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Review Article: Autism Spectrum Disorders

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New Therapeutic Options for Autism Spectrum Disorder: Experimental Evidences

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Autism spectrum disorder (ASD) is characterized by impairment in two behavioral domains: social interaction/communication together with the presence of stereotyped behaviors and restricted interests. The heterogeneity in the phenotype among patients and the complex etiology of the disorder have long impeded the advancement of the development of successful pharmacotherapies. However, in the recent years, the integration of findings of multiple levels of research, from human genetics to mouse models, have made considerable progress towards the understanding of ASD pathophysiology, allowing the development of more effective targeted drug therapies. The present review discusses the current state of pharmacological research in ASD based on the emerging common pathophysiology signature.

Key words: Autism, ASD, Pharmacotherapy, Treatment, Social behavior, Repetitive behavior

INTRODUCTION

Autism Spectrum Disorder (ASD) includes a group of developmental disabilities characterized by impaired social interaction and communication and the presence of repetitive and stereotyped behaviors as well as restricted interests [1]. In addition to these core symptoms, individuals with ASD often show a variety of additional impairments such as intellectual disability, epilepsy, motor deficits, hyperactivity, aggression, mood disorder, and sleep, sensory and gastrointestinal abnormalities [2]. Due to a lack of biological markers, the clinical diagnosis is solely based on behavioral observation and, being behavior a continuous domain, the final clinical phenotype among ASD patients can vary significantly. Although first thought to be mainly environmental and attributed to parental habits, today we know that ASD is largely genetic, however so far no major causative gene has been identified; rather studies have identified hundreds of risk genes, with either rare variants that are highly penetrant or common variants with small effect [3]. With this extraordinary genetic heterogeneity it is not surprising that no characteristic neuropathology has been conclusively identified for the disorder. However, the "many genes common pathways" hypothesis suggests that, although through different specific molecular mechanisms, the many genes associated with ASD will converge in their effect on the development and function of neural circuits involved in social cognition and language, core behaviors of autism [4]. The identification of these common neurobiological pathways will aid in the development of targeted therapies, which are currently absent in ASD. With an estimated prevalence of 1 in 68 individuals [5] the development of targeted effective drugs becomes a critical health issue. Despite the absence of targeted treatments, it is believed that as many as 75% of patients with ASD receive some kind of pharmacological treatment, which are mainly directed to treat non-core associated symptoms such as hyperactivity,

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irritability, aggression and self-injury [6]. Since there are no specific biological targets, drug prescription in ASD has been limited to testing compounds known to alleviate certain symptoms based on their approved use for other disorders, without necessarily understanding their neurobiological effect. As the underlying neurobiology of ASD is being discovered, targeted more efficient drug therapy is becoming possible. A comprehensive review of the emerging common neuropathology associated with ASD has been recently published [7]. The present review examines progress made in translation of the emerging signature of the neurobiology of ASD into more effective targeted therapies for the disorder.

NON-TARGETED TREATMENTS

In ASD, the co-occurrence of associated medical comorbidities is often the most preoccupying issue for families since it greatly affects their quality of life and makes behavioral interventions directed towards core social symptoms challenging. Among associated symptoms, aggression related behaviors (aggression, self injury, irritability) and hyperactivity/inattention are the most common and the ones receiving the most pharmacological attention (Table 1). In fact, in some cases improvements in social interaction have been observed as a secondary effect of an overall reduction in maladaptive behaviors and not a primary therapeutic effect of these medications. Also, it should be noted that, as there are no biomarkers for the disorder, improvement is based on evaluation of behavior by either a clinician or caregiver documenting severity and/or frequency of behavioral disturbances, being unbiased assessment sometimes challenging.

