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Enhancing AMPA to NMDA throughput as a convergent mechanism for antidepressant action

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Abstract

In this article, we first review current knowledge of the AMPA and NMDA glutamate receptors, their physiological properties and functions and their regulation by signaling cascades. We then discuss our hypothesis that the therapeutic effects of monoaminergic antidepressants and ketamine, an NMDA receptor antagonist, may be mediated by increased AMPA to NMDA throughput in critical neuronal circuits. We hypothesize that ketamine mediates this throughput directly, thus resulting in rapid antidepressant effects whereas monoaminergic antidepressants work indirectly and gradually; this may explain, in part, the delayed onset of several weeks to months that is observed with traditional antidepressants.

Introduction

Medications currently used to treat major depression include a variety of antidepressants that are primarily serotonergically and/or noradrenergically based. Although many people suffering from major depression benefit from existing antidepressant medications, these therapies have several limitations including treatment-limiting side effects, and incomplete response and remission rates. Recent data from a large effectiveness study in major depression involving approximately 2876 outpatients found that only 28% of patients achieved remission within 10–14 weeks. However, by 24 weeks 50% eventually did achieve remission with either a switch to another antidepressant or an augmentation strategy [1]. This study suggests that although people with major depression do benefit from antidepressants, it often takes up to 6 months and two antidepressant trials to achieve remission and a significant proportion of patients are still left unwell. The search for more tolerable and effective antidepressants has been underway for several decades. However, an area that has been neglected is the lag in onset of our current therapies. Since antidepressant medications often take weeks to months to achieve their full effects [2], patients remain vulnerable to the risk of self-harm. Sustainable pharmacological strategies with rapid onset profiles of hours or even days would therefore have an enormous impact on public health.

Finding a more direct target for depression

As noted above, a major limitation in the currently available monoaminergic antidepressants is their delayed onset of action. This delay has led to a heuristic 'initiation and adaptation' hypothesis, which posits that the delay may be due to the medications exerting their primary effects considerably upstream of their ultimate therapeutic targets. In this context, it is noteworthy that a growing body of data suggests that upon chronic administration, antidepressants attenuate expression and/or function of NMDA receptor subunits. This has led to an interest in glutamatergic-based potential therapeutics [3–5]. Substantiating this interest, recent clinical studies have shown that the NMDA antagonist ketamine exerts rapid antidepressant effects [6,7]. Specifically, robust, rapid and sustained (1–2 weeks) antidepressant effects have been observed after a single intravenous ketamine infusion in patients with major treatment-resistant depression [7]. Understanding ketamine's effects on the glutamatergic system, a drug that has been shown to have rapid antidepressant action, could possibly lead to insights in developing the next generation of more rapid-acting and effective therapies.

The direct antagonist effects of ketamine on the NMDA receptor complex are well known [8]. In addition to being a non-competitive NMDA antagonist, ketamine also induces rapid increases in extracellular levels of glutamate by facilitating the presynaptic release of glutamate [9], a process that is believed to be mediated by NMDA autoreceptors, and/or by GABAergic interneurons.

We hypothesize that the antidepressant properties of ketamine are mediated by the functional interplay of AMPA and NMDA receptors. In our model, ketamine increases presynaptic release of glutamate, which in turn preferentially activates AMPA over NMDA receptors due to NMDA receptor (NMDAR) blockade. The net effect of this action is increased glutamatergic throughput of AMPARs relative to NMDA receptors.

Glutamatergic system – AMPA and NMDA receptor families

Glutamate exerts its function via three classes of ionotropic glutamate receptors, including AMPAR (GluR1–4), NMDAR (NR1, NR2A–D, NR3A–B) and kainate receptors (KA, GluR5–7, KA1–2), as well as a class of metabotropic glutamate receptors (mGluRs, mGluR1–8) [10,11]. Ionotropic glutamate receptors are ion channels that allow sodium, potassium and calcium to flow into or out of the cell upon glutamate stimulation. Pore opening and subsequent influx of ions leads to changes in membrane polarization as well as the direct activation of intracellular signaling pathways. The second group, 'metabotropic receptors' are G-protein-coupled receptors that exert their action indirectly via second messenger pathways [10,11].

AMPA are composed of four homologous subunits (GluR1–4), which assemble into various heteromeric tetramers. In the adult hippocampus, most AMPAR contain GluR1 or GluR3 subunits in combination with GluR2, which confers calcium impermeability [10,11]. The NMDAR is present in the brain primarily as a tetramer, in which two subunits are obligatory glycine-binding NR1 subunits, whereas the remaining two are typically glutamate-binding NR2 subunits (Fig. 1). The NR1 subunit plays a particularly important

role in the targeting and transport of NMDARs from their site of assembly in the endoplasmic reticulum to sites on the cell surface. Each subunit consists of an extracellular amino-terminal and ligand-binding domain, three transmembrane domains plus a re-entrant pore loop, and an intracellular carboxyl-terminal domain [12] for all ion channel glutamate receptors.

