



REVIEW

Molecular Characterisation of the Mechanism of Action of Stimulant Drugs Lisdexamfetamine and Methylphenidate on ADHD Neurobiology: A Review

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ABSTRACT

Attention-deficit/hyperactivity disorder (ADHD) is a common childhood-onset neurodevelopmental disorder characterised by persistent inattention, hyperactivity and impulsivity. Moreover, ADHD is commonly associated with other comorbid diseases (depression, anxiety, bipolar disorder, etc.). The ADHD symptomatology interferes with subject function and development. The treatment of ADHD requires a multidisciplinary approach based on a combination of non-pharmacological and pharmacological treatments with the aim of ameliorating the symptomatology;

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among first-line pharmacological treatments are stimulants [such as methylphenidate (MPH) and lisdexamfetamine dimesylate (LDX)]. In this review we explored recent ADHD- and stimulants-related literature, with the aim of compiling available descriptions of molecular pathways altered in ADHD, and molecular mechanisms of current first-line stimulants MPH and LDX. While conducting the narrative review, we applied structured search strategies covering PubMed/MEDLINE database and performed handsearching of reference lists on the results of those searches. The aetiology and pathophysiology of ADHD are incompletely understood; both genetic and environmental factors have been associated with the disorder and its grade of burden, and also the relationship between the molecular mechanisms of pharmacological treatments and their clinical implications. The lack of comprehensive understanding of the underlying molecular pathology makes both the diagnosis and treatment difficult. Few published studies evaluating molecular data on the mechanism of action (MoA) of MPH and LDX on ADHD are available and most of them are based on animal models. Further studies are necessary to improve the knowledge of ADHD pathophysiology and how the MoAs of MPH and LDX differentially modulate ADHD pathophysiology and control ADHD symptomatology.

Keywords: Attention-deficit/hyperactivity disorder; Lisdexamfetamine; Mechanism of action; Methylphenidate; Molecular characterisation; Neurotransmitter imbalance

Key Summary Points

Several lines of evidence suggest that the neurotransmitter imbalance, neuroinflammation and defective immunoregulation, circadian system dysfunction and altered neural viability and neurodegeneration are pathophysiological processes related to attention-deficit/hyperactivity disorder (ADHD) disorder and their comorbidities.

We reviewed the literature for evidence that explains how the mechanisms of action (MoAs) of methylphenidate (MPH) and lisdexamfetamine dimesylate (LDX) act on these pathophysiological processes and control ADHD symptomatology.

More evidence has been found on the effect of MPH and LDX on neurotransmitter imbalance and neural viability and neurodegeneration. Regarding their role in neuroinflammation and defective immunoregulation as well as circadian system, there are few data available.

Despite the published studies on MPH and LDX, the few published molecular data available are based on animal models. Further studies are necessary to improve the knowledge of ADHD pathophysiology and how the MoAs of the MPH and LDX differentially modulate ADHD pathophysiology and control its symptomatology.

neurodevelopmental disorder characterised by a symptomatology based on persistent inattention, hyperactivity, and impulsivity that has been considered a progressively ceaseless condition [1]. The ADHD symptomatology interferes with subject function and development according to the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) diagnostic criteria [2] impairing daily function [3, 4]. Moreover, ADHD is commonly associated with other comorbid diseases (depression, anxiety, bipolar disorder, etc.) [5], and in adult age it is a risk factor for various mental disorders [6, 7]. Therefore, ADHD is a significant burden for the affected youngsters, adults, their families and society everywhere.

The worldwide prevalence of ADHD is around 4–12% in children and 2.5–5% in adults [8–11]. It is estimated that 15–50% of children diagnosed with ADHD carry its manifestations into adulthood, but ADHD symptoms are not as easily defined in adulthood as in childhood. Generally speaking, symptoms are likely to adapt when growing into adulthood. Hyperactivity and impulsivity in adults seem to be reduced, as hyperactivity is expressed as an inner tension, and impulsivity becomes more verbal than physical, while inattentiveness can be retained [12, 13].

ADHD, as with other psychiatric disorders, is not easy to diagnose. In this sense, their diagnosis has been criticized because it is not based on a biological testing, and it has been considered subjective [14]. There is no medical test that determines the presence of the condition. However, there are some manuals that establish its diagnosis criteria, the DSM-5 and the International Classification of Diseases 11th revision (ICD-11) being the most usual ones, which observe behaviour defects and study symptoms such as hyperactivity, inattentiveness and impulsivity [15]. One of the reasons that hinder the diagnosis and treatment of this disorder is the lack of complete understanding of the underlying molecular pathology [16]. However, the main features of the diagnosis are the presentation of developmentally inappropriate levels of hyperactive-impulsive and/or inattentive symptoms, or both combined, for at least 6 months and at different settings, with possible

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a common childhood-onset

impairment of life tasks. The disorder can affect highly intelligent people, often co-occurring with other psychiatric disorders [14].

Following a primary diagnosis of ADHD in a child, adolescent or adult, clinicians have at their disposal a wide range of non-pharmacological and pharmacological treatment options [17] which are administered in combination with a multidisciplinary approach [17, 18], as recommended by current treatment guidelines [19].

Non-pharmacological therapy includes several procedures as behavioural interventions, such as training of parent behaviour and/or social skills [20–22]; cognitive training, focused in reducing ADHD symptoms by improving performance in specific neuropsychological functions using electronic interfaces (computers, tablets, smartphones) or noncomputerized methods that allow performance reassessment so that training is adaptive [23–25]; neurofeedback based on improving self-control over brain activity patterns (real-time electroencephalogram (EEG) data monitoring of, for example, theta (vigilance) and beta (concentration and neuronal excitability waves) [26–28]; or coaching programs, focused on improving executive functions [29–32].

Pharmacologic treatment is based on stimulants [amphetamines, such as the prodrug lisdexamfetamine dimesylate (LDX), and methylphenidate (MPH)] and nonstimulants (atomoxetine, guanfacine, clonidine, bupropion, modafinil) [17]. Stimulants have generally been used as first-line pharmacologic treatment owing to a higher efficacy in symptomatology reduction compared to nonstimulant medications in all groups of age (children, adolescents and adults) [17, 33, 34]. In this sense, a systematic review and network meta-analysis carried out in 2018 using 133 double-blind randomised controlled trials positioned MPH in children and adolescents, and amphetamines in adults, as preferred first-choice medications for the short-term treatment of ADHD [34]. Non-stimulants are considered as second-line medication and are administered when stimulants are contraindicated or because of lack of response or intolerance [35].

Despite stimulant medications for ADHD being among the most effective drugs in psychiatry [36], there is a substantial placebo effect in subjects with ADHD [37], which could be explained by a synergy between placebo effects that influence the parent of the patient with ADHD and those acting on the clinician when interviewing the parent. Moreover, a nocebo effect affecting patient tolerability is reported, and it is mainly translated into dropouts due to adverse events or any other reason, and weight loss [37], indicating that explanation of potential adverse events due to medication must be expressed better to minimise nocebo effects.

Although the effects of stimulant medications are similar, they show different specific mechanisms of action (MoAs). Both MPH and amphetamines act by blocking presynaptic dopamine (DA) and norepinephrine (NE) transporters, thus increasing catecholamine transmission; however, amphetamines additionally increase the presynaptic efflux of DA [38]. In the case of LDX, the exact MoA in ADHD is not fully understood [39, 40]. It is presumed that is likely related to a blockage of the reuptake of NE and DA into the presynaptic neuron and an increase in the release of these monoamines into the extraneuronal space [39]. Despite this, the molecular mechanisms of stimulant medications are not fully understood. These mechanisms, or how patients respond to these drugs, can be further complicated by their interaction with patients' genotypes; in this sense, pharmacogenomics research seeks to explain how some drugs are more effective and or better tolerated for specific genotypes in improving patient outcomes [41]. Most of the pharmacogenomic markers for psychiatric drugs compiled by regulatory agencies to date are related to drug metabolism rather than to the mechanism of action [42]. Some stimulants, such as amphetamines, and non-stimulants, such as atomoxetine, have been reported to be substrates of cytochrome P450s, and their performance has been linked to CYP2D6 genotype [41, 42]. While MPH's US Food and Drug Administration (FDA) label does not contain pharmacogenomic biomarkers, a lot of research has been performed on the impact of genotype on MPH efficacy, and several biological

pathways have been suggested to potentially affect MPH mechanisms through patient genotype, mostly concerning monoamine pathways [43–48]. Although the research in this field has been increasing in recent years, and has the potential to affect prescription and possibly improve the outcomes of patients with ADHD [49], no clear guidelines have been developed and there is still much to understand in this regard [50]. Our review will not cover this issue further, as this aspect has been extensively reviewed by other authors [41, 51–53].

