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Review Article

What is the mechanism of Ketamine's rapid-onset antidepressant effect? A concise overview of the surprisingly large number of possibilities

S. E. Strasburger* PharmD, P. M. Bhimani* PharmD, J. H. Kaabe* PharmD, J. T. Krysiak* PharmD, D. L. Nanchanatt* PharmD, T. N. Nguyen* PharmD, K. A. Pough* PharmD, T. A. Prince* PharmD, N. S. Ramsey* PharmD, K. H. Savsani* PharmD, L. Scandlen* PharmD, M. J. Cavaretta* PharmD and R. B. Raffa*† PhD

Temple University School of Pharmacy, Philadelphia, PA, and †University of Arizona College of Pharmacy, Tucson, AZ, USA

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SUMMARY

What is known and objective: **Abundant clinical data now confirm that ketamine produces a remarkable rapid-onset antidepressant effect – hours or days – in contrast to the delayed onset (typically weeks) of current antidepressant drugs. This surprising and revolutionary finding may lead to the development of life-saving pharmacotherapy for depressive illness by reducing the high suicide risk associated with the delayed onset of effect of current drugs. As ketamine has serious self-limiting drawbacks that restrict its widespread use for this purpose, a safer alternative is needed. Our objective is to review the proposed mechanism(s) of ketamine's rapid-onset antidepressant action for new insights into the physiological basis of depressive illness that may lead to new and novel targets for antidepressant drug discovery.**

Methods: **A search was conducted on published literature (e.g. PubMed) and Internet sources to identify information relevant to ketamine's rapid-acting antidepressant action and, specifically, to the possible mechanism(s) of this action. Key search words included 'ketamine', 'antidepressant', 'mechanism of action', 'depression' and 'rapid acting', either individually or in combination. Information was sought that would include less well-known, as well as well-known, basic pharmacologic properties of ketamine and that identified and evaluated the several hypotheses about ketamine's mechanism of antidepressant action.**

Results: **Whether the mechanistic explanation for ketamine's rapid-onset antidepressant action is related to its well-known antagonism of the NMDA (*N*-Methyl-D-aspartate) subtype of glutamate receptor or to something else has not yet been fully elucidated. The evidence from pharmacologic, medicinal chemistry, animal model and drug-discovery sources reveals a wide variety of postulated mechanisms.**

What is new and conclusion: **The surprising discovery of ketamine's rapid-onset antidepressant effect is a game-changer for the understanding and treatment of depressive illness. There is some convergence on NMDA receptor antagonism as a likely, but to date unproven, common mechanism. The surprising number of other mechanisms, and the several novel biochemical aetiologies of depression proposed, suggests exciting new drug-discovery targets.**

Correspondence: Prof R. B. Raffa, 3825 E. Diablo Canyon Place, Tucson, AZ 85718, USA. Tel.: +1 610 291 7019; fax: +1 215 610 291 7019; e-mail: robert.raffa@gmail.com

At the time of writing, the first 11 authors were PharmD candidates. M. J. Cavaretta is Clinical Assistant Professor Temple University School of Pharmacy. R. B. Raffa is Professor Emeritus at Temple University School of Pharmacy and Adjunct Professor at University of Arizona College of Pharmacy.

WHAT IS KNOWN AND OBJECTIVE

The diagnostic criteria for major depressive disorder (MDD) as delineated in the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-V) require a series of symptoms including depressed mood, lack of interest or pleasure in activities, irritability, unintentional weight change, inability to concentrate, overwhelming feelings of guilt or worthlessness and suicide ideation (MDD is associated with high suicide rates if untreated).[1] It is one of the most widespread psychiatric illnesses, with an estimated prevalence of 10%–19% based on geographic location, differences in clinician diagnoses and reporting errors.[2] The prevalence rates for some countries, such as Japan, the Netherlands and Germany, are slightly lower (8%–12%).[3] For reasons not completely identified, the estimates for the United States are higher (18%). The wide variability might be biologically meaningful, due to underdiagnosis (mild cases of MDD may be self-limiting), underreporting[4] or other reason. Women are twice as likely as men to be diagnosed with MDD, but it is not clear whether this is due to physiological differences or sociological factors.[5] Whereas MDD can develop at any age, the most common age of onset is generally between 17 and 25, potentially requiring lifetime treatment with antidepressants.[6] The aetiology and clinical progression of MDD are multifactorial, including genetic and environmental influences.[7,8]

