Ampakines: Selective AMPA Receptor Modulators with Potential Benefits

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ÖZET

Ampakinler: Seçici AMPA reseptör modülatörlerinin olası faydaları

Glutamaterjik sistemin nöropsikiyatrik hastalıkların patofizyoloji ve tedavisindeki rolleri üzerine yapılan çalışmalara olan ilgi son yıllarda artmıştır. Eksitatör sinaptik iletimin ana mediyatörü olması sebebiyle, glutamatın beyin üzerindeki etkileri önemlidir. Glutamaterjik sistem, beyindeki iyonotropik ve metabotropik reseptörleri üzerinden gösterdiği geniş bir etki skalasına sahiptir. İyonotropik glutamat reseptörlerinden α-amino-3-hidroksi-5-metil-4-izoksazolpropiyonik asit (AMPA) tipi reseptörler glutamaterjik sistemin oluşturduğu aktivasyonda rol oynar. Ampakinler, yapısal olarak farklı, AMPA reseptörlere selektif pozitif modülatör etkisi olan, küçük moleküllerden oluşan bir gruptur. Kortikal ağlar arasındaki iletişimi sağlayarak öğrenme ve bellek üzerinde pozitif etki sağladıkları halde, BDNF (beyin kaynaklı nörotrofik faktör) gibi nörotrofik faktörlerini regüle etmeleri terapötik potansiyelleri hakkında fikir vermektedir. Duygudurum bozuklukları ve nörodejeneratif hastalıkların hayvan modellerinde umutlandırıcı sonuçlar veren birçok ampakin molekülünün klinik çalışmaları sürmektedir.
Anahtar sözcükler: Glutamaterjik sistem, AMPA reseptörleri, ampakinler, BDNF

ABSTRACT

Ampakines: Selective AMPA receptor modulators with potential benefits

The study of the glutamatergic neurotransmitter system has received attention during the last decade in the pathophysiology and treatment of neuropsychiatric disorders. Glutamate is the major mediator of excitatory synaptic transmission in brain, and thereby contributes to important brain functions. Its ionotropic and metabotropic receptors function in a great variety of activities. The ionotropic glutamatergic receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) can trigger the activation of the whole system. Ampakines are a structurally diverse family of small molecules that selectively and positively modulate AMPA receptors, and causing fast, excitatory transmission in the brain. They enhance learning and memory processes by intervening communication between cortical regions. Besides, their function in regulating some growth factors, such as BDNF (brain-derived neurotrophic factor), explains their wide range of therapeutic potentials. Ampakines have given successful results in animal models of mood and neurodegenerative disorders, yet their clinical studies continue for a couple of molecules.
Key words: Glutamatergic system, AMPA receptors, ampakines, BDNF

INTRODUCTION: GLUTMATERGIC SYSTEM AND AMPA RECEPTORS

The excitatory nature of glutamate in the mammalian brain and spinal cord has been recognized since 1950s. However, it took nearly 20 years to realize that glutamate is the main excitatory transmitter in the nervous system (1). During the decoding of glutamate action, it was assumed that glutamate postsynaptically acts on three different subtypes of ionotropic receptors, which are given their names after their preferred agonists: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate.

Although the activation of glutamatergic system receptors results in a marked excitation, they do not share exactly the same structural and functional properties. It can be said that AMPA receptor activation is necessary for functioning of NMDA receptors. Excitatory transmission, which causes fast and rapidly desensitizing excitation of many synapses, is mainly contributed by AMPA receptors. This activation is recognized as an early response to glutamate in synapse. This early activation allows the inward flow of Na^+, which provides depolarization of the neuronal membrane. Depolarization results in the release

of Mg^{2+} from NMDA receptor channel, providing passage of Ca^{2+} through the pore. The explained mechanism brings the co-expression of AMPA receptors with NMDA receptors at synapses. The excitatory postsynaptic potential (EPSP), which is produced by the inward Na^{+} ion flow after the AMPA receptor activation, is needed for the consistent activity of NMDA receptors. Structurally, AMPA receptors are composed of four subunits and GluA2 prevents the open channel from Ca^{2+} conductance, while only GluA1 and GluA3 subunit-containing AMPA receptors allow Ca^{2+} passage. Additionally, AMPA receptors have lower glutamate affinity than NMDA receptors, have faster kinetics and produce EPSPs to arrange the suitable occasions for NMDA receptor activation (2,3,4).

Differences in subunit expression, posttranscriptional changes, organization of postsynaptic densities, contribution of numerous signal transduction proteins, different splicing modifications provide a wide range of functional diversity and complexity to this type of receptors, through effects on desensitization time and receptor trafficking. As a result, they contribute to the processes of synaptic plasticity that are involved in learning, memory, excitotoxicity, and neuroprotection in divergent extents and through varied mechanisms. AMPA receptors, as initiator of excitatory response, are also worthy in physiological, pathological and therapeutic manners because of their unique properties (1,5,6).

