR) from being reabsorbed.[49]

The activities of ST-2223, E100, canagliflozin, and curcumin ultimately enhance ACh release in figure 2.

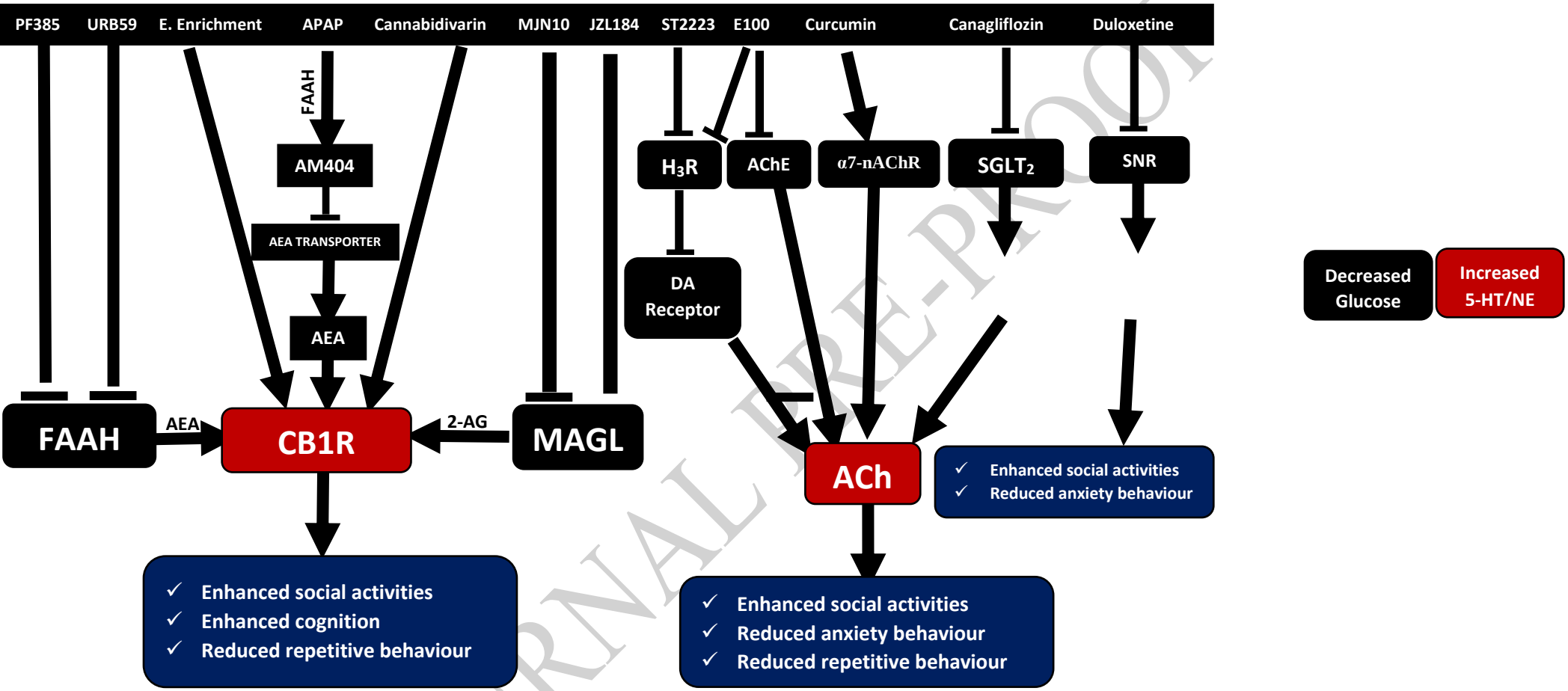

Figure 2 – The mechanism of actions of the studied therapeutic interventions and their phenotypic effects

Table 1 - Studies selected for systematic review of scientific investigation targeting cholinergic and endocannabinoid system as a therapeutic intervention for core autism associated phenotypes