The pathophysiology of MDD is still relatively poorly understood, but the historically prevailing theories derive from the monoamine hypothesis, and the majority of current pharmacotherapy targets a presumptive hypoactive monoamine system, primarily 5-HT (5-hydroxytryptamine, serotonin), but also norepinephrine or some combination of these. Current first-line antidepressant drugs include SSRIs (selective serotonin reuptake inhibitors) (e.g. citalopram, fluoxetine, paroxetine and sertraline), SNRIs (serotonin–norepinephrine reuptake inhibitors) (e.g. desvenlafaxine, duloxetine, levomilnacipran and venlafaxine), norepinephrine–serotonin inhibitor (mirtazapine) and a dopamine–norepinephrine reuptake inhibitor (bupropion). The

efficacy of these agents is initially limited during therapy, where suicide risk may be a primary concern, and remission is often not sustained in the long term, even if the agent is initially effective. The major issue with these agents is that it takes several weeks to months to produce positive effect, which leaves the patient susceptible when suicide is an imminent concern. Equally concerning is that a study conducted by the NIMH (National Institute of Mental Health) found that <33% of patients had achieved remission by the end of 12 weeks of therapy and only one-third of patients failed to achieve remission by the end of one year despite the use of four different agents.[9] A few compounds have been found to produce an antidepressant effect more rapidly than do the traditional pharmacotherapeutic options, but it still takes about 3–7 days until benefits are seen.[10]

Non-pharmacologic treatment includes cognitive behavioural therapy (CBT) and other therapies used in conjunction with pharmacotherapy,[11] but their use is sometimes limited by high cost or lack of coverage under health insurance plans. Electroconvulsive therapy (ECT) is currently largely out of favour in the United States, but is an option for severe persistent depressive disorder refractory to pharmacologic treatment. ECT is a rapid and effective therapy in such patients, with reported Hamilton Depression Rating Scale (HAM-D) scores in the single digits within 7 days (HAM-D score is a rating scale used to determine a patient's level of depression; single digit numbers correlate with a favourable response).[12] Success rates range between 50% and 90% depending on the number of ECT sessions per week and utilization of maintenance therapy afterwards. The risk of life-threatening response from ECT is 1 : 30 000, and most common cognitive deficits can be avoided through unilateral positioning of the electrode.[13] Of most immediate relevance, the results of ECT studies demonstrate that rapid-onset antidepressant action is feasible and that the affected biochemical pathway(s) can be targeted.[12]

Recent evidence shows convincingly (i.e. multiple tests by multiple independent investigators) that ketamine produces a rapid-onset antidepressant effect in animal models of depression and in human clinical trials.[14] In patients suffering from MDD, including treatment-resistant patients, clinical response upon administration of ketamine generally occurs within hours. Similarly, ketamine consistently produces rapid antidepressant-like effects in relevant rodent models of depression (e.g. the forced-swim and tail-suspension tests, novelty-suppressed feeding, sucrose preference, elevated plus maze, spontaneous or open-field locomotor and behavioural activity, and chronic mild stress tests).[14] However, it is unlikely that ketamine will become first-line therapy for most patients who have MDD because of its adverse effect profile. Nevertheless, its success raises questions about our current understanding of the biochemical pathophysiology of depression and casts doubt on the wisdom of current monoaminergic therapies.[14]

The mechanism of action of ketamine's rapid antidepressant effect is unknown, but is thought to be related, at least in part, to its antagonism at the NMDA receptor and also involve, directly or indirectly, other postulated pathways.[15] The quest for the mechanism of ketamine's antidepressant action has prompted study of other agents that share presumptive similar pharmacologic properties. Compounds currently being studied include the following: dextromethorphan, CERC-301 (formerly MK-0657, 4-methylbenzyl (3*S*,4*R*)-3-fluoro-4-[(2-pyrimidinylamino)methyl]-1-piperidinecarboxylate), cycloserine ((*R*)-4-amino-1,2-oxazolidin-3-one), lanicemine (formerly, AZD-6765 or (1*S*)-1-phenyl-2-pyridin-2-ylethanamine), AP5 (2-amino-5-phosphonopentanoic acid), dizocilpine (formerly MK-801, 5R,10S]-[+]-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine), traxoprodil (formerly CP-101,606, (1*S*,2*S*)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol), rapastinel (formerly GLYX-13, Thr-Pro-Pro-Thr-NH_2), Ro 25-6981 (αR-(4-hydroxyphenyl)-βS-methyl-4-(phenylmethyl)-1-piperidinepropanol, 2Z-butenedioate), memantine, LY-341495 (2-[(1*S*,2*S*)-2-carboxycyclopropyl]-3-(9*H*-xanthen-9-yl)-D-alanine), MGS-0039 ((1*R*,2*R*,3*R*,5*R*,6*R*)-2-amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3·1·0]hexane-2,6-dicarboxylic acid), amantadine, lamotrigine, and riluzole.[16–21] Dextromethorphan has pharmacologic properties in common with ketamine and has been reported to produce a rapid-onset antidepressant effect.[22] A recent study suggests that in addition to binding to NMDA receptors, dextromethorphan exerts some of its antidepressant action through sigma-1 receptors. If the results can be generalized, such findings and those from related investigation of other pathways would provide new leads for potential new classes of antidepressant medications.[23]