Modulation of AMPA receptors is a reasonable target in the treatment of abnormal excitatory neurotransmission in many neurological and psychiatric diseases. In epilepsy (7) and ischemia (8), AMPA receptor antagonists have been widely used. In the beginning, it was thought that NMDA receptors are playing a key role in the generation of seizures, leading to clinical studies of NMDA receptor blocking drugs in epilepsy. Use of these caused some disappointing results, but in time, it became appreciated that the AMPA receptor is actually the main mediator of excitatory neurotransmission in the central nervous system and moreover that AMPA receptors are critical to the generation and spread of epileptic activity. As drugs that selectively target receptors became available, it was possible to demonstrate that AMPA receptor antagonists have powerful antiseizure activity in *in vitro* and *in vivo* models. The same condition was also experienced during the attempts to amend the therapy of ischemia (9). Although drugs acting via AMPA receptors are in use, there are also a few concerns about targeting these receptors: AMPA receptor desensitization occurs quite quickly, and overactivation of AMPA receptors could induce convulsions and/or cell death to damage cerebral tissues. Therefore, less progress has been made with AMPA receptor agonists as drugs. Besides, use of AMPA agonists in disorders such as attention deficit hyperactivity disorder and schizophrenia would bring an accurate success in therapy. In theory, this could be achieved by direct activation or positive modulation of AMPA receptors. In practice, choosing positive modulation may solve the problem of overactivation of AMPA receptors during therapy. Certain drugs have become available in the past decade that can specifically target AMPA receptors and alleviate the problem of receptor desensitization, with the hope that such drugs could be useful in treating psychiatric and neurological disorders (10).

AMPAKINES

Classification

Ampakines are the family of drugs acting centrally as positive allosteric AMPA receptor modulators and comprising benzamide compounds. Four structural groups of ampakines have been identified so far (11):

I) Pyrrolidine derivative "racetam" drugs (e.g. piracetam, aniracetam): Aniracetam has been the prototype of this class, but there are still some concerns about the "selectivity" of these drugs.

II) CX- series having benzoylpiperidine and benzoylpyrrolidine structures (e.g. CX-516, namely Ampalex; CX-691, namely Farampator): Many drugs of this group are still under clinical investigation.

III) Benzothiazide derivatives (e.g. cyclothiazide, IDRA-21): Originally, these drugs are thiazide diuretics used especially as antihypertensive, but in 1990s cyclothiazide has been found to be a positive allosteric modulator of AMPA receptors (12) and in 2000s it has been discovered that it is negative allosteric modulator of GABAA receptors (13).

IV) Biarylpropylsulfonamides (e.g. LY-392, 098; LY-404, 187)

Mechanism of action

Ampakines are known to be possitive allosteric modulators of AMPA receptors, so that they reduce the

onset of AMPA receptor desensitization and/or deactivation to improve fast excitatory transmission. There are two cellular key factors, providing ampakines to show their effects: LTP (long-term potentiation) and BDNF (brain-derived neurotrophic factor). In this manner, ampakines are suitable to be used in cognitive deficits, because BDNF not only regulates induction of synaptic plasticity, but also contributes in the maintenance in the early and late phases of LTP (14,15). As a result, ampakines have been suggested to improve cognition in neurodegenerative disorders. As BDNF increase is also another subject, it also may have a use in a broad range of disorders, such as stroke, Alzheimer's disease and Parkinson's disease.

To show their postsynaptic activity, ampakines modulate postsynaptic AMPA receptors, which are bound to postsynaptic density (PSD)- a protein specialization attached to postsynaptic membrane serving as a signaling apparatus. Kinases and phosphatases in the PSD are released from PSD to provide triggering of additional signaling pathways. Ampakines show their effects through MAPK/ERK pathway, which regulates proliferation, gene expression, cellular differentiation, cell survival and apoptosis (Figure 1). Interestingly, AMPA receptor/MAPK pathway selectively inhibits excitotoxicity. On the other hand, the pathway did not protect against nonexcitotoxic pathologies (16). This finding indicates that AMPA receptors provide cellular maintenance and activate survival cellular signals to equipoise their own excitotoxic potential (16).