Functional regulation of AMPAR by phosphorylation

Recent studies in cultured neurons and brain slices have shown that the AMPAR subunits (GluR1, GluR2 and GluR4) can be phosphorylated at serine, threonine or tyrosine residues located on the intracellular C-terminal domain (Fig. 2, reviewed in [10]). Protein kinase signaling cascades, such as protein kinase A, protein kinase C, Ca^{2+} /calmodulin-dependent protein kinase II, and tyrosine kinases (Trks; or Src family kinases) are involved in site-specific AMPAR phosphorylation (Fig. 2, Table 1, [13–16]). Other glutamate (NMDAR) and G-protein coupled receptors (mGluRs, dopamine receptors DA1, 5-HT receptors) can also regulate AMPARs through protein phosphorylation and/or dephosphorylation mechanisms (Fig. 2, Table 1, reviewed in [10,11]). For example, stimulation of DA1 regulates GluR1 function via activation of PKA and the subsequent insertion of GluR1 into the neuronal membrane of cortical neurons [17]. It also has been reported that a growing body of evidence shows that protein phosphorylation directly modulates the electrophysiological (channel kinetics), morphological and biochemical (synthesis and subunit composition) properties of the AMPAR, as well as protein-protein interactions between the AMPAR subunits and various intracellular proteins (Table 1, [13–16]). These modulations underlie the major molecular mechanisms that ultimately affect many forms of synaptic plasticity in affective-associated neuronal circuitry [17,18].

Modulation of NMDAR functions by phosphorylation

NMDARs are targets for PKC and PKC-dependent tyrosine kinase cascades. There are two ways by which PKC modulates NMDA receptor – indirectly via Src family kinase (SFK) and by an alternative direct path. (1) Indirect path: via $\text{CAK}\beta$ then SFK cascades, PKC upregulates NMDAR function, by enhancing channel gating properties and increasing the number of receptors at the synapse (Fig. 3) [19,20]. Five members of SFK: Src, Fyn, Yes, Lck and Lyn, have been localized to the post-synaptic density [20]. SFK are important in regulation of NMDAR function through phosphorylation of specific sites on NMDA receptor subunits during synaptic plasticity and CNS development (Fig. 3 and Table 1). (2) Direct path: direct phosphorylation of NMDARs by PKC increases the sensitivity of the NMDAR to inactivation by intracellular calcium. In the hippocampus, PKC is a point of convergence for a variety of G-protein coupled and growth factor receptors at which to maintain control over NMDAR function and synaptic plasticity (Fig. 3 and Table 1) [19].

Striatal enriched tyrosine phosphatase (STEP) was recently shown to play an important role in regulation of synaptic function [21]. STEP down-regulates NMDAR, Fyn and MAP kinase activity by dephosphorylation of key activation sites [21]. It participates in several pathways, such as PKA, CaMKII and MAPK pathways in regulating synaptic function. G-

protein coupled receptors inactive STEP via PKA, whereas calcium activates STEP through a calcineurin-dependent mechanism (Fig. 3) [21].

Upon glutamate binding, influx of calcium through the NMDAR activates CaMKII. Ca^{2+} /calmodulin then promotes binding of CaMKII to NMDAR. CaMKII binds NR2B by its catalytic site and by the autophosphorylation site-binding pocket (APBP), a non-catalytic site. Mutagenesis of Glu-236, a residue in the APBP of CaMKII that likely interacts with NR2B, influences phosphorylation of NR2B [22]. After CaMKII and NMDAR bind to one another, continued calcium influx through NMDAR further activates CaMKII, which can subsequently phosphorylate AMPAR and other synaptic proteins that can facilitate long-term potentiation (Fig. 3 and Table 1) [23,24]. Subunit-specific regulation of NMDAR phosphorylation was summarized in Table 1.

AMPA/NMDA receptors are targets of antidepressant drugs

Recent data suggest AMPAR involvement in the mechanism of action of effective antidepressants. Studies have shown that chronic antidepressant treatment with norepinephrine and/or serotonin reuptake inhibitors (reboxetine, fluoxetine and desipramine) induce positive regulation of AMPAR surface expression by altering the phosphorylation levels of specific AMPAR subunits [25]. Acute fluoxetine treatment increases GluR1 phosphorylation at Ser831 and Ser845 (reviewed in [10]). In addition, chronic treatment with imipramine enhances hippocampal expression of GluR1 and GluR2/3 and GluR1 Ser 845 phosphorylation in rats [18,26]. Taken together, these results suggest that enhanced AMPA receptor function and/or inhibition of NMDA receptor function is associated with antidepressant effects.