The purpose of this review is to summarize the molecular evidence around the mechanisms of stimulant drugs on the molecular pathophysiology of ADHD in children and adults.

METHODOLOGY

While it is not a formal systematic review, we applied structured search strategies covering PubMed/MEDLINE database. In addition, we performed handsearching of reference lists in the articles identified through the structured searches.

To describe ADHD at the molecular level, we first explored the landscape of available molecular information on ADHD to obtain a picture of the pathophysiological processes involved. We reviewed indexed literature reviews in PubMed database, using the following search string (January 2020): (“Attention deficit hyperactivity disorder” [Title] OR “ADHD” [Title] OR “Attention-Deficit/Hyperactivity Disorder” [Title]) AND (“pathogenesis” [Title/Abstract] OR “pathophysiology” [Title/Abstract] OR “molecular” [Title/Abstract]) AND Review [ptyp]; we explored these results full-length, and reviewed their list of references as well as PubMed “related articles” to completely cover all published molecular pathophysiological knowledge.

Then, we explored the molecular information around first-line stimulant drugs, to identify described direct protein targets and drug-induced molecular changes. Thus, we performed a literature search in PubMed database considering the drugs’ generic and commercial names and the following search string (April

2020): (“molecular” [Title/Abstract] OR “mechanism” [Title/Abstract] OR “pathophysiology” [Title/Abstract] OR “pathogenesis” [Title/Abstract] OR “mode” [Title/Abstract] OR “action” [Title/Abstract] OR “signalling” [Title/Abstract] OR “signalling” [Title/Abstract] OR “expression” [Title/Abstract] OR “activation” [Title/Abstract] OR “inhibition” [Title/Abstract] OR “activity” [Title/Abstract]). Again, we explored these results full-length, and reviewed their list of references as well as PubMed “related articles” to identify relevant leads.

Finally, we used keywords from the ADHD pathophysiology description to identify published data relating these processes to the drugs and drug-related molecular changes. Equivalent searches and evaluation of the results were performed for both drugs. All the results were contextualised considering the potential clinical implications of these mechanisms as per the known role of the molecular mechanisms in behaviour regulation.

This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

ADHD AETIOLOGY AND PATHOPHYSIOLOGY

ADHD Aetiology

The ADHD aetiology is complex with a multiple pathophysiological entity involving multiple genetic and environmental factors, acting together, and creating a spectrum of neurobiological responsibility [54] (Fig. 1). In this sense, gender may play a role in ADHD aetiology with an incidence rate of 2:1 in boys versus girls [55].

Genetic Factors

Genetics has a great weight in ADHD, with a heritability ranging from 60% to 90% according to several studies carried out in twins [54, 56]. However, other studies using single-nucleotide polymorphism (SNP) analysis have estimated that SNP-based heritability ranged between 10% and 28% [57]. The variability in these

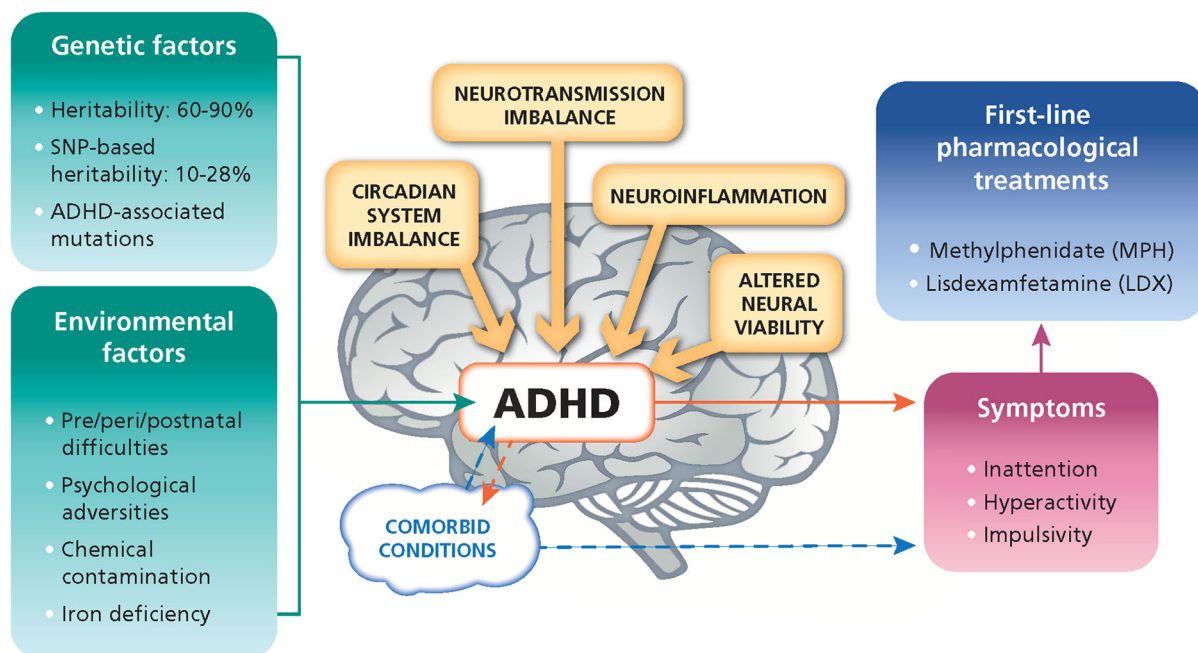


Fig. 1 ADHD aetiology, pathophysiological processes and symptoms scheme

percentages could explain the modulation in the ADHD manifestation, indicating a mixture of both dominant and recessive genes that work with complex patterns of polygenic transmission [54]. Another factor that supports an ADHD genetic background is the probability of developing ADHD being around 2–8 times more likely in the close relatives of patients with ADHD than relatives of unaffected individuals [58]. Several risk genes have been found in the human genome [59], but only a limited number of small impact size ADHD genes have been identified [60]. Genome-wide association studies (GWAS) have indicated genes playing a role in ADHD that could be linked to neuronal plasticity mechanisms (neuronal migration, cell adhesion and neuron proliferation) [61] or to deficiencies in the synthesis or release of neurotransmitters (NTs), mainly DA, but also serotonin and NE [56, 62]. However, ADHD-associated mutations have been reported to include a wide range of different physiological processes including circadian rhythm mechanisms [63].

Environmental Factors

The estimated involvement of environmental factors in ADHD pathogenesis is around 20–30% [64], highlighting prenatal, perinatal and postnatal difficulties, psychological adversities, exposure to chemical contaminants, or iron deficiency as factors that increase the probability of developing ADHD [54]; predisposing environmental factors increase the risk of developing ADHD based on a genetically or biologically determined vulnerability pattern.

Reduced maternal thyroid capacity, poor maternal wellness, maternal age, post-maturity of the foetus, long working hours, maternal stress, foetal stress, prenatal exposure to alcohol and tobacco, and antepartum haemorrhage are prenatal factors that could influence ADHD development [54, 58, 65]. Obesity and nutritional deficiencies (deficiency in the intake of essential fatty acids), lower serum levels of ferritin, and iron deficiency, as postnatal factors, have been suggested to promote abnormalities in the brain and thereby influence the onset of ADHD [66–69].

Regarding the influence of psychosocial adversity in ADHD disorder development, extreme parental discord, poor socioeconomic

status, paternal violence, inconsistent parent-child relationships, maternal psychiatric disability, parental disapproval, family anxiety and foster placement have been reported as family random factors associated with behavioural disorders in childhood [58, 70].

Environmental factors such as chemical contaminants (pesticides and harmful agricultural chemicals) may promote the neural structure impairments involved in ADHD [58].