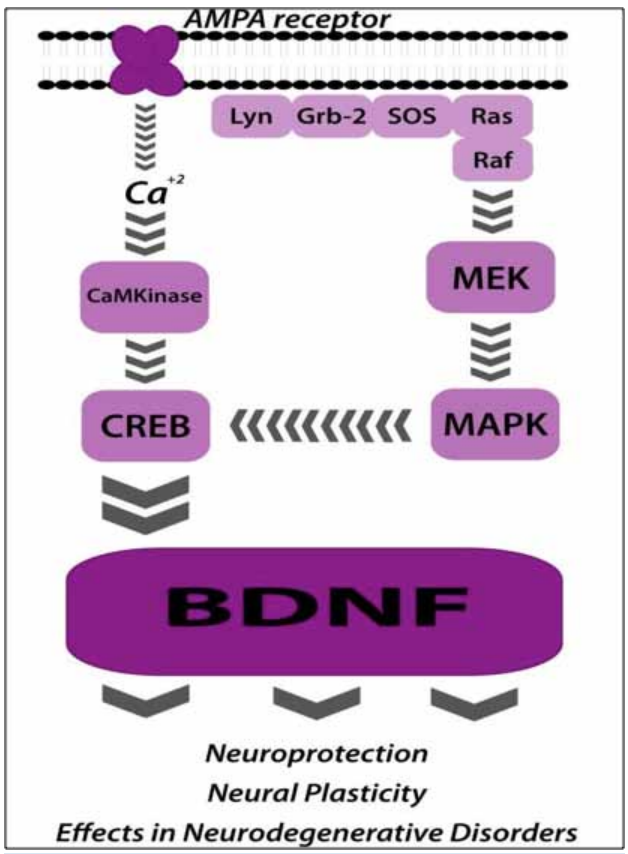

Figure 1: AMPA receptor activity may result in BDNF induction through both Ca^{2+}-dependent and -independent (MAPK) pathways. Subunit variety of AMPA receptors provides these two distinct activity pathways. Only GluR1 and GluR3 containing receptors provide Ca^{2+} influx and activation of Ca^{2+}-dependent pathway. (BDNF: brain-derived neurotrophic factor, CaM Kinase: Ca^{2+}/calmodulin-dependent protein kinase, cAMP: adenosine 30,50-cyclic monophosphate, CREB: Ca^{2+}/cAMP response element binding protein, Grb-2: growth factor receptor-binding protein 2, Lyn: tyrosine-protein kinase Lyn, MAPK: mitogen-activated protein kinase, MEK: MAPK kinase, Raf: serine/threonine-specific protein kinase, Ras: GTPase activating protein, SOS: son of sevenless)

Selectivity Issues

Ampakine effects differ according to the type of subunits of AMPA receptors. GluA2 and GluA4 subunits provide highest agonist binding with GluA1 providing lowest. GluA3 subunits in different areas act inconsistently in this content. Transmembrane AMPA receptor regulatory proteins (TRAPs) also alter the agonist binding capacity of receptors. γ-3 and γ-8 always exhibit higher binding of agonists. The subunits are also directed by TRAPs, so that receptor subunit type has been a secondary deciding factor.

The efficacy of ampakines varies greatly between brain regions. Effects on hippocampus and cerebellum have been found to be nearly three times greater than that in thalamus, brainstem, and striatum, with cortex being intermediate. This regional difference may be explained by receptor subunit and TARP expression. In hippocampus, GluA2 is dominant with TARPS γ-3 and γ-8, which expresses higher binding values almost twice those of thalamic combination, GluA4 with TARPS γ-2 and γ-4 (17, 18).

Potential Uses

The main advantage of ampakines in therapy is that they promote LTP and increase BDNF levels in most critical regions of the brain for neurodegenerative disorders and cognition, such as hippocampus and cortical areas (19.20). It is also found that, "24 h off / 24 h on" (spaced) ampakine treatment protocol can be used to sustain elevated

neurotrophin levels, so that neuroprotection may be ensured by endogenous neurotrophins (21).

Memory and cognition disturbances are improved by ampakine therapy. In neurodegenerative disorders, memory deficits and learning impairment are accompanying features. Today, researches are focused in preventing this accompany (Figure 2) and ampakines are a candidate group for it (22). Neurobiological studies have enlightened three routes by which ampakines attenuate disturbed behavior (23,24):

I) Impairments in cortical transmission strength cause cognitive disturbances, as well as aging and other neuropsychiatric disorders are triggering factors. Ampakines enhance transmission without causing serious side effects.
II) Ampakines lower the induction threshold and increase the magnitude of LTP. The link between LTP and memory explain why these drugs accelerate learning.
III) Ampakines enhance the production of neurotrophic factors, such as BDNF, by accelerating and strengthening excitatory input. BDNF exerts potent and acute effects on synaptic plasticity. This provides various behavioral effects associated with ampakines.