Therapeutic Intervention	ASD Model	Age/ Stage of the Animal Treatment	Brain Regions	Signaling System	Therapeutic effect on ASD model signaling system compared to untreated ASD model	Therapeutic effect on ASD model behaviours compared to untreated ASD model
Acetaminophen (100 mg/kg)	BTBR	Adult	Prefrontal Cortex (PFC)	CB1R	Non-significant difference	Enhanced social interaction and non-significant difference in the number of buried marbles
				AEA	Up regulated	
URB59 (1 mg/kg)	BTBR	Young adult	Forebrain	Anandamide	Up regulated	Enhanced social approach and non-significant difference in the time spent and the number of entries in open arm
				2-AG	Non-significant difference	
				FAAH	Down regulated	
ST-2223 (5 mg/kg)	BTBR	Adult	PFC, striatum, & hippocampus	ACh	Up regulated	Enhanced social approach
Curcumin (1 µM)	BTBR	Adult	CA1 region of the Hippocampus	a7-nACh Receptors	Potentiated a7-nACh Receptors	Enhanced sociability and social preference index
Duloxetine (4.5-6 dpf)	VPA-zebrafish	Juvenile-adult	Whole Brain	AChE	Down regulated	Enhancement of social activity and reduced anxity behaviour
Canagliflozin (10 mg/kg)	VPA-Sprague–Dawley	Infant	Cerebrum, and Cerebellum	ACh	Up regulated	Enhancement of social interaction and reduced anxity behaviour
E100 (10 mg/kg)	VPA-C57BL/6	Juvenile	Cerebellum	AChE	Down regulation	Enhancement of social interaction and reduced number of buried marbles and anxiety behaviour
E100 (5 mg/kg)	BTBR	Adult	Cerebellum	AChE	Down regulation	Enhanced social interaction, reduced number of buried marbles, reduced percentage escalation of shredded nestlet and reduced anxiety behaviour
				Anandamide	Up regulation	
				2-AG	Non-significant difference	
Cannabidivarin (20mg/kg)	VPA-Sprague Dawley rats	Juvenile-adult	Hippocampus	CB1R	Down regulated	Enhanced sociability, and social preference. Enhanced short-term recognition memory and decreased grooming time
				CB2R	Up regulated	
				FAAH	Down regulated	
				MAGL	Down regulated	
				NAPE- PLD	Non-significant difference	
				DAGLα	Non-significant difference	
Environmental enrichment (1 h a day for 20 days)	BTBR	Adult	Cerebellum	CB1R	Down regulated	-
PF3845 (10 mg/kg)	VPA-Sprague–Dawley rats	Juvenile	Prefrontal Cortex	Anandamide	Up regulated	Non-significant difference in the time spent in the open arm and Non-significant difference in the number of entries and the time spent in inner Zone of open field test
				2-AG	Non-significant difference	
MJN110 (5 mg/kg)	VPA-Sprague–Dawley	Juvenile	Prefrontal Cortex	Anandamide	Non-significant difference	Significant decrease in the time spent in the open arm & significant decrease in the number of entries and the time spent in inner Zone
				2-AG	Up regulated	
JZL184 (10mg/kg)	VPA-Wistar rats	Juvenile	Hippocampus	CB1R	Down regulated	Decreased escape latency, and time of platform crosses in Morris water maze test. Increased sociability & preferential index in Social interaction. Decreased number of buried marbles in Marble burying test. Decreased grooming time in Self- grooming test.
				CB2R	Down regulated	
				NAPE- PLD	No significant difference	
				FAAH	Down regulated	
				DAGL	Down regulated	
				MAGL	Up regulated	
			Prefrontal Cortex	CB1R	No significant difference	
				CB2R	Down regulated	
				NAPE- PLD	No significant difference	
				FAAH	No significant difference	
				DAGL	Down regulated	
				MAGL	Down regulated	

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S/N	Title, authors and year
1	Acetaminophen differentially enhances social behavior and cortical cannabinoid levels in inbred mice Gould et al., 2012[30]
2	Enhancement of Anandamide-Mediated Endocannabinoid Signaling Corrects Autism-Related Social Impairment. Cannabis Cannabinoid Res Wei et al., 2016[31]
3	Experimental Studies Indicate That ST-2223, the Antagonist of Histamine H3 and Dopamine D2/D3 Receptors, Restores Social Deficits and Neurotransmission Dysregulation in Mouse Model of Autism. Eissa et al., 2022[16]
4	Curcumin Potentiates α7 Nicotinic Acetylcholine Receptors and Alleviates Autistic-Like Social Deficits and Brain Oxidative Stress Status in Mice. Jayaprakash et al., 2021[33]
5	Duloxetine ameliorates valproic acid-induced hyperactivity, anxiety-like behavior, and social interaction deficits in zebrafish Joseph et al., 2022[35]
6	Canagliflozin alleviates valproic acid-induced autism in rat pups: Role of PTEN/PDK/PPAR-γ signaling pathways Elgamal et al., 2023[34]
7	The Dual-Active Histamine H3 Receptor Antagonist and Acetylcholine Esterase Inhibitor E100 Alleviates Autistic-Like Behaviors and Oxidative Stress in Valproic Acid Induced Autism in Mice Eissa et al., 2020a[25]
8	Simultaneous Blockade of Histamine H3 Receptors and Inhibition of Acetylcholine Esterase Alleviate Autistic-Like Behaviors in BTBR T+ tf/J Mouse Model of Autism Eissa et al., 2020b[26]
9	Cannabidivarin Treatment Ameliorates Autism-Like Behaviors and Restores Hippocampal Endocannabinoid System and Glia Alterations Induced by Prenatal Valproic Acid Exposure in Rats. Zamberletti et al., 2019[27]
10	Effects of environmental enrichment and sexual dimorphism on the expression of cerebellar receptors in C57BL/6 and BTBR + Itpr3tf/J mice Monje-Reyna et al., 2022[29]
11	Increasing Endocannabinoid Tone Alters Anxiety-Like and Stress Coping Behaviour in Female Rats Prenatally Exposed to Valproic Acid Thornton et al., 2021[32]
12	Alterations of the endocannabinoid system and its therapeutic potential in autism spectrum disorder Zou et al., 2021[28]