Despite recent advances in the genetics and molecular biology of depression and modest improvement in therapeutic options, there remains a critical need for improved – namely rapid-onset – antidepressant therapies. Ketamine's demonstrated ability to induce a rapid onset of antidepressant effect shows that a clinically meaningful level of improvement can be achieved much earlier than that provided by current therapies.[24] Additionally, ketamine appears to work via a different mechanism of action, suggesting that the currently accepted knowledge surrounding the pathophysiology of MDD may be suboptimal and, further, that it is possible to develop improved novel classes of antidepressants.

METHODS

A literature and Internet search was conducted to identify current information available on rapid-acting antidepressants and their mechanism of action. Key search words included, for example, 'ketamine', antidepressant', 'mechanism of action', 'rapid acting', 'dextromethorphan', combinations of these and others. The search terms were selected to obtain information that would encompass the well-known, as well as the less well-known, basic science information about the pharmacologic properties of ketamine; to identify recent clinical trials undertaken to test the efficacy of ketamine as an antidepressant; and, in particular, to identify and summarize the hypotheses about the mechanism of ketamine's antidepressant action. The 'hits' arising from these searches were used as sources and starting points for further searches. Additional non-published sources were obtained, for example, from poster presentations at scientific meetings. All were reviewed, evaluated and summarized.

RESULTS

Glutamate and NMDA receptor antagonists

Various studies implicate glutamate in the pathophysiology of MDD, making it a potential target for antidepressant action:[25] patients with depression have higher serum levels of glutamate than do non-depressed subjects;[26] there is a positive correlation between plasma glutamate levels and the severity of depressive symptoms in patients with MDD; elevated glutamate levels are reduced during antidepressant treatment;[26] some current antidepressant agents have effects on NMDA receptor function; and most recently, ketamine, which is a non-competitive NMDA receptor antagonist, has been shown to have rapid antidepressant activity.

There are several metabotropic (G protein-coupled) and ionotropic (ligand-gated ion channel) glutamate receptors. The metabotropic glutaminergic (m-Glu) receptors to date have not been a successful target for the treatment of depression.[24] The three ionotropic glutamate receptors are as follows: NMDA (*N*-methyl-D-aspartate), AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate), and kainate. Within the NMDA receptor is an internal binding site for Mg^{2+} ion, which creates a voltage-dependent block, and the removal of which depends on AMPA receptor activity. The NMDA subtype of glutamate receptor has been implicated in MDD. As summarized in a recent review,[9] MDD is associated with decreased synaptic connectivity in prefrontal cortex and hippocampus, and increased synaptic activity in other parts of the brain such as the amygdala and the nucleus accumbens. Decreased synaptic activity is hypothesized to result from abnormally high and sustained levels of extracellular glutamate throughout the brain. NMDA receptors are located both intra- and extrasynaptically. Activation requires co-activation of glutamate and glycine sites. When NMDA receptors are activated inside the synapse, they help promote synaptic formation and cell survival; but excess extracellular glutamate that binds to extrasynaptic NMDA receptors leads to synaptic atrophy and neuronal death because it enhances excess influx of Ca^{2+}. Decreased levels of synaptic glutamate result in less binding to synaptic AMPA receptors and decreased levels of the neurotrophic factors BDNF (brain-derived neurotrophic factor) and mTORC1 (mechanistic target of rapamycin complex 1). Low serum levels of BDNF and mTORC1 are common in patients with MDD.[9] Although some studies have suggested that ketamine's antidepressant effect might be due to modulation of the AMPA receptor, evidence suggests that rapid activation of AMPA receptor signalling pathways may be secondary to antagonism of the NMDA receptor.[9]