AMPA/NMDA modulators have efficacy in the animal behavioral models for mood disorders

Preclinical studies have shown that drugs exerting positive allosteric modulation of AMPAR, called AMPAkinases also display antidepressant-like effects. Several classes of these small benzamide compounds have been developed, including benzylpiperidines (CX-516, CX-546), pyrrolidones (piracetam, aniracetam), benzothiazides (cyclothiazide) and biarylpropylsulfonamides (LY392098, LY404187) [27]. The antidepressant-like efficacy of AMPAkinases was shown to be similar to standard antidepressants in animal models of depression [7]. AMPAkinases have also been demonstrated to augment the effectiveness of standard antidepressants in animal models of depression [28].

Similar to AMPAkinases, chronic treatment with NMDA antagonists induces antidepressant-like effects in different animal models of depression [29]. Ketamine, a noncompetitive NMDAR antagonist, showed significant antidepressant-like effects in animal models (reviewed in [11]). NMDAR antagonists such as MK-801 and AP-7 have been shown to have antidepressant properties in animal models of depression, including the forced swim test (FST), the application of inescapable stressors and tail suspension test (TST), and in the learned helplessness model of depression. The antidepressant effects of NMDA antagonists are comparable to the tricyclic antidepressants (reviewed in [30]). Interestingly, the

antidepressant effects of ketamine seem to depend on the activation of AMPA-mediated synaptic activity (discussed in the next section). Similar to chronic treatment with diverse monoamine-based antidepressants, AMPA/kines were shown to enhance the expression of BDNF, which has been shown to have antidepressant efficacy in animal models of depression [27]. Interestingly, the AMPAR antagonist NBQX inhibited an increase in BDNF levels whereas NMDA receptor blockers did not negatively regulate this neurotrophin, which suggests specific positive regulation induced by AMPAR pathways in the expression and release of BDNF [31]. In summary, an increase in AMPA/NMDA throughput, which indicates AMPA activation and NMDA inhibition, appears to be associated with antidepressant action.

The NMDA antagonist ketamine in treatment-resistant major depression

Preclinical and clinical research indicates that the NMDAR complex may also be involved in the therapeutic mechanisms of antidepressant and possibly in the pathophysiology of depression (reviewed in [30]). In a double-blind placebo-controlled crossover study, we found that a single intravenous infusion of ketamine (0.5 mg/kg over 40 min) in patients with treatment-resistant major depression resulted in a rapid onset of antidepressant effects within hours. Seventy-one percent met response criteria (50% decrease in Hamilton depression rating scales (HDRS) scores), and 29% achieved remission (< 7 HDRS) at 24 h following the infusion of ketamine. Six (35%) subjects maintained response to ketamine for at least 1 week. Mild perceptual disturbances occurred in most patients; overall the study medication was well tolerated [7].

Although there is much preclinical information on the antidepressant-like properties of AMPA potentiators in depression, to date, there are no published studies with these types of compounds on their efficacy in mood disorders [32]. One trial is currently underway at NIMH to determine whether an AMPA potentiator (ORG24448) is efficacious in major depression <http://clinicaltrials.gov/ct/show/NCT00113022?order=1>. We review below our preclinical findings with AMPAR.

Function interplay between NMDA and AMPA receptors: does increasing glutamatergic throughput of AMPA relative to NMDA result in rapid antidepressant response?

As discussed above, we found that a single-intravenous infusion of the non-competitive NMDA antagonist ketamine, resulted in rapid (within 2 h), robust and relatively sustained (1–2 weeks) antidepressant effects in patients with treatment-resistant major depression [7]. Using ketamine as a pharmacological tool could possibly lead to insights in developing the next generation of rapid-onset therapies.