When it comes to the findings in this systematic review, research that assessed how different therapeutic interventions affected components of the endocannabinoid and cholinergic systems in models of autism was our major focus. The behavioral activities associated with the three core symptoms of ASD were also highlighted to ascertain the phenotypic impact of the therapeutic interventions (Table 1).

Discussion

The etiology of autism is complex and involves a variety of factors, such as genetic, epigenetic, environmental, and immunological contributors and has a heterogeneous nature.[1,4] Abnormal changes in molecular signaling pathways, neuronal synapses, the immune environment, and brain functional connections are the ultimate manifestations of autism.[50] An enormous regulatory network in the body maintains homeostasis and physiology through neuronal signals originating from the endocannabinoid and cholinergic systems.[5-7] ASD has been linked to dysregulated cholinergic and endocannabinoid systems. In addition to playing a significant role in various events in brain development, the cholinergic and endocannabinoid systems are vital to a number of physiological processes and neuroadaptive responses, such as movement control, learning and memory, nociception, reward, and endocrine function.[8,9]

Therapeutic Regulation of the Endocannabinoid System

Recent evidence from research on humans and animals refers to the EC system's role in the etiology of ASD. Patients with ASD have been found to have a reduced level of ECs

in their bloodstream as well as altered EC receptors and enzymes.[5,51,52] Human evidence showing changes in many ECS components in the brains of hereditary and environmental models of autism is supported by animal studies.[53-56] It has been observed that pharmacological manipulation of EC signaling can improve certain animal phenotypes associated with ASD.[31,57-59] This suggests that targeting the endocannabinoid system may be advantageous in mitigating the symptoms of ASD. In line with literature data, we found that cannabidivarin, environmental enrichment, and JZL184 reversed the excessive up-regulated CB1 receptors in the autistic animal models with a positive impact on the autistic behaviors.[27,28,29] Acetaminophen, URB59, E100, PF3845, and MJN110 increased the level of endocannabinoid (anandamide) and ameliorate the associated autistic behaviors except PF3845, which has no significant difference in the assessed behavioral activities.[25,26,30,31,32] Cannabidivarin, JZL184, and URB59 decreased the level of hydrolytic enzyme (FAAH), which is the enzyme responsible for the hydrolysis of anandamide, while Cannabidivarin and JZL184 downregulated MAGL, which is a serine hydrolase that plays a crucial role in catalyzing the hydrolysis of monoglyceride 2-arachidonoylglycerol (2-AG) into glycerol and fatty acids.[27,28,31] The downregulation of the hydrolytic enzymes of endocannabinoids by cannabidivarin, JZL184, and URB59 enhanced cognition, social activities, and repetitive behaviors.[27,28,31] The reversal of the control level of the components of the endocannabinoid system identified in the reviewed literature indicates that the endocannabinoid system is a strong therapeutic target for ameliorating core ASD phenotypes in clinical trials.

The results from this review indicate that pharmacological modulators of the endocannabinoid system may offer therapeutic potential in ASD. The results of down-

regulating CB1 receptors, increased degradation of EC hydrolytic enzymes, and compensatory upregulation of EC signaling molecules corroborated the reversal of ASD-associated phenotypes to the control level. The behavioral findings in the ECS were characterized by reduced repetitive and stereotypical behaviors in the marble burying and self-grooming tests, reduced hyperactivity in the open field test, increased sociability and social preference in the three-chamber test, enhanced short-term recognition memory in the novel object recognition test, and improved cognitive functioning in the Morris water maze test. The review of research papers that assessed EC components was important to encourage the identification of potential targets for improved therapeutic treatments in ASD.

Therapeutic Regulation of the Cholinergic System

Clinical investigations have indicated that abnormalities in brain cholinergic neurotransmission may be a major factor in the behavioral aspects associated with ASD.[60] As a result, this review focused on how novel multiple-active test substances (curcumin, ST-2223, canagliflozin, duloxetine, and E100) modulate the cholinergic system's brain components in ASD behavioral symptoms that are shown in both genetic and environmental models of autism.

