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Mechanism of action of guanfacine: a postsynaptic differential approach to the treatment of attention deficit hyperactivity disorder (ADHD)

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The treatment of ADHD has focused on the use of psychostimulants drugs such as methylphenidate or amphetamine and derivatives, or not stimulants agents, such as atomoxetine. These agents act mainly on catecholaminergic presynaptic mechanisms. Recently the European Medicines Agency (EMA) has approved another not psychostimulant drug, guanfacine extended release (ER), as a new option to the treatment of ADHD, which acts at postsynaptic level. Guanfacine stimulates postsynaptic alpha-2A adrenergic receptors so it inhibits the production of cAMP and closes HCN channels enhancing the effectiveness of the signal of the pyramidal neurons of the prefrontal cortex (PFC), thus improving working memory and attention. In addition, stimulation of the alpha-2A receptors promotes growth and maturation of the dendritic spines of pyramidal neurons of the medial PFC, that are associated with brain function such as learning and memory. In contrast with psychostimulants or atomoxetine, guanfacine mimics noradrenaline stimulation of postsynaptic receptors alpha-2A on the PFC.

Keywords: Guanfacine, ADHD, alpha-2A receptors, Psychostimulants, Non-stimulants

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Mecanismo de acción de la guanfacina: un abordaje postsináptico diferencial del tratamiento del trastorno por déficit de atención e hiperactividad (TDAH)

El tratamiento del TDAH se ha centrado en el empleo de fármacos psicoestimulantes, como metilfenidato o derivados de anfetaminas, o no estimulantes, como la atomoxetina. Estos agentes actúan principalmente sobre mecanismos catecolaminérgicos presinápticos. Recientemente la Agencia Europea de Medicamentos (EMA) ha aprobado otro fármaco

no psicoestimulante, la guanfacina de liberación prolongada (LP), como una nueva opción al tratamiento del TDAH, que actúa a nivel postsináptico. Guanfacina estimula receptores adrenérgicos alfa-2A postsinápticos, por lo que inhibe la producción de AMPc y cierra los canales HCN potenciando la eficacia de la señal de las neuronas piramidales de la corteza prefrontal (CPF) y mejorando la memoria de trabajo y la atención. Además, la estimulación de los receptores alfa-2A promueve el crecimiento y la maduración de las espinas dendríticas de las neuronas piramidales de la CPF medial, relacionadas con funciones cerebrales como el aprendizaje y la memoria. En contraste con los psicoestimulantes y la atomoxetina, la guanfacina imita las acciones de la noradrenalina por estimular receptores alfa-2A postsinápticos de la CPF.

Palabras clave: Guanfacina, TDAH, Alfa-2A, Psicoestimulantes, No-estimulantes

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a heterogeneous neurobiological condition that affects neurodevelopment. Etiology of ADHD is unknown. There are multiple genetic and environmental factors that result in a state of vulnerability characterized by dysfunction of catecholamines, dopamine (DA) and norepinephrine (NE), at central level, and mainly in the prefrontal cortex (PFC). This dysfunction is the main target for drug treatment of ADHD¹.

Drugs available in Spain for the treatment of ADHD include psychostimulants such as methylphenidate, both as immediate-release tablets and in various extended-release galenic forms, and lisdexamfetamine dimesylate, an extended-release prodrug of dexamfetamine. Atomoxetine, a selective norepinephrine reuptake inhibitor (SNRI), is also available as a "non-psychostimulant" drug². Despite the recognized efficacy of these agents, some patients do not respond to them, while others may experience adverse effects or have comorbid conditions advising against their use, and

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in still other cases the family is reluctant to use them. Thus, the search for alternatives continues³.

Guanfacine is approved by the FDA (Food and Drugs Administration) for the treatment of ADHD in children and adolescents aged 6 to 17 years. Recently (23/08/2015), the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) recommended marketing of INTUNIV[®], extended-release guanfacine, for the treatment of ADHD (EMA/CHMP/450088/2015). The pharmacological characteristics that differentiate guanfacine, a drug with a predominant postsynaptic action, from the drugs currently available to treat ADHD prompted us to conduct this review.

MATERIALS AND METHODS

The most relevant articles found in a literature search in Pubmed/MEDLINE, Google-Scholar, and the literature resources of the University of Alcalá library using English and Spanish terms related to "ADHD", "hyperactivity", "attention deficit", "guanfacine", "alpha-2 adrenergic agonists", and "non-stimulants" were selected. The literature references of articles identified were also reviewed to find additional references. A search was also made in the drug agencies AEMPS, EMA, and FDA on extended-release guanfacine until November 15, 2015.