Some studies report that the non-competitive NMDA receptor antagonist dizocilpine has antidepressant activity when administered alone or in combination with other antidepressants.[27] A small open-label trial reported that the non-competitive open-channel NMDA receptor antagonist memantine (used in the treatment of moderate-to-severe Alzheimer disease) produces significant improvement in depressive symptoms within 1 week. Memantine has also been reported to significantly decrease the baseline level of depression in patients with comorbid alcohol dependence.[26] However, in another report, memantine did not improve depressive symptoms in patients with MDD. Therefore, the actual efficacy of memantine as an antidepressant has yet to be determined.

The tricyclic antidepressant desipramine and surprisingly fluoxetine, a selective serotonin reuptake inhibitor (SSRI), both have some NMDA receptor antagonist properties.[25] An animal study involving administration of imipramine with amantadine, a non-competitive NMDA receptor antagonist, suggested antidepressant activity (reduced immobility time compared to either treatment alone).[25] The study also examined combinations with antidepressants from different classes. Most combinations showed a positive effect, suggesting that using a combination of an NMDA receptor antagonist with current antidepressant therapies might produce improved antidepressant response.

Some other antidepressant therapies have been shown to involve the NMDA receptor:[28] for example, electroconvulsive shock and chronic antidepressant treatments modulate NMDA receptor subtypes and expression of NMDA receptor subunits. Lithium deactivates an NMDA receptor subunit, which may contribute to neuroprotection; chronic medications that work at the NMDA receptor lead to changes in the monoaminergic modulatory systems, which may further suggest the involvement of the NMDA receptor in depression.

Recent clinical trials of several new NMDA receptor antagonists have demonstrated potential effectiveness. For example, infusions of AZD6765 (lanicemine), a low-affinity open-channel NMDA receptor antagonist, were shown to promote a rapid antidepressant effect compared to placebo.[26] In a double-blind crossover study (N = 22), depression scores improved significantly in as little as 80 min and lasted several days.[29] Unlike ketamine, AZD6765 produced no adverse effects and no difference in psychotomimetic or dissociative effects. Another randomized placebo-controlled study of 3-week duration (N = 152) reported that repeated administration of lanicemine at 3-day intervals provided sustained antidepressant effects.[30] Ro 25-6981 ((α*R*,β*S*)-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinepropanol maleate), an antagonist of an NMDA receptor subtype (GluN2B),[31] was shown to produce rapid (within 24 h) antidepressant effects in rats.[32] MK0657 (CERC-301), an orally bioavailable NMDA receptor 2B subtype antagonist (NR2B),[33] is generally well tolerated in humans. A randomized study of this drug in patients with resistant MDD showed significant antidepressant effects in as early as 5 days when compared with placebo. GLYX-13 (Rapastinel), a partial agonist at the NMDA receptor glycine modulatory site,[3] produced rapid (within 15 min) and long-lasting (at least 2 weeks) antidepressant effects in patients who had failed in conventional antidepressant treatment. NRX-1074, an orally active agent, is more than 1000-fold more potent than GLYX-13 and produces rapid (within 24 h) and long-acting antidepressant-like effects in animal models.[26]

Another proposed mechanism posits agonism at $sigma_1$ receptors. The results from a recent animal model study suggested that at least part of dextromethorphan's mechanism of action is through that pathway.[23] In this study, mice were injected with imipramine as a positive control, normal saline, dextromethorphan or one of two known selective $sigma_1$ receptor antagonists (BD1063 and BD1047). The mice were tested using forced-swim tests and locomotor-activity tests before and after injections of the drugs.[23] Both imipramine and dextromethorphan had an effect on mouse behaviour, whereas normal saline and the $sigma_1$ antagonists did not. The mice that received the dextromethorphan injection were then given an injection that contained a combination of dextromethorphan and a $sigma_1$ antagonist. The combination had much less effect on mouse behaviour than did dextromethorphan alone.