Alzheimer's disease (AD) is distinguished by Aβ plaques, neurofibrillary tangles, synaptic loss and neuronal death. In AD, cognitive decline is an enormous problem. There were some imprecise findings, pointing that presence of Aβ plaques or tangles cause cognitive decline. On the other hand, there are numerous elderly people, who do not have impaired cognitive functions, but have Aβ plaques and tangles. The truth is that, Aβ plaques may disrupt normal memory and learning through negative regulation of synaptic properties. In the presence of soluble Aβ plaques, LTP is impaired and LTD (long-term depression) is facilitated. Soluble plaques also increase AMPA receptor internalization via clathrin-mediated endocytosis, which resembles LTD mechanism. As a result of this, overall excitatory transmission decreases, which is related to the loss of dendritic spine and synapse loss (10, 25, 26). It cannot be directly understood, if spine loss caused directly by AMPA receptor internalization. Researches to date have explained that soluble Aβ plaques bind to AMPA receptors to lead Ca^{2+} activation of calcineurin for clathrin-mediated AMPA receptor internalization. This endocytosis weakens the synapse and causes dendritic spine shrinkage, which alters the neuronal circuit (25).

By this time, some ampakines have been tried in animals and humans against cognitive impairment in AD. LY451395 showed reversal of age-related memory deficits and increased the performance in object recognition test in rats. However, in clinical trials, it was administered for 8 weeks to AD patients with mild/moderate cognitive impairment and provided no significant improvement compared to control group (27). This distinction between rats and humans may be caused by pharmacokinetic differences of species, insufficient concentrations of given drug, or too short treatment period.

Dopamine-related disorders, such as schizophrenia and hyperactivity are also in the target list of ampakines. They suppress abnormal behavior induced by amphetamine. This suggests that ampakines may be used in ***schizophrenia***. Ampakines also reduced ***hyperactivity*** in dopamine transporter-lacking mice. These effects can be explained as follows: ampakines increase glutamatergic activity in the forebrain and curtail effects of aberrant levels of activity in non-glutamatergic projections arising in the lower brain in monoamine systems (28).

Depression has many novel hypotheses in recent years, which render the disease to be cured by various therapy options. Positive allosteric modulators of AMPA receptors could function as a novel class of antidepressant agents. However, this idea conflicts with a number of ideas (29):

I) Depression is mainly linked with monoamine neurotransmitters and their increase with antidepressant treatment ameliorates the situation.
II) Glutamate levels have been reported to be increased in patients with major depression.
III) Antidepressant treatment is linked with NMDA receptor blockade.

To eliminate this contradiction, some other aspects have been identified as follows and ampakines are thought to be effective in treatment of depression. Evidences are:

I) AMPA receptor potentiators increase BDNF levels in relevant brain areas (20,21).
II) AMPA receptor poteniators increase cell proliferation in the hippocampus in vitro and in vivo (30).
III) AMPA receptor potentiators were studied in acute and chronic models of antidepressant activity and have been found effective in stress and depression models,

such as tail suspension test and forced swim test (28).

IV) AMPA receptor potentiators and conventional antidepressants demonstrate mutual synergy in animal models (29).

V) Widely used antidepressant drugs, like fluoxetine, activate AMPA receptors (31).

Stem cell-based applications give promising results in *in vitro* and *in vivo* testings in recent years. The subventricular zone (SVZ) is the main cerebral area containing neural stem cells with brain repair potential. Identification of new pharmaceutical compounds with neurogenesis-enhancing properties is important as a tool to promote neuronal replacement based on the use of SVZ cells. Ampakines are also thought to be effective on SVZ cells. Accordingly, CX546 treatment triggered an increase in proliferation in the neuroblast lineage and prevented apoptosis. Short exposure to CX546 increased axonogenesis and dendritogenesis. Altogether, it is showed that ampakine CX546 promotes neurogenesis in SVZ cell cultures and thereby may have potential for future stem cell-based therapies (32).

CONCLUSION

Today, from among ampakine drug groups, only "racetam" drugs are in use around the world. Because they were not specifically intended as ampakines, they were classified as nootropics. Among them, aniracetam and piracetam have been widely used. Other chemical classes are still under preclinical or clinical investigation.

Although ampakines give promising results in preclinical studies, initial clinical trials have not proven very successful. A reason for this failure could be their low potency. Production of more potent ampakines did not solve the problem and they have been still unsuccessful in clinical trials for the treatment of neurological disorders. The lack of tools and biomarkers to determine how ampakines modulate the deactivation and/or desensitization of AMPA receptors in vivo is a huge problem. Additionally, although mechanism of action of ampakines has been identified, but there is only less evidence about the morphological changes at synapses following a long ampakine treatment. As ampakines induce LTP, it is usually said that spine formation is the main goal of these molecules. However, it should be remembered that both spine formation and spine loss are crucial for effective learning and memory functions. The complicated nature of learning and memory process may also be a reason for the clinical unsuccess of ampakines and more detailed researches are needed to understand all effects of ampakines in synapses. Despite a couple of problems in clinical settings, ampakines promise new therapeutic solutions for a variety of neuropsychiatric disorders.

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