To date, the only drugs that are approved by the United States Food and Drug Administration (FDA) to treat symptoms in patients with autism are risperidone (approved in 2006) and aripiprazole (approved in 2009), both atypical antipsychotics used to treat irritability, hyperactivity and aggression. Although conventional (typical) antipsychotics, such as haloperidol, a potent dopamine antagonist, were first used to treat disruptive behaviors in patients with autism, severe side effects (dyskinesia and dystonia) were reported with long term treatments making them unsuitable in most cases [8]. Still, short term treatment with typical antipsychotics is commonly used in cases of severe aggressive bouts. The development of second generation (atypical) antipsychotic drugs allowed for a treatment of aggressive behavior with fewer and milder side effects. Most atypical antipsychotics act on the serotonin and dopamine systems and, in addition, have affinity for a wide range of other receptors including adrenergic, histaminergic and cholinergic [9]. This broad targeting of receptor systems is likely the cause of the variability in their efficiency and adverse effects associated to these drugs. Risperidone is the most widely used and has been shown to improve symptoms of irritability, aggression, hyperactivity, self-injury and stereotipies, although common side effects include weight gain, drowsiness and sedation, drooling, tremor, and dizziness [10, 11]. Aripripazole is a newer atypical antipsychotic shown to improve irritability in patients with autism with usually milder side effects that involve weight gain, fatigue and somnolence, gastrointestinal symptoms and motor restlessness [12]. Other atypical antipsychotics such as clozapine and olanzapine have been used with limited benefits.

Stimulants commonly used to treat Attention Deficit and Hiperactivity Disorder (ADHD) have long been used to treat inattention, impulsivity and hyperactivity in patients with ASD.

Table 1. Most common pharmacological treatments in ASD

Class	Action	Drug	Targeted symptoms
Antipsychotics	Typical*	Haloperidol	Aggression/irritability
	Atypical**	Risperidone	Aggression/irritability
		Aripripazole	Aggression/irritability
Stimulants	DRI	Methylphenidate	Hyperactivity/inattention
Antihypertensive	α2AR agonists	Guanfacine	Hyperactivity/inattention
		Clonidine	Hyperactivity/inattention
Antidepressants	NRI	Atomoxetine	Hyperactivity/inattention
	SSRI	Fluoxetine	Repetitive behavior/impulsivity
		Citalopram	Repetitive behavior/impulsivity
CNS depressant	MT receptors	Melatonin	Sleep problems

*Typical: first generation antipsychotics, mainly act as dopamine D2 receptor antagonist.
**Atypical: second generation antipsychotics, mainly act as dopamine D2 and serotonin 2A receptors antagonists.
Abbreviations: DRI: dopamine reuptake inhibitor, SSRI: selective serotonin reuptake inhibitor, NRI: norepinephrine reuptake inhibitor, α2AR: Adrenergic alpha-2 receptor agonists, MT: melatonin.

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According to the fifth edition of the American Psychiatric Association's Diagnostic and Statistical Manual (DMS-V), ASD and ADHD are no longer mutually exclusive [1], therefore patients with autism no longer need to be treated off-label for ADHD symptoms. The psychostimulant methylphenidate is currently the most commonly used and has been shown to improve hyperactivity and inattention [13]. Other ADHD approved drugs such as the antidepressant atomoxetine, a norepinephrine reuptake inhibitor [14], and the antihypertensives guanfacine and clonidine, both α-2A adrenergic receptor agonists, have also shown moderate improvements in hyperactivity and inattention [15].

Other antidepressants, mostly selective serotonin reuptake inhibitors (SSRI), are widely used in the treatment of maladaptive behaviors in ASD. The most commonly used are fluoxetine and citalopram [16]. Initial evidence that they improved repetitive behaviors and compulsivity generated the belief that these behaviors in ASD were similar to the ones observed in Obsessive Compulsive Disorder (OCD), since SSRIs are the standard treatment in OCD. However, consequent trials have shown a low level of effectiveness [17] and the presence of undesired side effects such as hyperactivity, agitation, insomnia, and aggression have been reported, raising questions about their suitability in the treatment of repetitive behaviors in ASD.

Sleep problems are reported in up to 55% of children with ASD [2], and it's usually associated with worsening of behavioral symptoms. Melatonin related drugs are one of the most commonly used pharmacotherapies to aid in sleep function, ameliorating some of the associated behavioral problems in these patients [18].