GUANFACINE. POSTSYNAPTIC ALPHA-2 ADRENERGIC AGONIST FOR THE TREATMENT OF ADHD

Guanfacine is a phenylacetyl guanidine derivative (IUPAC name: N-diaminomethylidene-2-(2,6-dichlorophenyl) acetamide) which acts as a selective agonist of central alpha-2 adrenergic receptors. Alpha-2 agonists were initially used in therapeutics as antihypertensive drugs, but have been used off-label for years as an alternative to psychostimulants or in children with behavioral problems, tics, or sleep disorders, and also for Tourette syndrome, migraine, nicotine dependence⁴, and opioid withdrawal syndrome⁵.

Case reports and open label and controlled studies with immediate-release (IR) guanfacine providing promising results in the treatment of ADHD have been published since 1955⁶. This stimulated pharmaceutical development of extended-release (ER) guanfacine (INTUNIV[®]), which would promote drug compliance. This preparation contains guanfacine hydrochloride in an inactive matrix including polymers and organic acids that delays dissolution in the bowel and therefore absorption, thus increasing plasma half-life to allow for a single daily dose. A clinical research program has shown the efficacy of the drug for the

treatment of ADHD both as monotherapy and add-on therapy⁷.

THE PREFRONTAL CORTEX AS PHARMACOLOGICAL TARGET IN THE TREATMENT OF ADHD: IMPORTANCE OF CATECHOLAMINES

Among the multiple anatomic and functional abnormalities found in ADHD, special mention is deserved by those seen in PFC area much more affected in comparison to healthy controls and other neuropsychiatric patients⁸. The PFC is the most evolved brain region in humans, but is also the most vulnerable. Its circuits store information that allows for elaborating from abstract thinking the "working memory", or mental schemes that guide behavior, thinking, and affect. The PFC mediates the "executive functioning" that allows for planning and making decisions, for which PFC filters distractions and focuses attention to achieve the planned objectives⁹.

The importance of catecholamines DA and NE is such that their depletion with 6-OH-DA or reserpine causes functional impairment similar to that induced by resection. In ADHD there is dysfunction of the DA pathways that is related to lack of attention, impulsiveness, intolerance to waiting for rewards and short reward duration¹⁰. The NE pathways originating from *locus coeruleus* (LC), which are distributed throughout virtually the whole CNS, are involved in states of attention and alert, emotions, sleep, and adaptation to stress situations. Phasic LC discharges occur in response to relevant stimuli, which allows for focusing attention. LC discharges in ADHD are tonic, increasing background noise, which makes it difficult to detect the relevant signals to focus attention¹¹.

The dendritic spines on pyramidal neurons in PFC have alpha-2A adrenergic and D1 dopamine receptors involved in functioning of PFC cell networks. NE has a high affinity for the postsynaptic alpha-2A receptor and enhances cognitive function, reinforces synaptic impulses, and promotes connectivity of prefrontal cell networks. Complementarily, stimulation of D1 receptors facilitates "fine tuning" by reducing background noise in neuronal networks and disregarding inappropriate connections. PFC function can be represented as an "inverted U" and needs an optimum catecholamine concentration. Catecholamine deficiency results in poor control of impulsiveness with hyperactivity, attention deficit, and poor planning and organization, three characteristic symptoms of ADHD. By contrast, excess catecholamines in PFC, e.g. in situations of stress or overdosing with psychostimulants, stimulates alpha-1 and beta adrenergic receptors, which causes functional blockade of PFC¹².

Although DA was initially considered more important, multiple experimental and clinical arguments support the importance of NE in the pathophysiology and treatment of ADHD, as summarized below.

To date, all drugs approved for the treatment of ADHD, both psychostimulants and non-stimulants, have an influence on noradrenergic function and improve PFC functionality^{13,14}.

Some polymorphisms of the dopamine beta-hydroxylase gene¹⁵, which are associated to deficient NE synthesis, or of the alpha-2A receptor gene-2A¹⁶, are related to symptoms of ADHD and to decreased orbitofrontal blood perfusion, which may increase the risk of ADHD¹⁷.

Functional PFC impairment caused by catecholamine depletion improves after administration of alpha-2 adrenergic agonists¹⁸. By contrast, administration of yohimbine, an alpha-2 blocker, collapses discharges from cell networks in the PFC, impairs working memory and impulse control, and causes hyperactivity^{19,20}.