Comorbid Conditions

ADHD disorder is usually accompanied by other comorbid conditions. In fact, patients with ADHD are frequently diagnosed with another neuropsychiatric conditions, which can mask ADHD [71].

A significant correlation has been demonstrated between intellectual disability and ADHD onset [72]. Then, intellectual development that is conditioned by cognitive and behavioural problems associated with ADHD [72] precedes the onset of ADHD. In this sense, a specific polymorphism of the brain-derived neurotrophic factor (BDNF) is significantly associated with both ADHD and intellectual disability [73]. In fact, intellectual disability appears before ADHD and it might condition the presence of ADHD.

A wide variety of other neurodevelopmental disorders and cognitive and behavioural problems have been found in ADHD: dyslexia, reasoning disorder, dyscalculia, written language expression disorder, language difficulties, motor control difficulties and also autism spectrum disorders [72].

Furthermore, ADHD has demonstrated a high degree of comorbidity of other psychological and mental conditions such as insomnia, anxiety, conduct disorder, sleep disturbance, oppositional defiant disorder, binge eating disorder, tic disorder, bipolar disorder, personality disorder, drug and substance dependence and mood disturbances, among others [72, 74]. Most of these conditions/disorders usually appear later in development and can manifest as a result of ADHD, but this is not always the case with early stage behavioural problems directly related to ADHD [58].

Pathophysiological Processes and Specific Molecules Involved in ADHD

ADHD pathophysiology is largely unknown. There are several causative cellular and molecular processes related to the ADHD development, among which NT imbalances or alterations in neuronal plasticity and neuronal survival are very well described. Nevertheless, other less studied processes are gaining relevance as regards their involvement in ADHD, such as the role of neuroinflammation and alteration of circadian rhythms.

Neurotransmitter Imbalance

The prefrontal cortex (PFC), caudate and cerebellum are the main brain regions responsible for ADHD development [75]. These regions are involved in controlling consciousness, feelings, impulses, attitudes and behaviour [76]. Slower maturation or decreased size of the PFC and also diminished PFC, caudate or cerebellum activation have been found in patients with ADHD [77, 78]. The pathway function within such regions is highly responsive to the neurochemical setting and is regulated by NTs such as DA, NE, serotonin (5-HT), glutamate and glutamate/gamma-aminobutyric acid (GABA) [75]. Deficiencies in catecholamines such as NE and DA [75, 79, 80] or dysregulation of such NTs, which are essential for normal brain function, including executive and attentional functions [33], are among the causing physiological alterations that have been reported to explain ADHD development.

DA Deficiency DA plays an essential role in mediating regulation of the cortical system, memory, mood, vascular structure, anticipation of events, motivation, behaviour inhibition, decision-making and problem-solving [16, 81, 82]. Dysregulation of the dopaminergic system is related to the ADHD symptoms and signs [54]. This system is involved in motivation and reward/avoidance behaviours; in this sense, lower DA levels in the PFC could lead to less motivation, resulting in inattention. This might also be the reason for the promiscuous reward-

seeking behaviours found in hyperactive-impulsive patients with ADHD [83].

DA interacts with five different receptors localized in postsynaptic neurons [75], which are dysregulated in several brain regions of patients with ADHD. In fact, the dysfunction of DA receptors (DRD1–5) and DA transporter (DAT-1) is the main reason for the altered activity in the dopaminergic system, playing a significant role in the pathogenesis of ADHD [84]. Moreover, DAT-1 is a DA transporter whose function is the reuptake of extracellular DA in the synaptic space [83]. Increased density of DAT-1 in patients with ADHD has been reported, resulting in inadequate extracellular DA levels in these brain regions. MPH and amphetamine-derived compounds (AMPH), the two most widely used drugs for ADHD, act on DAT-1 by inhibiting its transporter function and thereby increasing the levels of extracellular DA [75].

NE Deficiency NE, also known as norepinephrine (NA), is an important NT in behaviour control, playing a key role in high-level cognitive processes such as working memory and inhibitory response, which appear to be disrupted in ADHD [79], producing irritability and anxiety [85]. NE is also involved in regulating attention [86], in particular those related to emotional and neutral emotions [87], and this signalling network has been found altered in different brain areas in patients with ADHD [88]. NE-mediated signal transduction speed and duration are affected by NE transporter (NET) protein, as it is involved in NE reuptake [79]. Abnormalities in NET function contribute to ADHD development by decreasing the extracellular NE levels. Therefore, treatments for ADHD, such as MPH and AMPH, increase the reservoir of NE and thereby attenuate the hyperactivity and impulsivity experienced in ADHD [75].

Serotonin (5-HT) Imbalance The imbalance of excitatory serotonin function has also been related to ADHD development [89, 90]. Serotonin dysfunction plays a mediator role in ADHD-related hyperactive and impulsive behaviours [91] inducing impaired control of

impulses, violent behaviour, concentration and appetite [89]. Moreover, serotonin regulates DA activity through its receptors 5-hydroxytryptamine receptor 1B (5-HT_{1B}) or 5-hydroxytryptamine receptor 2A (5-HT_{2A}). The dysfunction of these receptors can lead to problems of serotonin–DA dynamics, resulting in ADHD symptoms [89, 90]. In addition, other proteins related to 5-HT metabolism, such as tryptophan 5-hydroxylase 2 (TPH2) and nitrous oxide synthase 1 (NOS-1), which reduces the serotonin transporter (SERT or SLC6A4) cell surface localization increasing the levels of extracellular serotonin, might be involved in the pathophysiology of ADHD [90, 92].

Glutamate Imbalance Glutamate signalling is the most common excitatory synapse NT [93] and deficiencies in this signalling has been associated with ADHD symptoms [94], although it is unknown whether ADHD is associated with differences in glutamate levels [95–97].

GABA Imbalance Alteration of glutamate/GABA equilibrium has been related to a diminished capability to focus on demanding tasks [98] and to result in DA dysregulation, causing inhibition of defective pyramidal neurons and a decrease shift in excitation towards tasks regulated in the PFC [99, 100]. In this sense, higher glutamate levels and reduced GABA levels have been observed in children with ADHD, which indicates a bias towards neuron excitation that might be implicated in hyperactivity induction, although without a firm conclusion [101].

Neuroinflammation and Defective Immunoregulation

Neuroinflammation is hypothesized to affect brain growth and thereby raises the risk of neurodevelopmental disorders through pathways such as glial activation, increased oxidative stress, problems related to neuronal growth and impaired NT activity [60]. In ADHD, low levels of cortisol, an important hormone in the hypothalamus–pituitary–adrenal (HpA) axis, have been observed in children [102–105]. The HpA axis is a hormonal system that controls stress responses [106] and affects several hormonal behaviours [107, 108]. Furthermore, HpA

axis involvement in typical ADHD comorbidities has been reported [109]. This promotes an imbalance in pro-inflammatory T helper 1 cell (Th1)/anti-inflammatory T helper 2 cell (Th2) cytokine profile making a more pro-inflammatory environment in ADHD [60, 102], which can be related to a defective regulatory T cell (T_{reg}) regulation. Pro-inflammatory cytokines such as tumour necrosis factor beta ($TNF\beta$), interferon alpha ($IFN\alpha$), interleukin-1 (IL-1), IL-5, and IL-16 are found upregulated, whereas the anti-inflammatory cytokines IL-4 and IL-13 are downregulated, resulting in an inflammatory process that causes neuronal damage and contributes to ADHD symptomatology [60]. This dysfunction of the immunoregulation by T_{reg} cells and imbalance in the Th1/Th2 response might be a crucial factor in the development of allergies in children with ADHD [110], or a factor relating Th2-mediated hypersensitivity to ADHD development [111, 112].

Circadian System Dysfunction

Circadian rhythm regulates some biological activities such as sleep and mood [113] through various mechanisms such as the transcription-translation feedback loop (TTFL), which includes two transcription activators: circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like protein 1 (BMAL1). Both activators create a heterodimer, which inhibits the transcription of repressor proteins Period 2 (PER2) and cryptochrome (CRY) that regulates the daily rhythms of locomotor activity, metabolism and behaviour [114]. Mutations in CLOCK gene have been associated with ADHD, indicating that the disrupted circadian pathways could exacerbate the symptoms of this disease [113], including ADHD-associated aggressiveness symptoms [115]. Moreover, the circadian system regulates dopaminergic system and therefore DA production is subjected to circadian rhythm variations [113]. In this sense, the dopaminergic DA receptor 4 (DRD4) gene is strongly involved in translating light into electrical signals in the retina, and there is a strong circadian pattern in its transcription [63].