In addition to the above described drugs, many other compounds such as anticonvulsants, cholinesterase inhibitors, opioid antagonists and others have been tested in patients with ASD with limited or low evidence of their benefits.

ASD NEUROBIOLOGY: IN SEARCH FOR TARGETED TREATMENTS

The integration of findings obtained through multiple levels of research, from human genetic studies to transcriptomic and neuropathological analyses of postmortem brain, has translated into considerable progress towards the understanding of ASD pathophysiology [7]. In addition, animal models based on human genetic findings have been key in the understanding of gene function as well as in the development and evaluation of the effectiveness of pharmacological treatments (Fig. 1). The traditional concept that abnormalities occurring during brain development are permanent and thus neurodevelopmental disorders irreversible, has been challenged in the past few years by studies on mouse models of neurodevelopmental disorders including Rett syndrome [19], fragile X syndrome [20], neurofibromatosis type 1 [21], Down's syndrome [22], and tuberous sclerosis [23] when showing that brain activity and ultimately the associated cognitive and behavioral deficits can be restored in the mature brain. This has also been shown to be true for autism, where an increasing number of studies in mouse models have shown that certain behavioral and molecular defects can be reversed in the mature mouse brain, paving the way for clinical trials in human patients. Thus, if a dysfunction in a neurochemical signaling pathway is identified, targeted pharmacological therapies aiming to restore or compensate these imbalances could be effective. A summary of the published clinical trials for targeted treatments in ASD can be found in Table 2. Experimental findings in mouse models are currently leading drug development towards the following emerging common affected neurobiological mechanisms in ASD: synaptic transmission and serotonin and oxytocin signaling.

SYNAPTIC TRANSMISSION

A large number of the genes identified as associated with ASD encode proteins involved in synaptic transmission [24]. Dendritic spines are the sites of most glutamatergic excitatory neurotransmission and their development, maturation and plasticity are critical for correct synapse function [25]. Alterations

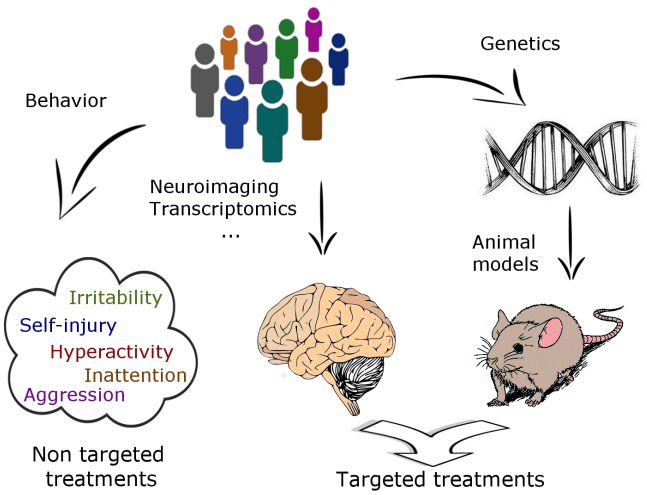

Fig. 1. Schematic representation of the strategies used to guide ASD treatment. Non-targeted treatments are directed towards treating symptomatology rather than underlying neurobiology and allow for management of associated maladaptive behaviors (left). On the other hand, the integration of multiple research approaches, from human studies to animal models based on human genetic findings converge in a deeper understanding of ASD pathophysiology guiding the development of novel more focused targeted treatments.

Table 2. Clinical trials of targeted pharmacological treatments in ASD

Class	Drug	Action	Results	Ref.
Growth factor	IGF-1	IGF1 receptor	Improved social behavior	[29]
			Improved repetitive behavior	[29]
Small molecule	Memantine	NMDAR antagonist	Improved language	[34, 35]
			Improved social behavior	[34, 35]
			Reduced repetitive behavior	[36]*
			Reduced hyperactivity	[36]
			Reduced irritability	[36]
Antibiotic	D-cycloserine	NMDAR agonist	Improved social behavior	[40, 41]
			Reduced repetitive behavior	[42]
Small molecule	Arbaclofen	GABA(B)R agonist	Improved social behavior	[54, 55]
			Reduced irritability	[55]
Hormone	Oxytocin	OXT receptor	Improved social behavior	[70-74]