From these and other studies it may be inferred that endogenous production of NE and its action on alpha-2A receptors appear to be needed for regulation of the PFC both at experimental level and in humans^{14,21}.

PHARMACODYNAMIC ASPECTS OF GUANFACINE RELEVANT TO ITS MECHANISM OF ACTION IN THE TREATMENT OF ADHD

In different experimental and clinical models, guanfacine improves working memory and regulates attention, cognitive performance, and behavioral inhibition. These effects are independent from a sedative action^{9,14}. In monkeys, guanfacine also promotes delay in the most important rewards as compared to immediate rewards, and improves impulse control²². Iontophoretic delivery of guanfacine in dorsolateral PFC increases delay in neuronal discharges, essential for enhancing working memory²³. In neuroimaging studies in primates and humans, guanfacine increased blood flow in PFC and improved in parallel working memory without affecting areas unrelated to cognitive performance²⁴.

Guanfacine: Differential receptor profile as postsynaptic alpha-2A agonist

Guanfacine has behavioral and neurophysiological effects because it acts as an agonist to postsynaptic alpha-2A adrenergic receptors⁹. These receptors are predominantly located in human brain, mainly in the dendritic spines of pyramidal PFC neurons, and are coupled to a Gi protein that

inhibits adenylate cyclase, which decreases production of cAMP (cyclic adenosine monophosphate).

Guanfacine is the most selective alpha-2A agonist available, and its affinity for alpha-2A receptors is approximately 60 and 20 times greater than for alpha-2B and alpha-2C receptors respectively²⁵. Guanfacine has a high affinity for postsynaptic alpha-2A receptors¹⁴, as destruction of presynaptic NE neurons by neurotoxin DSP-4 does not modify its effects²⁶.

Clonidine, also approved by the FDA to treat ADHD, is a poorly selective alpha-2A agonist because it binds to the whole spectrum of alpha-1, alpha-2, and beta adrenergic receptors, and also to histamine and imidazoline receptors. Moreover, clonidine binding to presynaptic alpha-2A receptors is approximately 10 times greater as compared to guanfacine^{9,27}.

Selectivity of guanfacine for postsynaptic alpha-2A receptors explains its greater effectiveness for enhancing PFC functions¹⁸ and its better tolerability, as it does not induce the sedation, drowsiness, and hypotensive effect characteristic of clonidine^{28,29}.

There is ample evidence showing that guanfacine preferentially stimulates postsynaptic alpha-2A receptors in PFC. Thus, BRL44408, a selective alpha-2A antagonist, blocks the effects of guanfacine in the ADHD model of "young spontaneously hypertensive rat", while this does not occur with alpha-2B or alpha-2C antagonists²⁶. On the other hand, genetic mutation of the alpha-2A receptor impairs working memory and nullifies cognitive enhancement induced by guanfacine³⁰. However, guanfacine is effective in mice with no alpha-2C receptor¹⁸. Neurophysiologically, guanfacine inhibits excitatory glutamate transmission in pyramidal cells in PFC. This effect is antagonized by yohimbine, which does not affect presynaptic neuronal discharge³¹.

These and many other data suggest that stimulation of postsynaptic alpha-2A receptors by guanfacine would be equivalent to the "switch" that activates the "neuronal hard disk" (transduction mechanisms) of the neuron eventually responsible for its pharmacological effects.

Guanfacine: Transduction mechanisms after postsynaptic alpha-2A stimulation

Microcircuits of pyramidal cells in layer 3 of PFC are interconnected by NMDA glutamate receptors, located in dendritic spines, that provide the "raw fuel" for their discharges. Next to these are alpha-2A receptors, which improve connectivity of neuron of common characteristics, coordinating the "signal" to create the working memory and behavioural inhibition, essential for PFC function³².

In addition, this system is negatively modulated by the HCN (*hyperpolarization-activated cyclic nucleotide gated*) channels, also located in the dendritic spines of pyramidal neurons. HCN channels, dependent on cAMP, mainly allow efflux of potassium and other cations, which results in hyperpolarization that decreases discharges and interconnections of pyramidal neurons, with dispersion of synaptic impulses. Increases in cAMP caused, for example, by uncontrollable stress, open the HCN channels and impair connectivity in PFC, which loses information and shows impaired performance¹⁴. By mimicking the action of NE on postsynaptic alpha-2A receptors, guanfacine inhibits adenylate cyclase and decreases cAMP levels. As a result, HCN channels are closed, hyperpolarization disappears, efficacy of the synaptic impulse increases, and connectivity of pyramidal neurons, and thus PFC function, is recovered^{9,23,32}.