ADHD also involves a delayed sleep-phase symptom (sleep disturbances) typical of sleep

onset insomnia [116] and other behavioural symptoms related to ADHD [63]. In children with ADHD, irregular sleep cycles, troubled sleep initiation, reduced sleep effectiveness with greater wakefulness periods and increased nocturnal activity have been observed, among others [117]. Although delayed sleep-phase is not enough to developed ADHD symptoms, it has been reported that treatments for sleep problems have promoted a reduction of ADHD symptoms [63]. Moreover, a deficit in melatonin levels has been found in adults and children with ADHD [117], and melatonin has been used as a treatment for children with sleep problems diagnosed with ADHD [118].

Sleep disturbances and deficit of alertness have also been found to be linked to abnormal eating behaviours, including binge eating, which is a common ADHD comorbidity; this link is hypothesized to involve the hypocretin/orexin system through hypoactivation of hypocretin/orexin neurons from perifornical and dorsomedial hypothalamic neurons (involved in arousal) and overactivation of hypocretin/orexin neurons from lateral hypothalamus (involved in reward seeking, including feeding) [119]. Understanding these pathways can lead to the development of therapies for patients with ADHD that improve their wakefulness and reduce abnormal reward-seeking behaviours (such as binge eating).

Altered Neural Viability and Neurodegeneration

Neuronal network morphogenesis is based on an effective equilibrium between neuroplasticity and neurodegeneration, as well as the formation of new cells through proliferation/survival signalling and cytoskeletal structures [120]. ADHD seems to present a morphogenesis dysregulation [92, 121], with a high number of neurophysiological difficulties caused by neuronal network dysfunctions in the brain. It has been observed that the inability to modulate neural connections can promote a reduction of brain volumes and inefficient neural networks [92]. These impairments might be the result of neurodegeneration, neurogenesis problems, altered proliferation-differentiation balance, deficient neurotrophic factors

and, thereby, loss of neuronal viability [92]. Besides, oxidative stress results in a notable problem in terms of neuronal damage which can lead to all of the defects mentioned above [92].

There are several proteins and molecular processes involved in neuronal survival, including trophic factors, NTs or neuronal function regulators. One such protein involved is BDNF, which stimulates neuronal development and maintenance, regulates NT function and contributes in neuronal plasticity processes, such as long-term potentiation and learning [122]. BDNF dysregulation is associated with ADHD. Neurotrophin-3 (NTF-3) has been also related to neuron survival [92]. Furthermore, it has been reported that NTF-3 promotes bone marrow neural stem cell proliferation and differentiation into cholinergic neurons [123]. Glutamate and some of its receptors are also involved in neurogenesis and proliferation [92]. Alterations in glutamate receptors (GRM-1, GRM-5, GRM-7 and GRM-8) have been associated with ADHD [124]. Other proteins related to proliferation and neurodevelopment that are altered in ADHD are cyclic AMP response element-binding protein (CREB), cyclin D1 and NOS-1, cadherin-13 (CADH13) and E3 ubiquitin-protein ligase parkin (PARK2) [92].

Among the molecular processes involved, it has been shown that patients with ADHD present an ineffective oxidative stress response [125]. In this sense, paraoxonase-1 (PON-1) (antioxidant agent) has been reported to be downregulated in patients with ADHD [126]. Also, it has been shown that ephrin stimulates excitatory synapses development [127–129] and is closely related to neurogenesis, plasticity and learning [93]. Moreover, it has been suggested that ephrin family members have a role in ADHD-related synaptogenesis alterations [92, 130].

MOLECULAR MECHANISM OF STIMULANT TREATMENTS

In order to obtain a full picture of the MoA of stimulants (MPH and LDX), one must consider not only direct targets but also other molecular

mediators known to be modulated by the drugs. In the following sections, known and well-established drug targets are reviewed, along with other molecular changes induced by either MPH or LDX, which might be valuable information to help fully understand the molecular consequences of stimulant treatment and how these mechanisms impact clinical observations.

Methylphenidate (MPH)

MPH is a NE–DA reuptake inhibitor; therefore, it causes an increase of catecholamines in the synaptic cleft [shown by positron emission tomography (PET) imaging] [131]. It is the first-line pharmacological treatment recommended in current guidelines [132, 133] for children of at least 6 years of age and adults with ADHD. MPH is the most commonly prescribed medication to treat ADHD [134, 135].

MPH has several formulations, including novel modified release systems to extend its effect. The main slow release systems are Concerta®, an osmotic release system (OROS technology) with 22% immediate and 78% extended release; Medikinet®, modified release (also known as Medikinet XL® or Medikinet Retard®), which is based in a multiarticular beads release, that combines 50% immediate and 50% extended release [136]; Equasym XL® which is based on capsules containing 30% immediate and 70% extended release beads [137]; and Rubicrono®, an extended release formulation based on MPH hydrochloride [138].

Drug Molecular Targets and Drug-Induced Molecular Changes

MPH has a psychostimulant effect through different direct target proteins, and several drug-induced molecular changes have also been described for MPH.

MPH has been reported to modulate with pharmacological action three human protein targets (Table 1): it acts as an inhibitor of DAT (SLC6A3) [139] and NET (SLC6A2) [139] (both transporters of DA and NE, respectively), and as an agonist for the 5-hydroxytryptamine (serotonin) receptor 1A (HTR1A) [140, 141]. All these

Table 1 MPH target proteins and drug-induced molecular changes identified

Protein name	Gene name	Effect	References
Target proteins			
5-Hydroxytryptamine receptor 1A (HTR1A)	HTR1A	↑	[117, 118]
Sodium-dependent dopamine transporter (DAT)	SLC6A3	↓	[116]
Sodium-dependent noradrenaline transporter (NET)	SLC6A2	↓	[116]
Drug-induced molecular changes			
Alpha-2A adrenergic receptor	ADRA2A	↑	[119]
Alpha-2B adrenergic receptor	ADRA2B	↑	[119]
Beta-1 adrenergic receptor	ADRB1	↑	[120]
Apoptosis regulator BAX	BAX	↑	[124]
Calcium/calmodulin-dependent protein kinase type IV	CAMK4	↑	[125]
D1A dopamine receptor	DRD1	↑	[119]
D1B dopamine receptor	DRD5	↑	[119]
D2 dopamine receptor	DRD2	↑	[121]
Glutamate receptor 1	GRIA1	↑	[120]
Interleukin-1 beta	IL1B	↑	[125]
Ras-related C3 botulinum toxin substrate 1	RAC1	↑	[126]
Tumour necrosis factor	TNF	↑	[125]
Adenylate kinase isoenzyme 1	AK1	↓	[125]
BDNF/NT-3 growth factors receptor	NTRK2	↓	[122]
Cyclic AMP-responsive element-binding protein 1	CREB1	↓	[125]
Cytochrome <i>c</i> ₁ , haem protein, mitochondrial	CYC1	↓	[124]
Mitogen-activated protein kinase 3	MAPK3	↓	[125]
Proto-oncogene c-Fos	FOS	↓	[125]
Apoptosis regulator Bcl-2	BCL2	↑↓	[124]
Brain-derived neurotrophic factor	BDNF	↑↓	[122, 123]
Caspase-3	CASP3	↑↓	[124]

targets are involved in the presynaptic signalling; therefore, MPH has a role in regulating three main NT pathways: DA, NE and serotonin (Fig. 2).