The direct antagonist effects of ketamine on the NMDAR complex are well known [8]. In addition to being a non-competitive NMDA antagonist, ketamine also facilitates the presynaptic release of glutamate [9]. This increase in glutamate release then preferentially favors AMPAR over NMDAR because the NMDARs are blocked by ketamine; the net effect is an increased glutamatergic throughput of AMPA relative to NMDA. Notably

antidepressants, upon chronic administration, have also been shown to enhance AMPAR surface levels [18,26]. In addition, AMPA potentiators have antidepressant-like properties in animal models [28]. We have therefore postulated that the therapeutic effects of both monoaminergic antidepressants and ketamine may be mediated by increased AMPA to NMDA throughput in critical neuronal circuits. The difference in the rapidity of onset of ketamine versus monoaminergic-based antidepressant (hours versus weeks/months, respectively) might be owing to the fact that traditional anti-depressants increase AMPA to NMDA throughput indirectly and gradually after a series of downstream signaling changes have occurred while ketamine does this more directly. Indeed, in animal behavioral studies, we found that in the forced swim test (FST), a test that has fairly high predictive validity in identifying antidepressant compounds, ketamine decreased the immobility time as also occurs with standard antidepressants [33,34]. In the same test, NBQX, an AMPA/Kainate antagonist, had no effects in FST when given alone. When NBQX was given immediately before ketamine and imipramine, it abolished the decrease in immobility time with ketamine but not imipramine [31,35]. This finding suggests, at least in animal models, that the antidepressant-like properties of ketamine are mediated in part by AMPAR throughput.

Concluding remarks

Although there are many antidepressants available for the treatment of our patients, it is evident that there are limitations in what they offer. Recent preclinical and clinical studies indicate that the functional interplay between AMPA and NMDA receptors in how they modulate glutamatergic throughput may be particularly important to the onset of rapid antidepressant effects. We hypothesize that antidepressant effects are mediated by increased AMPA to NMDA throughput in critical neuronal circuits and that ketamine does this directly – by increasing glutamate release and concurrently blocking postsynaptic NMDA receptors, whereas monoaminergic antidepressants do this indirectly and gradually by producing delayed effects on AMPA and NMDA receptor phosphorylation. Indeed, data by our group supports our hypothesis. Furthermore, more direct targeting of AMPA to NMDA throughput might be a strategy for treatment-resistant depression as this strategy would bypass defects in critical circuits, which monoaminergic antidepressants require for their therapeutic effects. This line of research holds considerable promise and might lead to the next generation of rapid-acting antidepressants, which could help to reduce the initial morbidity and mortality associated with the delay of current treatments.

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Figure 1.

Structure of NMDA receptor. NMDAR is a tetramer comprised of two NR1 and two NR2 subunits. This 3D schematic shows a cross-section of the NMDAR through the channel pore. The NMDAR is composed of four domains: amino terminal domain (ATD), agonist binding domain, transmembrane domain and intracellular C-terminal domain (not shown). The NR1 subunit binds glycine or D-serine and the NR2A/B subunit binds glutamate. Activation of the NMDAR requires binding of both glycine (or D-serine) and glutamate, as well as simultaneous depolarization of the ion channel by AMPA or kainate receptors, which relieves the Mg²⁺ block. Under these conditions, the NMDAR channel will open and permit the entry of both Na⁺ and Ca²⁺ and the exit of K⁺. Within the ion channel, two additional binding sites have been identified: the sigma (σ) site (holding Mg²⁺) and the phencyclidine (PCP) site. The hallucinogenic drug PCP and ketamine, bind the PCP site and are considered noncompetitive receptor antagonists, which inhibit NMDAR channel function. Several other endogenous ligands interact with the NMDAR at different sites on the molecule and can have modulatory effects on its function. The endogenous polyamine spermine interacts with the NR2B subunit of the NMDAR and potentiates channel opening; paradoxically, it (and other polyamines) can also block the channel, reducing its conductance. Zinc also inhibits the NMDAR in a subtype-specific manner; receptors containing NR2A are inhibited by far lower concentrations of zinc than are those containing NR2B. Receptor activity is also modulated, at least in vitro, by redox state and pH. The 3D structure was cited from Dr Huggins' article [55].

Figure 2.

Regulation of AMPAR trafficking by signaling cascades. AMPAR trafficking and channel properties are regulated by phosphorylation of C-terminal domain and subsequent alteration of protein–protein interactions. (1) Phosphorylation of GluR1 at the PKA site S845 facilitates insertion of GluR1 into the neuronal membrane and is often associated with induction of long-term potentiation. Dephosphorylation of GluR1 by protein phosphatases (e.g. calcineurin and PP1) targets GluR1 to recycling endosomes, where re-phosphorylation by PKA can occur, with subsequent reinsertion into the neuronal membrane. (2) CREB has been shown to be important for AMPAR synthesis at the synapse. (3) In response to stimulation, CaMKII translocates to postsynaptic sites from dendrites and phosphorylates GluR1 at Ser831 to enhance AMPAR single channel conductance, as well as to aid in its delivery to the synapse (reviewed in [10]). (4) Ras mediates activity-evoked increases in synaptic GluR1 and GluR4 containing AMPARs via a pathway that requires p42/44 MAPK activation (reviewed in [10]). (5) It was recently shown that GSK-3 β mediates NMDAR-induced GluR1/2 internalization (Professor Yu-Tian Wang, pers. commun.). Phosphorylation of Ser880 by PKC on GluR2 causes a switch from receptor retention at the membrane by binding to ABP/GRIP, to receptor internalization by binding to PICK 1 to release the AMPAR from anchoring proteins and initiate receptor internalization (reviewed in [10]).