NMDA receptor antagonists such as ketamine and dextromethorphan are known to bind to and inhibit the actions of extrasynaptic NMDA receptors. This enhances glutamate release in the synapses, which binds to AMPA receptors, which leads to increased production of BDNF and mTORC1 and increases neuronal plasticity.[9] BDNF is a protein that is involved in the proliferation, differentiation and survival of neuronal cells, which contributes to synaptic plasticity and connectivity in the adult brain. The single nucleotide polymorphism Val66Met has been associated with reduced BDNF activity, and this allele also has a proposed effect on ketamine's antidepressant mechanism of action: in mice that have a knock-in of the human Val66Met polymorphism, the antidepressant action of ketamine is blocked, and humans with this polymorphism have a decreased response to ketamine.[35] This implies that at least a part of ketamine's antidepressant mechanism of action is through BDNF release, because patients with the Val66Met polymorphism have lower amounts of BDNF release in the brain.[35] Elevated BDNF and mTORC1 levels are also observed after a few weeks of therapy with traditional antidepressants (which corresponds to the time

course of the clinical effect), and ketamine promotes synaptogenesis by inhibiting the protein eEF2 (eukaryotic translation elongation factor 2), which decreases BDNF production.[9] An increasing level of BDNF may be a large part of the antidepressant activity of NMDA receptor antagonists.[16] Supporting this link, about 25% of the population has a BDNF Val66Met polymorphism – and this allele blocks activity-dependent release of BDNF and a markedly decreased response to treatment with ketamine.[16] BDNF release is believed to induce mTORC1 signalling and leads to increased synapse formation. It is also believed that ketamine needs to induce mTORC1 signalling through this pathway in order to have an antidepressant effect, because several studies have shown that ketamine's actions are blocked by the mTORC1 inhibitor rapamycin.[36]

The NMDA receptor and current antidepressant therapy (e.g. SSRI, SNRI)

Interestingly, current antidepressant pharmacotherapy has been linked to the NMDA receptor.

The role of glutamate in MDD was suspected when it was reported that tricyclic antidepressants have functional effects on the NMDA receptor by blocking the ion channel associated with the receptor.[15] These findings prompted a number of questions about how current therapies (especially SSRIs and SNRIs) may involve NMDA function (Fig. 1). It is summarized as follows:[15,37]

- Citalopram (an SSRI) increases glutamate levels.[38]
- But escitalopram (an SSRI) yielded no change in glutamate or glutamine despite improvement in HAMD scores.[39]
- Changes in both NMDA and AMPA receptors accompany fluoxetine (an SSRI) treatment.[40]
- NMDA receptors are downregulated following treatment of animals with citalopram (an SSRI) or fluoxetine (an SSRI).[41,42]
- NR2B levels decrease after induction of depression in a rat model; venlafaxine (an SNRI) treatment prevented the reduction of NR2B expression, but escitalopram (an SSRI) treatment did not.[37]

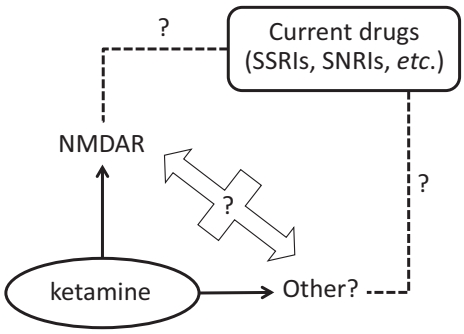

Fig. 1. Ketamine and current antidepressants might share common mechanistic pathways – or not. Is there some shared physiological mechanisms relating ketamine's antidepressant action with the mechanism of the current neuronal monoamine reuptake inhibitor antidepressants? Neither the SSRIs (selective serotonin reuptake inhibitors) nor the SNRIs (serotonin/norepinephrine reuptake inhibitors) have affinity for the NMDA receptor (NMDAR), but perhaps they influence NMDAR activity indirectly. Alternatively, ketamine and current antidepressants might share some other, as yet unidentified, pathway(s).

NMDA receptor antagonists. Memantine and amantadine have been the most tested NMDA antagonists for possible rapid-acting antidepressant effects, but the trials showed variable response rates with an onset in the range of weeks rather than hours or days.[43–48] The trials did lead to investigation of other compounds that have varying activities at the NMDA receptor. Recent studies of new NMDA receptor antagonists in clinical trials demonstrate the possible effectiveness of such targets in depression. Infusions of Lanicemine (AZD6765), a novel low-affinity open-channel NMDA receptor blocker, produced an antidepressant effect within 3 days of administration, and in one trial within 80 min.[18] A randomized study of MK0657 (now CERC 301), an orally bioavailable NMDA receptor subtype antagonist, produced significant antidepressant effect in patients with resistant MDD in as early as 5 days when compared with placebo in a small population of patients.[19] A phase II trial of this compound was recently completed.[49] Another agent with NMDA antagonist activity, riluzole, demonstrated an antidepressant effect in small, open-label trials.[50,51] All NMDA receptor antagonists tested (ACPC [1-aminocyclopropanecarboxylic acid], AZD6765 [lanicemine], ketamine, memantine, MK-801 [dizocilpine] and RO 25-698) have produced statistically significant reductions in tetrabenazine-induced ptosis in mice, showing that this is a class effect.[52]