Besides these direct targets, the indirect modulation of several other proteins has been reported for MPH. First, other NT pathways have been reported to be stimulated by MPH,

including adrenergic, DA and glutamate receptors (e.g. alpha-2A adrenergic receptor [ADRA2A] and alpha-2B adrenergic receptor [ADRA2B], beta-1 adrenergic receptor [ADRB1], DA receptor 1A [DRD1], DA receptor 1B [DRD5], DA receptor 2 [DRD2]) and glutamate receptor 1 (GRIA1) [142–144]. Furthermore, MPH has been reported to modulate neurotrophic factors

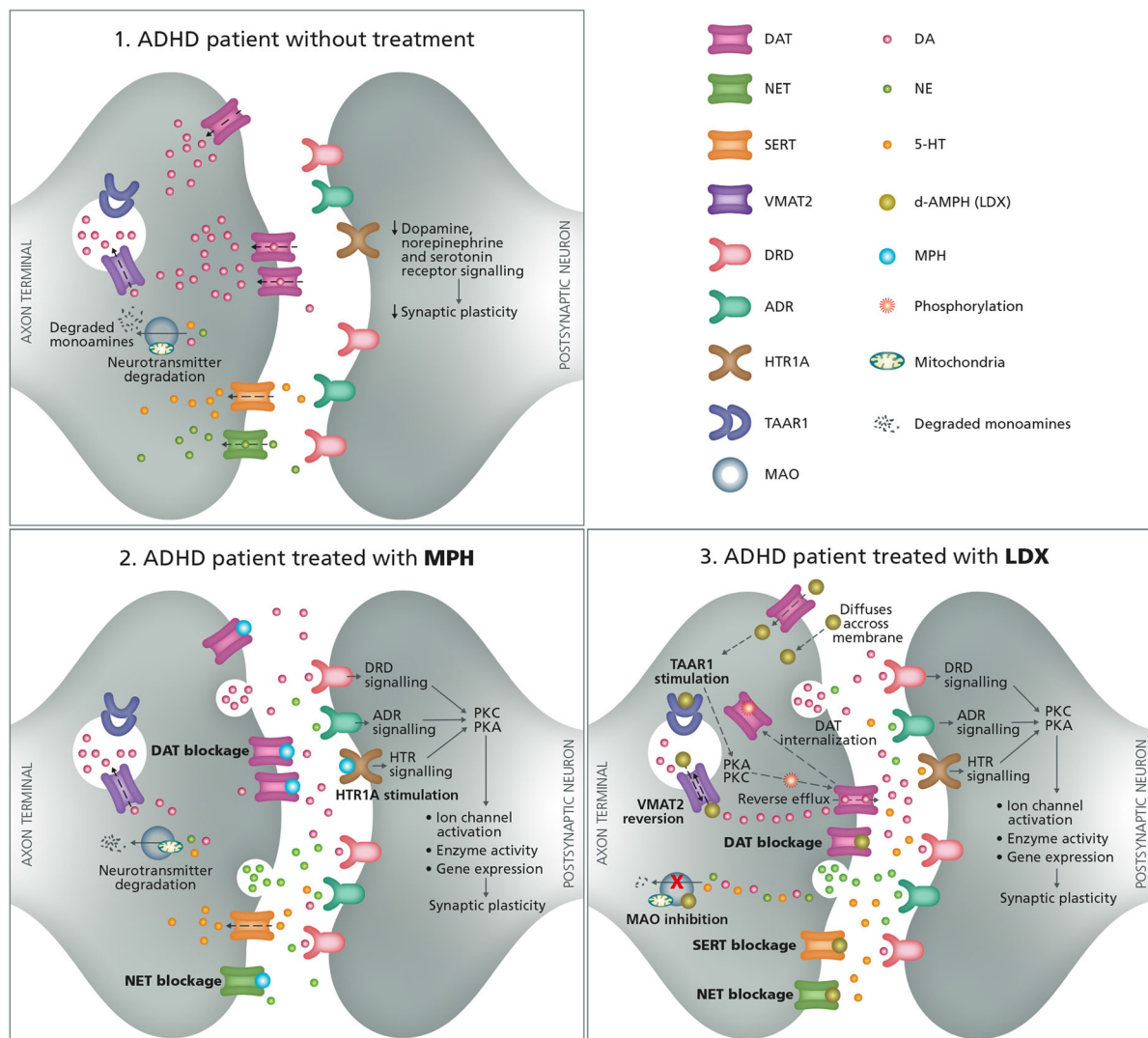


Fig. 2 Mechanism of LDX/MPH in the synapse: 1. Patient with ADHD without treatment. 2. Patient with ADHD treated with MPH 3. Patient with ADHD treated with LDX

involved in neuronal survival and plasticity, such as BDNF and its BDNF/NT-3 growth factor receptor (NTRK2); the effect of MPH over these factors is unclear, since some experiments report inhibition [145], whereas others report activation, improving BDNF plasma concentrations in children with ADHD [146].

MPH is also able to modulate other more general cellular processes, such as apoptosis, which might also impact neuronal survival. The apoptosis-related proteins reported to be modulated by MPH include apoptosis regulator Bcl-2 (BCL2), apoptosis regulator BAX (BAX), caspase-

3 (CASP3) and cytochrome c_1 , haem protein, mitochondrial precursor (CYC1) [147]. Interestingly, canonical inflammation mediators, such as interleukin-1 beta (IL-1B) and TNF are induced in MPH treatment, suggesting a potential immunomodulatory role for the drug, although the evidence does not readily support it. CREB1, involved in synchronization of circadian rhythmicity, has also been shown to be modulated by MPH treatment [148]. Finally, MPH has been reported to modulate intracellular mediators and transcription factors involved in neuronal signalling, including

adenylate kinase isoenzyme 1 (AK1), mitogen-activated protein kinase 3 (MAPK3), proto-oncogene c-Fos (FOS), calcium/calmodulin-dependent protein kinase type IV (CAMK4) and Ras-related C3 botulinum toxin substrate 1 (RAC1) [149].

Lisdexamfetamine Dimesylate (LDX)

LDX is the first stimulant prodrug indicated and recommended by guidelines in children at least 6 years of age when the MPH treatment is not optimal [132, 150], and also as first-line treatment in adults [133, 151] and for binge eating disorder [40] in the USA. Elvanse® (Vyvanse® in the USA) or LDX is a long-term release prodrug of dextroamphetamine (D-amphetamine) [152]. LDX is itself pharmacologically inactive, but following oral administration it is converted by rate-limited enzymatic hydrolysis to L-lysine and D-amphetamine [152]. The active form of the drug has a central nervous system stimulating activity by the primary inhibition of DAT, NET, trace amine-associated receptor 1 (TAAR1) and vesicular monoamine transporter 2 (SLC18A2), among other targets, therefore regulating the reuptake and release of catecholamines (primarily NE and DA) on the synaptic cleft [153].

Once administrated, LDX is converted to the active drug D-amphetamine, through cleavage of the L-lysine (an essential amino acid) in the bloodstream carried out by the erythrocytes. All the subsequent analysis was carried out taking into account the active compound, since the parental prodrug is biologically inactive [154].

Drug Molecular Targets and Drug-Induced Molecular Changes

LDX has been reported to modulate with pharmacological action of seven human protein targets (Table 2). On the one hand, it acts as activator of TAAR1. On the other hand, six protein targets are inhibited by LDX: synaptic vesicular amine transporter (SLC18A2), DAT (SLC6A3), NET (SLC6A2), SERT (SLC6A4) and amine oxidase [flavin-containing] (MAO) A and B [153–157]. All of these targets are involved in the presynaptic signalling, and LDX therefore

has a role in regulating three main NT pathways: DA, NE and serotonin. Moreover, LDX has a role as a neuromaintenance agent since it inhibits MAOA and MAOB oxidative enzymes (Fig. 2).

LDX has been reported to indirectly modulate several proteins. First, LDX induces activation of the known dopaminergic and adrenergic receptors DA receptor 1A (DRD1), DA receptor 1B (DRD5), DRD2, ADRA2A, ADRA2B and alpha-2C adrenergic receptor (ADRA2C) [155, 158, 159], contributing to neurotransmission signalling. Furthermore, LDX has been reported to modulate several inflammation mediators such as TNF, IL-4, IL-6 and IL-10 [160]. As it occurs with MPH, TNF is induced upon LDX treatment, suggesting a potential immunomodulatory role for the drug. Other cytokines stimulated by LDX are IL-4, IL-6 and IL-10, with IL-4 and mainly IL-10 showing an anti-inflammatory profile, suggesting LDX may have a role in the reduction of inflammatory response.