Figure 3.

Regulation of NMDAR functions by phosphorylation. NMDARs are regulated by several signaling cascades. (1) G-protein coupled receptors interact with and liberate α and $\beta\gamma$ subunits, leading to activation of phospholipase C (PLC). PLC activation induces DG and IP₃ production, leading to PKC activation and intracellular calcium release. PKC and calcium promote activation of CAK β , and its association with SFK. Phosphorylation of NMDAR by SFK (indicated by red color) will either enhance NMDAR channel gating or synaptic localization. The phosphorylation sites of SFK are indicated by red color. PKC also directly phosphorylates NMDA receptors (sites marked in green) [56]. (2) Initial calcium influx and subsequent formation of the Ca²⁺/calmodulin complex promotes binding of CaMKII to NMDAR. Continuous calcium influx through NMDAR further activates CaMKII, which phosphorylates NMDAR, AMPAR and other proteins important for long-term potentiation [23]. (3) Stimulation of G-protein coupled receptors (such as dopamine receptor 1) leads to cAMP synthesis and activation of PKA. PKA releases the inhibition of Fyn from RACK1 and also prevents STEP from interacting with its substrates to promote tyrosine phosphorylation of NMDAR. (4) Calcium influx and activation of calcineurin (PP2B) leads to dephosphorylation of STEP and thereby activation of STEP, which dephosphorylates NMDAR at their SFK sites [21].

Table 1

Phosphorylation of AM PA and NMDA and receptors and regulation of their functions

Receptor	Phosphosite	Kinase	Functional regulation	Refs
AM PA receptors				
GluR1	S845	PKA	Insertion of GluR1 onto the neuronal surface, increasing channel open probability	[13,16]
	S831	CAMKII/PKC	Trafficking of GluR1 to the synapses. Increasing single channel conductance	[13,36–38]
	S818	PKC	Promoting GluR1 synaptic incorporation	[14]
GluR2	S863	PKC	Regulating GluR2 internalization	[19]
	Y876	Src	Tyrosine phosphorylation of GluR2 dissociates GluR2 from GRIP1/2	[39]
	S880	PKC	Inhibition of the association of GluR2 to GRIP	[40]
GluR4	T830	PKC	Unknown	[41]
	S842	PKC/PKA/CaMKII	Driving AMPA receptor to the synapses	[41]
NMDA receptors				
NRI	S890	PKC α	Dispersion of surface-associated cluster of NRI, membrane localization	[42]
	S896	PKC γ	Enhancing NMDAR delivery to the synapses	[42,43]
	S897	PKA	Phosphorylation is significantly reduced in schizophrenia samples	[42,44]
NR2A	Y402	Pyk-2	Li inhibits pNR2A Y402 increase after ischemia	[45]
	Y416	Src	Li inhibits pNR2A Y416 increase after ischemia	[45]
	Y1292	Src and Fyn	Controlling NMDA receptor current modulation	[46]
	Y1325	Src and Fyn	Unknown	[46]
	Y1387	Src and Fyn	Controlling NMDA receptor current modulation	[46]
	S1232	Cdk5	Increasing spontaneous NMDA mediated Excitatory Post Synaptic Currents (EPSCs)	[47,48]
	S1291 S1312	PKC PKC	Necessary and sufficient for insulin induced potentiation of NR2A receptors	[49]
	S1416	PKC	Inhibiting of α CaMKII-binding and promoting dissociation of α CaMKII. NR2A complex	[50]
NR2B	S1303	PKC CaMKII	Phosphorylation of NR2B at Ser (1303) by CaMKII inhibits glutamate binding and promotes slow dissociation of preformed CaMKII.NR2B complexes	[51]
	S1323	PKC	Potentiation of EPSCs	[49]
	Y1336	Fyn	Enhanced by Eupherin B	[52]
	Y1472	Src	Potentiation of EPSCs, NMDA receptor insertion to the synapses; Li inhibits Src and NR2B S1472 phosphorylation	[52,53]
	S1480	CK-2	Phosphorylation leads to decreased surface expression of NR2B	[54]