This functional hypothesis for guanfacine has significant experimental support. The coexistence and proximity of HCN channels and alpha-2A receptors in dendritic spines of pyramidal neurons in the dorsolateral PFC has been shown by electron microscopy with immunohistochemical techniques²³. Moreover, the permanent activity and discharges from neuronal PFC networks are enhanced when cAMP levels are decreased with ZD7288, a blocker of HCN channels, or with stimulation of alpha-2A receptors with guanfacine. By contrast, increases in cAMP levels, either directly with Sp-cAMPS (cAMP analogue) or indirectly by blocking alpha-2A receptors with yohimbine, or through inhibition of phosphodiesterase-4, impair PFC connections and function and also blocks guanfacine actions^{18,23}.

These data suggest that the neuronal transduction cascade triggered by guanfacine (postsynaptic alpha-2A stimulation; cAMP decrease; closure of HCN channels) facilitates discharges from pyramidal neurons, strengthens connectivity and enables PFC to more effectively regulate attention, behavior, and emotions¹⁴.

The PFC has a balance mechanism modulated by alpha-2A receptors. In order to restrict potential excessive impulses due to alpha-2A stimulation, a sharp decrease in cAMP reduces activity of PKA (protein kinase A). This activates PP1 (protein phosphatase 1), which reduces activation of the AMPA glutamate receptor. Excitatory impulses therefore decrease as a mechanism to protect the working memory that would be activated by NE excess, for example in stress situations³¹. This mechanism has also been noted with guanfacine, which in low doses enhances excitatory impulses through closure of HCN channels, but at high doses attenuates excitatory impulses by inhibiting activation of AMPA receptors²³.

Guanfacine: Neuroprotection of dendritic spines after alpha-2A stimulation

Integrity of dendrites, where alpha-2A and glutamate receptors and HCN channels are located, is essential for normal PFC function. It has been shown that over a half of genes implicated in ADHD are related to proteins directly involved in dendritic growth³³. Moreover, changes in dendrite branching and spine number and length in PFC are related to impaired attention and behavioral flexibility³⁴.

In cultures of PFC neurons, guanfacine promoted development³⁵, branching, and maturation of dendritic spines. This trophic effect, which is associated to increased levels of the postsynaptic protein PSD95, with no change in levels of the presynaptic protein synapsin, was antagonized by yohimbine³⁶. Guanfacine, acting as agonist of postsynaptic alpha-2A receptors, increases dendrite trophism in PFC.

Moreover, in animals subject to hypobaric hypoxia, in which dendritic spines deteriorate, treatment for 7 days with guanfacine increased dendritic branching, with longer spines at the expense of immature spines³⁷. In rats subject to chronic stress, daily administration of guanfacine prevented loss of dendritic spines in pyramidal neurons of layers 2/3 of the PFC. In this study, protection and density of dendritic spines correlated to cognitive performance³⁸.

CONCLUSIONS

The mechanism of action of guanfacine in the treatment of ADHD has not been fully elucidated, but there is adequate experimental evidence showing that stimulation of postsynaptic alpha-2A receptors is the main target of its pharmacological and therapeutic effects. Stimulation of these receptors decreases cAMP levels, allows for closure of HCN channels, and strengthens connectivity and functionality of the PFC to more effectively regulate attention, behavior, and emotions. In addition, guanfacine promotes dendritic branching of pyramidal cells in PFC, which has been related to improved cognitive performance. While the drugs currently available for ADHD are characterized by acting through presynaptic mechanisms, guanfacine is the first drug approved for the treatment of ADHD which has a direct and selective action on postsynaptic alpha-2A receptors. This differential mechanism of action most likely explains guanfacine efficacy and tolerability in the clinical setting, and increases the spectrum of action in ADHD.

CONFLICTS OF INTEREST

C. Álamo has received in the last 12 months fees for participating in continuous training courses or grants for

participating in congresses from Janssen, Lundbeck, Juste, Shire, Grunenthal, Rovi, Otsuka, Normon, and Baxter.

F. López-Muñoz has received in the last 12 months fees for participating in courses, congresses, or research projects from Lilly, Janssen, AstraZeneca, Alter, Lundbeck, and Normon.

J Sánchez-García currently has no conflicts of interest and is a former employee of Shire.

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