MPH vs LDX: Similarities and Differences

Similarities

MPH and LDH have similar targets and act in the same way: both promote the inhibition of DAT and NET [153, 156, 157]. Regarding the indirect drug-induced molecular changes, both drugs promote the activation of DRD1, DRD5, ADRA2A and ADRA2B, then modulate the dopaminergic and adrenergic signalling, therefore regulating the reuptake and release of catecholamines (primarily NE and DA) on the synaptic cleft [131, 153].

Likewise, TNF is indirectly activated by both drugs, which implies a potential immunomodulatory role for MPH and LDX. Although the role of the effect of both drugs on TNF is unknown, it has been reported that TNF α promotes astrocyte activation [161]. In an ADHD comorbid disease, major depressive disorder, activated astrocytes can reduce glutamate on the synaptic cleft via excitatory amino acid transporters (EAATs) and convert it to glutamine in the cytosol [162]. In this sense, higher glutamate levels have been observed in children

Table 2 LDX target proteins and drug-induced molecular changes identified

Protein name	Gene name	Effect	References
Target proteins			
Trace amine-associated receptor 1	TAAR1	↑	[132]
Amine oxidase [flavin-containing] A	MAOA	↓	[131, 133]
Amine oxidase [flavin-containing] B	MAOB	↓	[131, 133]
Sodium-dependent dopamine transporter (DAT)	SLC6A3	↓	[130, 133, 134]
Sodium-dependent noradrenaline transporter (NET)	SLC6A2	↓	[130, 133]
Sodium-dependent serotonin transporter (SERT)	SLC6A4	↓	[130, 134]
Synaptic vesicular amine transporter	SLC18A2	↓	[130, 132]
Drug-induced molecular changes			
Alpha-2A adrenergic receptor	ADRA2A	↑	[132, 136]
Alpha-2B adrenergic receptor	ADRA2B	↑	[132, 136]
Alpha-2C adrenergic receptor	ADRA2C	↑	[132]
D1A dopamine receptor	DRD1	↑	[132, 135, 136]
D1B dopamine receptor	DRD5	↑	[132, 135, 136]
Interleukin-4	IL4	↑	[137]
Interleukin-6	IL6	↑	[137]
Interleukin-10	IL10	↑	[137]
Tumour necrosis factor	TNF	↑	[137]

with ADHD [101]. Then the activation of TNF could improve the synaptic signalling. On the other hand, in vitro studies carried out in astrocytes have shown that cytokines such as TNF, among others, support the production of neuroprotective mediators [163]. More studies are necessary to understand the role of the effect of both drugs on TNF in ADHD.

Differences

MPH and LDX have different target profiles that could underlie differences in their MoAs. MPH activates HTR1A [140, 141], which induces a partial release of DA, thereby improving the presynaptic signalling. LDX inhibits the activity of two other important proteins, not shared with MPH, involved in the monoaminergic system described in ADHD: SERT [164] and MAO [165]. The inhibition of DAT1, targeted by

both drugs, leads to no reuptake of the DA to the cytosol [166]; LDX also reduces monoamine degradation by MAO inhibition [167, 168]. LDX is also a specific agonist of TAAR1 [169], a protein involved in DA pathway modulation [170]. Another target protein activated by LDX is SLC18A2 (also known as VMAT2) that contributes to regulating the reuptake and release of catecholamines (primarily NE and DA) on the synaptic cleft [153].

More differences were found among drug-induced molecular changes for both drugs. MPH has a broader range of modulation encompassing dopaminergic and adrenergic neurotransmission, inducing the activation of ADRB1 and DRD2 [143, 144] and modulating proteins involved in neuronal survival such as BCL2, BAX, CASP3 and CYC1 [147], acting as an immunoregulator owing to its effect on TNF

[148] and affecting circadian rhythm through CREB1, involved in its synchronization [148]. The modulation of LDX involves dopaminergic and adrenergic neurotransmission, inducing upregulation of ADRA2A, ADRA2B, ADRA2C, DRD1 and DRD5 [155, 158, 159], and immunomodulation by modulating several interleukins, mainly IL-4 and IL-10 that present an anti-inflammatory profile [160].

When considering these differences, it is important to remark that the information available may be biased, and therefore it must be interpreted cautiously, as it only informs on data reported for one of the drugs.

IMPACT OF MECHANISMS ON CLINICAL OBSERVATIONS

MPH and LDX show an impact on the aforementioned causative processes related to ADHD development (NT imbalance, neuroinflammation and defective immunoregulation, circadian system dysfunction and altered neural viability and neurodegeneration) owing to both drugs' activity in ADHD symptomatology control.

Neurotransmitter Imbalance

MPH

MPH exerts its action promoting the downregulation of DA and NE transporter (DAT and NET) (protein targets) [139]. This leads to an increase of DA and NE levels in the prefrontal cortex (synaptic cleft) that control hyperactivity and deficits in inhibitory behaviour (improvement in attentional deficit and cognitive functioning) [139, 171]. Nevertheless, the molecular mechanisms underlying their action are poorly understood. The interaction of MPH with several proteins involved in the neurotransmission process could explain its MoA (Fig. 3).

Regulation of Presynaptic Receptors DA accumulation after MPH blockade of DAT induces disinhibition (upregulation) of the presynaptic receptor DRD2 (dopaminergic neuron) and activates D1 receptors on the postsynaptic neuron that uptake DA, continuing the

neuronal transmission and therefore improving attention, focus and organized thoughts and actions [172, 173].

On the other hand, MPH activates HTR1A and DRD2 (and potentially other G-coupled receptors) that might have a reinforcing feedback role on MPH modulation of the NT imbalance through further regulation of DAT1 [174]. Moreover, HTR1A induces a partial DA release which in turn improves the presynaptic signalling [140, 141].

Regulation of Postsynaptic Receptors In PFC neurons, MPH indirectly activates the postsynaptic DRD1 and DRD5 [142] that uptake DA and transmit the signal in postsynaptic neurons [175]. The stimulation of these receptors probably leads to the GRIA1 receptor phosphorylation promoted by MPH, as described by Pascoli et al. [143].

MPH also upregulates ADRA2A, ADRA2B and ADRD1B receptors located in postsynaptic neurons. The assessment of the effect of MPH on the two major signalling pathways (cAMP-dependent protein kinase [PKA] and extracellular signal-regulated kinase [ERK]) in PFC [143] showed no activation of ERK2 phosphorylation. However, the MPH effects on these signalling pathways were totally inhibited using ADRD1B blockers, showing that ADRD1B mediates the phosphorylation of PKA signalling through MPH in the PFC [143]. Moreover, it has been shown that MPH exerts excitatory actions on PFC neurons by activating ADRA2 [175, 176]. In this sense, NE neurotransmission seemed also to be modulated via ADRA1 and ADRA2 [143].

LDX

LDX controls the neuronal transmission, both at presynaptic (direct) and postsynaptic (indirect) levels. This results in an amplification of DA activity and an improvement in attentional deficit and cognitive functioning, as well as a reduction in hyperactivity (Fig. 3).

Regulation of Presynaptic Receptors LDX inhibits DAT and NET transporters leading to an increase in DA and NE levels in the synaptic cleft [153, 156, 157]. LDX can also promote the increase of DA in the synaptic cleft by activating