In the animal model of ASD, a reduction in ACh leads to significant changes in grooming and rearing patterns of behavior and duration, [61-63] a rise in repetitive-stereotyped movements over time, [61,64] social deficits, and an increase in anxiety-like behaviors. Downregulation of ACh, believed to be a neurotransmitter involved in neuronal

development in the brain,[65] has been linked to behavioral alterations in autistic patients.[66] In both human and animal ASD research, there was an increase in the expression of the AChE protein.[24,67] According to Kim *et al*. [61], AChE expression increased in cultures that received VPA treatment. Research by Friedman *et al*. [67] recorded that there were changes in the level of choline-containing compounds in many brain regions of ASD patients.

In line with the papers reviewed in this study, curcumin potentiates α7-nACh receptors, ST-2223, and canagliflozin increases the level of ACh, which ultimately alters the ASD-associated phenotypes by enhancing social activities.[33,34,36,] Specifically for the VPA-autistic Sprague- Drawley rat model treated with canagliflozin, reduced grooming and rearing, reduced time spent in the close arm, and increased time spent in the open arm were observed in the elevated plus maze test, while reduced locomotion and grooming and increased time spent in the central area were recorded in the open field test.[34] AChE, which is an enzyme that catalyzes the breakdown of acetylcholine, was down-regulated in various autistic models after treatment with duloxetine and E100.[25,26,35] The tests for the behaviors associated with the ASD phenotype were assessed in the autistic animal models treated with E100, and enhanced social activities, reduced number of buried marbles, increased time spent and number of entries in the open arm, and decreased percentage escalation of shredded nestlets were recorded.[25,26] Several lines of evidence suggest that endocannabinoid and nicotinic cholinergic systems are implicated in the regulation of different physiological processes,[68] including cognition, social activities, anxiety, and repetitive behaviors. A crosstalk between these two systems is substantiated

by the overlapping distribution of cannabinoid and nicotinic ACh receptors in many brain structures.[68]

The primary phenotypes linked to autism spectrum disorder are shown by the dysregulated components of the cholinergic and endocannabinoid systems.[5-7] The ability of CB1 receptors to inhibit the release of acetylcholine, which is mediated by both acetylcholine receptors, causes synaptic impairments in autism due to the abnormally excessive expression of CB1 receptors in several brain regions. The way these systems interact lends credence to the theory that in a number of neuropsychiatric disorders, one mechanism regulating synaptic activities is the control of cholinergic activity through activation of the CB1 receptor.[69] The brain's excitatory-inhibitory balance is influenced by cholinergic signaling. Long-term potentiation (LTP), which promotes a depolarization state, is induced by nAChRs that are postsynaptically or presynaptically situated, and they can increase intracellular Ca2+ release to affect synaptic plasticity.[70] It has been reported that autistic people have altered levels of nAChRs in different brain areas.[71] Reportedly, the cerebellum, parietal, and frontal cerebral cortex showed reduced expression levels of α4β2 nAChRs among individuals with ASD.[72,73,74] It was shown, however, that the granule cell layer of the cerebellum had elevated α7nAChR subunit expression, but Purkinje cells and the molecular cell layer did not show the same effect. Research by Ray et al.[75], however, found that the paraventricular nucleus (PV) and nucleus reuniens (Re) had decreased neuronal α7 and β2 nAChR IR-y, and that PV had lost its α7 neuropil IR-y. The endocannabinoid system maintains major significance among the neuromodulatory systems that regulate cholinergic neurotransmission. In postmortem human samples from autistic patients as well as in animal models of cognitive

impairment and cholinergic lesion models, alterations in endocannabinoid signaling have been reported.[76] Numerous lines of evidence indicate that the neuropathological basis of psychiatric disorders as well as the regulation of other physiological processes, including reward, are associated with the endocannabinoid and nicotinic cholinergic systems.[77] The overlapping distribution of nicotinic acetylcholine and cannabinoid receptors in many brain regions suggests an interaction between these two systems.[77] As a result, the nicotinic cholinergic and endocannabinoid systems offer a viable pharmacological target for the development of effective therapeutic interventions to treat the neuropsychiatric phenotypes linked to autism. This review outlined the impact of therapeutic modulators on targeting the systems for ameliorating the core symptoms of ASD and directing the development of therapeutic interventions with crosstalk potential between the cholinergic and endocannabinoid systems.

Conclusion

Alteration of the brain components of the cholinergic and endocannabinoid systems is significant in ASD-related behavior, with the results of this review indicating that pharmacological modulators of the cholinergic and endocannabinoid systems may offer therapeutic potential in ASD, pre-clinical trials of the combinations of some of the therapeutic interventions are advised to assess its effectiveness and safety.

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Authors' contributions

All the authors contributed to the study concept and design, the acquisition, analysis, or interpretation of data, and the drafting of intellectual input in the manuscript. All authors read and approved the final manuscript.

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