*When administered with risperidone as adjunctive therapy comparing with risperidone-only treatment.

in dendritic spines, including spine density, morphology and/or dynamics have been identified in postmortem studies of ASD patients, as well as in studies of animal models of autism [26]. Accordingly, drugs that enhance spine maturation represent a possible therapeutic option. One such medication is the insulin-like growth factor 1 (IGF-1), which regulates synapse formation. IGF-1 has been shown to promote the formation of mature excitatory synapses in neurons generated from induced pluripotent stem cells from patients with Phelan-McDermid syndrome (PMS), a complex neurodevelopmental disorder associated with ASD [27], as well as to reverse several phenotypes in the mouse model of the syndrome, deficient for Shank3 [28]. A pilot study of IGF-1 treatment in children with PMS recently showed significant improvement in social impairment and restrictive behaviors with no serious adverse effects reported [29]. The fact that synaptic transmission might represent a common pathophysiological pathway in ASD has led to the use of glutamatergic and GABAergic agents in preclinical models, with reasonable success. The most common genetic cause of autism, accounting for about 1% of cases, is Fragile X syndrome (FXS). FXS is caused by the absence of the protein encoded by the FMR1 gene (FMRP), an mRNA binding protein involved in protein synthesis through translational repression [30]. The mouse model of FXS, knockout for the Fmr1 gene, shows increased density in dendritic spines and altered spine morphology [31]. In addition, Fmr1-knockout mice show enhanced signaling through group I metabotropic glutamate receptor type 5 (mGluR5) [32]. Decreasing mGluR5 activity in this mouse model, by crossing it with mutant mice for mGluR5, rescued protein synthesis, dendritic spine alterations, and multiple behavioral phenotypes [20]. Since mGluR5 and the ionotropic glutamate receptor NMDA show a positive reciprocal regulation, where activation of either one of them potentiates the response mediated by the other one and, in a similar way, antagonism of either one of them indirectly inhibits the function of the other [33], modulators of both types of receptors constitute a promising pharmacotherapy in ASD. Memantine is an NMDA receptor antagonist approved by the FDA for use in other neurological disorders such as Alzheimer's disease. Interestingly, memantine treatment in cultured cerebellar granule cells from Fmr1 knockout mice rescued dendritic spine density and maturation and restored the excitatory synapses to a normal range [34]. Several studies have investigated its effect in patients with autism with significant improvements reported in language and social behaviors [35, 36] as well as with a reduction in repetitive behaviors, hyperactivity and irritability when administered together with risperidone as adjunctive therapy [37]. Interestingly, Chung and collaborators [38] have recently shown that either NMDA antagonism through memantine or mGluR5 antagonism through MPEP rescues social deficits as well as NMDA hyperactivity shown in IRSp53 knockout mice, a gene linked to ASD in humans. Conversely, mice knockout for another autism susceptibility gene, Shank2, show decreased NMDA receptor function and treatment with either the NMDA agonist D-cycloserine or an mGluR5 positive allosteric modulator restores NMDA activity and social behavior [39]. In fact, a small pilot study in patients with autism has recently shown an improvement in social and repetitive behaviors after treatment with D-cycloserine [40-42].

As opposed to the mouse model of FXS, the mouse model of another syndromic form of ASD, Tuberous Sclerosis (TSC), presents with downregulation of mGluR5 signaling and, consequently, synaptic and cognitive defects in these mutants are corrected by treatments that modulate mGluR5 in opposite