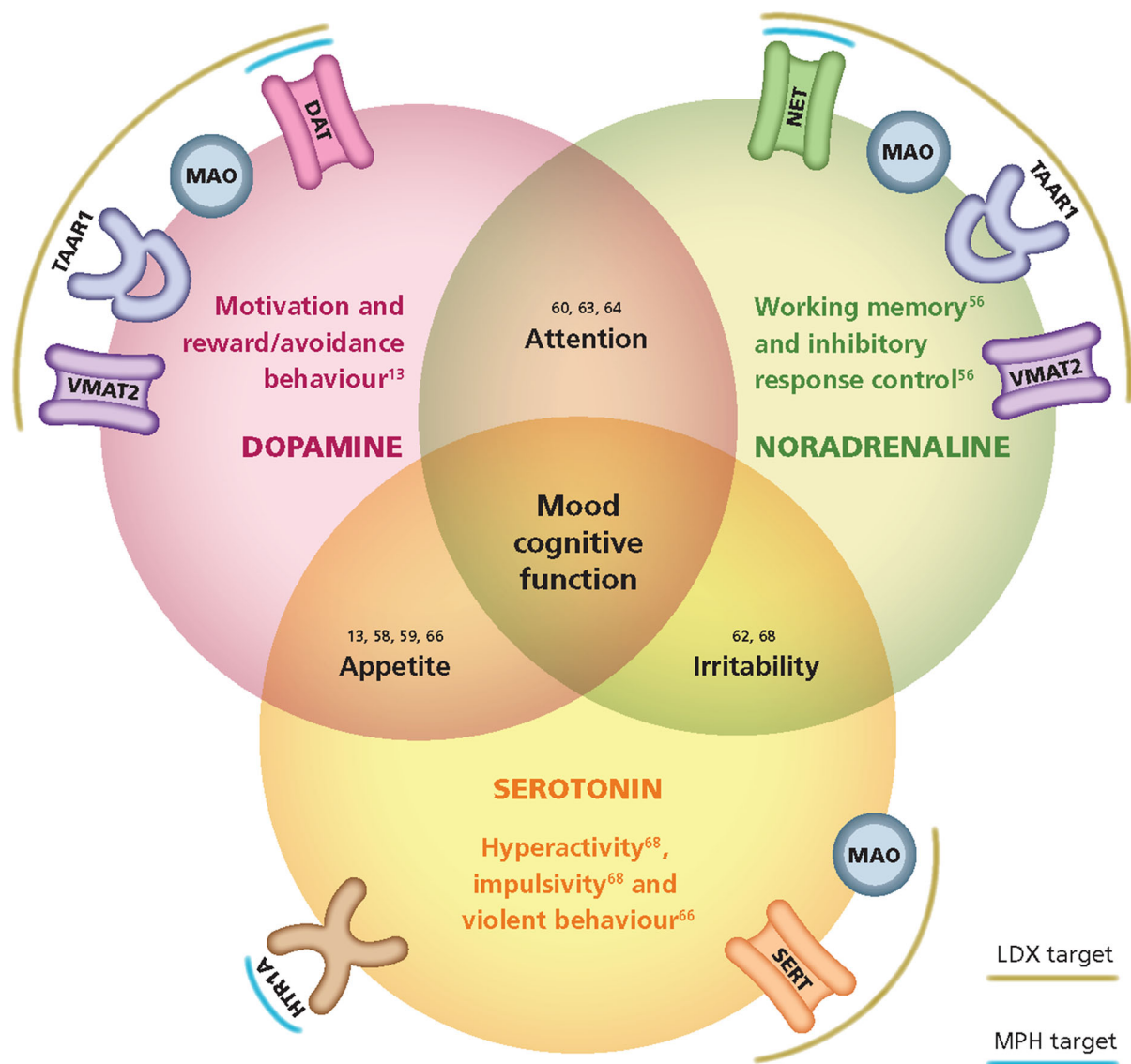


Fig. 3 Relationship between function and behavioural effects of monoamines. Direct targets of the stimulant drugs are indicated in each monoaminergic pathway

protein TAAR1, which produces the efflux of monoamine NTs, mainly DA, from storage sites on presynaptic neurons [155, 177]. TAAR1 activation leads to intracellular cAMP signalling that results in PKA and PKC phosphorylation and activation [178–180]. This PKC activation decreases DAT1 [181], NET1 [182] and SERT [183] cell surface expression, intensifying the direct blockage of monoamine transporters by LDX and improving the neurotransmission imbalance in ADHD. In addition, PKC might induce p38 MAPK-mediated inactivation of

AKTs [184, 185], proteins essential for DAT cell surface redistribution due to AKT induction of actin reorganization, regulating the dopamine efflux impaired in ADHD [186].

The increase of DA promotes the dopaminergic transmission by regulating (inhibiting) other proteins: VMAT2, whose inhibition induces the DA liberation from vesicular storage and the concomitant release of cytosolic DA via DAT reverse transport [33, 187], SERT (with weaker affinity), and MAOA and MAOB [153].

Regulation of Postsynaptic Receptors LDX amplifies dopaminergic transmission in the mesolimbic and mesocortical tracts (two major pathways in cognition and memory) [155]. The cognitive-enhancing effects of amphetamine occur through the dopaminergic transmission activation which, in turn, is mediated by both D1 dopamine receptors and α 2-adrenoceptors in postsynaptic neurons in PFC [188].

The DRD1, DRD5, ADRA2A, ADRA2B and ADRA2C are activated downstream by LDX [155, 158, 159]. The effects on D1 receptors increase the locomotor activity [189], and the stimulation of ADRA2 receptors results in a profound inhibition of the spontaneous firing rate of locus coeruleus neurons [190] that could improve the efficacy of LDX on symptom control. Moreover, the indirect activation of α 1-adrenoceptors and DRD1 increases the catecholaminergic neurotransmission [191].

The effect in PFC of D-amphetamine activation of DRD and ADRA2 receptors on the two major signalling pathways (PKA and ERK) has been also analysed [143]. D-Amphetamine activated the phosphorylation of both pathways through ADRD1B [143]. ADRA blockers did not inhibit the ERK phosphorylation although its effect was slightly reduced; this reflects the major role of DRD in ERK activation by D-amphetamines with a slight contribution of ADRA2 [143]. Moreover, D-amphetamine phosphorylates GRIA and, as with MPH, it could derive from the stimulation of dopamine D1/D5 receptors [143].

Probably minimal differences such as ERK2 phosphorylation and the action on locus coeruleus neurons by LDX could serve as a differentiator to determine the efficacy level of each drug.

Neuroinflammation and Defective Immunoregulation

Neuroinflammation is hypothesized to increase the risk of neurodevelopmental disorders and impaired NT activity [60].

MPH

MPH presents a pro-inflammatory profile. Several studies have exposed that the long-term use of MPH induces the DA neuron loss and microglia activation causing an increase in pro-inflammatory markers TNF α and IL-1 β , among others, resulting in the activation of neuroinflammation and triggering a neurodegenerative process [192, 193]. Therefore, their long-term use must be considered the result of loss of DA transmission and, in turn, efficacy.

On the other hand, the use of MPH could create dependence or abuse [194, 195]. The chronic abuse of MPH also induces the expression of inflammatory cytokines, such as TNF α and IL-1 β [148, 196, 197]. With MPH at 10 mg/kg, an increase of the inflammatory markers in the amygdale has been observed [148].

LDX

It is important to remark that the inflammatory profile of LDX has not been evaluated in an ADHD context. It has been assessed in ADHD comorbid disease, such as bipolar disorder in animal models [160], in which LDX increased the pro-inflammatory cytokines TNF α and IL-6, and also the anti-inflammatory cytokines IL-4 and IL-10 in the frontal cortex, striatum and serum [160, 198]. However, a recent study did not show LDX having an effect on IL-10 (anti-inflammatory cytokine, upregulated to control the inflammatory response duration and intensity) neither on TNF α nor on IL-1 β (pro-inflammatory cytokines) [199]. On the other hand, MAOA inhibition and TAAR1 activation by LDX (both direct targets of LDX) might influence MAPK3 activation, which is crucial for the downstream modulation of a large number of proteins involved in many biological processes, such as neuroinflammation [200]. MAPK3 can induce the expression of some anti-inflammatory cytokines (e.g. IL-2, IL-10), downregulated in patients with ADHD, via NF κ B (NFKB1) activation [200–202].

Recent evidence suggests the involvement of the immune system in several psychiatric conditions [203–205]; one of the most studied in this respect is depression [206–208]. Given the role of LDX in modulating inflammatory and immunomodulatory molecules in psychiatric

condition settings, a possible role of LDX in modulating the immune system in other psychiatric conditions, such as depression or ADHD, could be expected. Thus, more studies are required to understand the inflammatory profile of LDX in ADHD.

Circadian System Dysfunction

MPH

As previously exposed, ADHD is related to a delayed sleep-phase [116]. MPH increases the activity at mid-to-late night and leads to a delay in sleep relative to the light–dark cycle [209]; but the mechanisms of MPH involved in the circadian rhythm are unknown. One study in ADHD that investigated markers of oxidative stress and inflammation showed that MPH downregulated c-FOS (gene involved in circadian rhythm) [148]. Another study that assessed the impact of MPH on CLOCK gene protein expression showed that MPH increased c-Fos expression. Although the authors did not consider its influence on the circadian functioning of the cerebral cortex, MPH causes a widespread increase in neuronal activation throughout the region [210].