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directions, or interestingly, when mice are bred to carry both mutations [43]. Therefore, the use of mGluR5 agonists might be suitable in some forms of ASD. In addition, the TSC genes (TSC1 and TSC2) are upstream of the mechanistic target of rapamycin (mTOR) pathway, which is critical for protein synthesis. Protein synthesis within synaptic spines is necessary for neuronal plasticity and is required for proper cognitive function. Several genes upstream the mTOR pathway have been associated with ASD, including TSC1/TSC2, PTEN and NF1, and mutations in these genes cause hyperactivity of the mTOR pathway. Accordingly, mTOR inhibitors, such as rapamycin, have been tested for their effectiveness in alleviating behavioral symptoms in ASD, successfully improving behavioral deficits in mouse models of TSC and PTEN [44]. Currently, two mTOR inhibitors are approved by the FDA for TSC treatment, everolimus and vigabatrin, although their effects in ASD remain to be elucidated [45]. Even though the possibility of adverse events occurring in patients receiving such treatments needs to be carefully assessed, modulation of mTOR is considered a promising target for drug development in ASD and clinical trials are underway.

In addition to an altered glutamatergic synaptic transmission, dysfunction in the GABAergic system is considered an emerging signature of ASD [46]. As a consequence, an altered balance between excitatory and inhibitory neurotransmission (E/I imbalance) has been proposed to contribute to the pathogenesis of the syndrome [47]. In mice, elevation, but not reduction of cellular E/I balance within the medial prefrontal cortex was found to elicit impairments in social behavior, and compensatory elevation of inhibitory cell excitability partially rescued social deficits [48]. Several genetic mouse models of ASD show a reduction in the number of cortical GABAergic interneurons, especially the parvalbumin subtype, including Fmr1 [49], Cntnap2 [50], Cadps2 [51] and En2 [52]. Thus, increasing GABAergic signaling might improve behavioral outcome by compensating a potentially excessive glutamatergic neurotransmission. As in the case of glutamate, both ionotropic and metabotropic GABA receptor subtypes are of interest as targets of therapeutic agents. The metabotropic GABA(B) receptor agonist arbaclofen, showed promising results in a preclinical study of FXS [53] and clinical trials in humans with FXS reported improvement in social function [54, 55]. Modulators of the ionotropic GABA(A) receptor, such as the positive allosteric modulator clonazepam, have also proven to ameliorate symptoms in pre-clinical models of neurodevelopmental disorders associated with autism [56, 57]. Further research is needed to determine the safety and efficacy of these drugs in humans.

SEROTONERGIC SYSTEM

Alterations in the functioning of the serotonin system were among the first biochemical changes found in individuals with ASD, with increased serotonin levels found in whole blood in up to 45% of patients [58] together with decreased serotonin receptor 5HT2A binding in hyperserotonemic individuals [59]. Variants in genes involved in the serotonin system, such as the serotonin transporter (SLC6A4) [60] and the monoamine oxidase A gene (MAOA) [61], involved in the degradation of serotonin, have been proposed as linked to ASD in humans. In support of this, mice with mutations in these genes present with abnormal serotonergic transmission and social deficits [62, 63]. The serotonin system is involved in many neurobiological processes, including brain development; therefore it is plausible that defects in this system would affect circuits important for ASD related behavior. Conversely, the most common serotonin related drugs used in ASD are SSRIs and, as described above, their effectiveness is not clear in most cases. It is possible that the variability in the response to SSRIs is due to a dysfunction of the serotonin system at different levels (receptor, transport, processing etc) [64] and thus, there is a potential for developing drugs affecting the serotonin system at more specific levels.

OXYTOCIN

Perhaps the molecule that has received the most attention as a potential treatment for social deficits in ASD is the neuropeptide oxytocin (OXT). OXT is produced in the hypothalamus and is involved in the modulation of a broad range of social behaviors in mammals including maternal behavior, mother-infant bonding, pair bonding and social memory and recognition [65]. Animal studies have shown that in mice, OXT is required for the rewarding properties of social stimuli [66] and, accordingly, mice with a compromised OXT system, knockout for either the OXT gene, the OXT receptor gene, or a gene involved in OXT release (CD38), all show social deficits [67]. In humans, it has recently been shown that OXT plasma concentration and polymorphisms in the OXT receptor gene drive individual differences in social cognition in both ASD and normal populations [68]. Also, genetic variation in CD38 has recently been associated with a differential response in social eye cues in infants [69]. Therefore, variation in the OXT system seems to contribute to social phenotypes in humans, regardless of medical diagnosis. It is not surprising that a number of clinical trials have recently been conducted to test the efficacy of OXT treatment in the social domain of patients with autism, with very promising results [70-74]. A recent systematic