Other hormones involved in circadian rhythm are serotonin and melatonin [211, 212]. Healthy children have higher 5-HT concentrations in the morning compared with patients with ADHD, with very similar concentrations in the evening [212]. In that study, MPH treatment induced a decrease of 5-HT levels in the evening (without changes in the morning); the effect of MPH on melatonin concentrations was a decrease in the morning and an increase in the evening, normalizing these values to those observed in the non-ADHD population [212].

It is interesting to note that the role of MPH as an inhibitor of DAT1 [174] and 5-HT1A [141] leads to inhibition of circadian clock regulators CRY1 and CRY2. These CLOCK components CRY1 and CRY2, implicated in the ADHD-associated dysregulation of the circadian system [213], are modelled through AMPK activity by MPH improving the impairment of this system [214]. This mechanism could explain the effect of MPH in circadian rhythm regulation.

LDX

We did not find evidence on whether the MoA of LDX induces improvements in circadian rhythm in ADHD.

Altered Neural Viability and Neurodegeneration

MPH

Neuronal Survival Motaghinejad et al. showed that MPH downregulates CREB1 [148], a major transcription factor in brain development and neurogenesis [215], and its product BDNF, which acts downstream in the CREB pathway signalling [148]. This mechanism observed with the use of amphetamines leads to neurodegeneration and lower cell survival [216].

Other proteins related to neural viability are MAPK3 and AK1, both downregulated by MPH [148]. These proteins act upstream in the CREB pathway signalling favouring CREB/BDNF phosphorylation and the activation of this signalling pathway [148].

BDNF has a relevant role in synaptogenesis regulation and stimulates neuronal development and maintenance [122, 217]. Disruption of BDNF and its downstream signals has been found in ADHD correlating with severity of ADHD symptoms [218, 219]. MPH increases plasma concentrations of BDNF in children with ADHD, although in that study the authors found that lower baseline plasma BDNF levels have a positive effect on hyperactivity symptoms [146]. It has been postulated that higher levels of BDNF could compensate dysfunctions of DA and 5-HT systems observed in neurodevelopmental disorders [122, 220], suggesting that higher levels of BDNF in subjects with ADHD are related to more profound NT dysfunction [146]. On the other hand, more recent studies have shown that the expression of BDNF is downregulated by MPH [145, 148]. The use of neuroprotective agents reverses the effects of MPH, increasing the expression of CREB and BDNF [148, 221].

c-FOS, as does BDNF, acts downstream in the CREB pathway signalling [148]. The role of MPH on c-FOS is unclear. It has been described that

c-FOS is downregulated by MPH [148]. However, it has also been described that MPH increases c-FOS level in a study that assessed the impact of MPH on circadian rhythm [210] and c-FOS expression in the medial septum (area intimately connected to the hippocampus) [222].

Apoptosis Control Another way of acting on neural viability and neurodegeneration is through apoptosis control. BAX (pro-apoptotic) and BCL2, CASP3 and CYC1 (anti-apoptotic) proteins have been regulated by MPH, although the MoA of MPH remains elusive as MPH effects were dependent on age and brain area [147].

Oxidative Stress Several studies have shown that MPH modulates oxidative stress in the brain [223, 224], either increasing or reducing reactive oxygen species (ROS) depending on age, drug dose and brain region, and relate that to neural viability. In this sense, Coelho-Santos et al. demonstrated that MPH promotes ROS generation via activation of RAC1-dependent NADPH oxidase (NOX) and c-Src activation [149].

Another protein related to oxidative stress is CAMK4, whose expression is activated by MPH; it has been suggested that CAMK4 increase could amplify calcium influx and be involved in the increase of oxidative stress and inflammation [148].

Taken in combination, MPH could induce apoptosis and neurodegeneration through its action on the CREB signalling pathway and oxidative stress generation through RAC1 and CAMK4. Whether these pro-neurodegenerative effects can be related to MPH misuse is not clear.

LDX

Oxidative Stress LDX causes oxidative imbalance through increased lipid peroxidation, protein oxidation and alterations in the activity of antioxidant enzymes in some brain areas [225].

LDX increases the thiobarbituric acid-reactive substances in the cerebellum, hippocampus and cerebral cortex, although its MoA is unknown [225]. One plausible MoA related to

the oxidative stress caused by LDX is the regulation of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). In this sense, two studies have shown that the levels of these enzymes were decreased by LDX in neuropsychiatric disorder models [225, 226].

CONCLUSIONS

Based on the literature published, the MoAs of both LDX and MPH (current first-line pharmacological treatments) have an effect in the network of the specific molecules involved in the described pathophysiological motives of ADHD.

Regarding NT imbalance, LDX and MPH are related to the increase of central DA and NE activity in brain regions promoting the down-regulation of DAT and NET at presynaptic level. Activation of DRD and ADRA by LDX and MPH at postsynaptic level is involved in DA and NE transmission. LDX regulates more receptors (TAAR1, VMAT2, SERT, MAOA and MAOB) than MPH in neurotransmission, and minimal differences, such as ERK2 phosphorylation and the action on locus coeruleus neurons by LDX, could differentiate efficacy between both drugs. The regulation of TAAR1 is also related to MAPK3 activation, crucial in the downstream modulation of the neuroinflammation inducing the expression of some anti-inflammatory cytokines (e.g. IL-2, IL-10). In this regard, neuroinflammation and defective immunoregulation have been observed in ADHD. Long-term use of stimulant medication could cause loss of DA transmission and, therefore, efficacy. The studies published show MPH as a drug with a pro-inflammatory profile mainly due to its activation of cytokines TNF α and IL-1 β . LDX promotes the activation of pro-inflammatory cytokines such as TNF α and IL-6, although it also promotes the expression of the anti-inflammatory cytokines IL-4 and IL-10 in the frontal cortex, striatum and serum. On the contrary, TNF α promotes astrocyte activation reducing glutamate on the synaptic cleft, which could improve the synaptic signalling. More studies are necessary to understand the effect of both drugs on TNF in ADHD. Also, altered

neural viability and neurodegeneration have been observed in ADHD. Mechanisms of neuron survival and oxidative stress are involved in neurodegeneration. It has been shown that MPH could have an effect on neurodegeneration and oxidative stress modulation through several mechanisms. Apoptotic enzymes have been related to MPH although its MoA in that regard remains unclear. Moreover, MPH has been shown to promote ROS via RAC1 and CAMK4 activation, although the evidence regarding whether MPH use indeed induces or reduces oxidative stress is not clear. The relationship between LDX and neurodegeneration has only been shown through increasing oxidative stress by downregulating the level of antioxidant enzymes CAT, GSH-Px and SOD. Lastly, circadian system dysfunction is also observed in ADHD. In this regard, the mechanisms of action of MPH involved in the circadian rhythm are unknown, although it has been shown that the use of MPH in children with ADHD normalizes serotonin and melatonin levels to those of non-ADHD population. Nevertheless, no evidence has been found on the effect of LDX on the circadian rhythm in ADHD.

Both LDX and MPH have improved the neuronal signalling that leads to an increase of DA and NE levels in the prefrontal cortex, by acting on DAT and NET and improving attentional deficit and cognitive functions. However, LDX acts on more targets than MPH. Regarding neuroinflammation and defective immunoregulation control, it seems that LDX has a greater effect related to its interaction with TAAR1 and MAOA, and its promotion of anti-inflammatory cytokines such as IL-10. MPH, as well as LDX, promotes the activation of IL-1 and TNF but the role of TNF must be studied in more depth. Regarding neural viability and neurodegeneration, it seems that both drugs have a deleterious effect resulting in increased oxidative enzyme levels. Lastly, MPH shows more evidence as a potential option for treating circadian clock impairments in patients with ADHD.

Despite the studies published on the effect of MPH and LDX on pathophysiological processes and their specific molecules involved in ADHD, there are few data published on the subject and

some are based on investigations run in animal models. Further studies are necessary to improve the knowledge of the ADHD pathophysiology and to identify how the MoAs of the MPH and LDX, as current first-line pharmacological treatments, control ADHD symptomatology.

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