review of the published randomized controlled trials until 2013 reports that 85% of them found statistically significant differences in outcome related to social behavior between placebo and OXT treated groups, with eye gaze and facial emotion recognition being the domains more frequently improved [75]. In addition, some of these studies provide evidence of a neurobiological basis for improvement in social behavior through restoration of brain activity in specific areas. In particular, Aoki et al. [70] have recently shown that a single dose of intranasal OXT mitigated autistic behavioral deficits through the restoration of activity in the ventromedial prefrontal cortex, as demonstrated with functional magnetic resonance imaging (fMRI) during a socio-communication task. In addition, Watanabe et al. [71] have reported that a 6-week intranasal administration of OXT significantly improved social reciprocity in patients with ASD and fMRI showed that this improvement was accompanied by an OXT induced enhancement of resting-state functional connectivity between anterior cingulate cortex and dorso-medial prefrontal cortex. Therefore, there is a great interest in the investigation of OXT as a potential target for therapeutic treatment in ASD. A key issue is identifying which forms of ASD would benefit the most from OXT treatment, since detrimental effects could potentially occur by an overstimulation of this signaling pathway. There is evidence that some forms of ASD could potentially have an associated dysfunction in the OXT system. Genetic variation in the OXT receptor gene and CD38 has been linked to the disorder in some studies [76, 77]. Also, lower levels of peripheral OXT in patients with ASD have been found in some cohorts [78, 79], although higher levels have also been reported in other studies [80, 81]. In addition, Green and collaborators [82] found lower levels of the peptide but higher levels of its precursor, which suggests a dysfunction in its processing. This heterogeneity likely indicates dysfunction in the OXT system at different levels (i.e. synthesis, processing, storage, and release), adding to the challenge of developing a clinically useful biomarker. Research in genetic animal models are uncovering a potential dysfunction in the OXT system in some genetic forms of ASD, other than the directly related to the OXT system, which suggests that this could be a more extensive deficit than originally thought. For instance, the Fragile X mouse model, Fmr1 knockout, has been reported to show lower OXT immunoreactivity in the hypothalamus [83]. Interestingly, recent studies have reported improvement of social cognition in patients with Fragile X treated with intranasal OXT, and OXT is being considered as a potential treatment for social anxiety in this syndrome [84]. The Cntnap2 knockout mouse model of ASD, a gene involved in a syndromic form of ASD called Cortical Dysplasia and Focal Epilepsy syndrome [85] also shows lower OXT levels in brain, and both exogenous administration and activation of endogenous OXT release restored social behavior in this model [86]. Therefore, investigating potential impairments in the OXT system in other forms of ASD would be worthwhile. Animal models are also critical in helping understand the mechanism whereby OXT ejects its effects. OXT has been found to be involved in the perinatal excitatory to inhibitory shift of GABA during fetal and early postnatal periods [87], a process that is disrupted in the Fmr1 mouse model [88]. In the adult brain, OXT stimulates fast-spiking parvalbumin interneuron activity [89], therefore OXT could potentially compensate for GABAergic deficits found in these mouse models. Although research into the potential therapeutic application of OXT is still in the early stages, the OXT system is one of the most promising targets for improving social function. Possibly, analysis of OXT peripheral levels and OXT receptor sequence could become, to some degree, useful biomarkers to identify the most responsive individuals. In addition, OXT is known to interact with other neurotransmitter systems [67], a deeper understanding of the circuits involved will help develop therapeutic approaches based on manipulating this system.

In summary, despite the high heterogeneity in both the phenotype and the etiology of ASD, the integration of findings in recent studies at multiple levels of research have allowed a deeper understanding of ASD pathophysiology, identifying convergent mechanisms and allowing the development of more effective targeted drug therapies.

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