NMDA receptor agonists. Interestingly, some studies have reported that some NMDA receptor agonists produce a rapid-onset antidepressant effect. This observation led to a search for alternative strategies, including even testing NMDA receptor agonists. As recently reviewed,[24] Rapastinel (GLYX-13) (Thr-Pro-Pro-Thr-NH_2), a selective weak partial agonist of the glycine site of the NMDA receptor, had positive effects on mood and depression; a glycine transporter inhibitor was more efficacious than citalopram in depression in animal models, and high doses of D-cycloserine, an NMDA agonist, produced an antidepressant effect.

NMDA receptor partial agonists. A small trial using the NMDA partial agonist D-cycloserine (D-4-amino-3-isoxazolidinone) reported some effect on MDD, but failed to find a significant difference vs. placebo, possibly due to the small study size.[53] GLYX-13 (rapastinel) was tested as a single IV bolus dose and had an effect within 2 h. The trial had a large placebo effect nearly equal to that of GLYX-13, but there was a significant increase in duration of the antidepressant effect in the study arm.[54] A large, phase II trial of GLYX-13 in refractory MDD (N = 371) has been completed.[55] And a phase II trial of apimostinel (NRX-1074) is underway.[56] Overall, the results from animal studies and early clinical trials have shown promise for this mechanism. The current clinical trials will be crucial in determining the potential of NMDA partial agonists in MDD.

NMDA receptor subtypes. A study was conducted to determine the importance of the NMDA receptor subtype GluN2B (*GRIN2B*) in depression-like behaviour. The study generated cortex- and principal neuron-specific GluN2B knockout (KO) animals. Each group was injected with either saline or ketamine. The KO animals displayed a large decrease in depression-like behaviour compared to the wild-type animals in two standard tests (the forced-swim test and the tail-suspension test.[57] This implies that subtypes of the NMDA receptor might be responsible for ketamine's antidepressant action.

Postulated other mechanisms of action

Kynurenine pathway. A proposed mechanism of ketamine's rapid-onset antidepressant action is its inhibition of quinolinic acid, an

activator of inflammatory pathways, and a metabolite of the kynurenine pathway.[58] Inhibitors of the kynurenine pathway, a major tryptophan metabolism pathway, are neuroprotective.[59] And alterations in kynurenine metabolism have been linked to depression.[60] Some components of this pathway are agonists, and others antagonists, of the NMDA receptor.[61] Influencing these pathways has been suggested as a possible explanation, or goal, for a rapid-acting antidepressant action.[62]

Patients with MDD have increased levels of the pro-inflammatory cytokines IL-6 and TNF-alpha. These cytokines individually activate both indoleamine 2,3-dioxygenase (IDO) and kynurenine monooxygenase (KMO). IDO promotes the inactivation of endogenous 5-HT and promotes conversion of tryptophan into kynurenine. Kynurenine is metabolized to form kynurenic acid and quinolinic acid. Kynurenic acid is an endogenous NMDA receptor antagonist; quinolinic acid is an endogenous NMDA receptor agonist. In human astroglial cells, there is a balance between quinolinic acid and kynurenic acid levels that prevents the neurotoxic effects associated with higher quinolinic acid levels.[63,64] Suicidally depressed patients have increased levels of quinolinic acid compared with healthy patients,[63] implicating the kynurenine pathway in MDD. Ketamine reduces IL-6 and TNF-alpha levels, slowing down cytokine-mediated deactivation of 5-HT and cytokine-mediated conversion of kynurenine into quinolinic acid.[63]

Gpr39. GPR39 KO mice showed significantly increased immobility time in the forced-swim test compared to that of wild-type controls.[65] Chronic administration of monoaminergic-based antidepressants (escitalopram, imipramine) caused a significant reduction in immobility time compared to wild-type controls injected with saline; however, these drugs were ineffective in GPR39 KO mice. Only NMDA antagonists (MK-801 and ketamine) were active in the GPR39 KO mice. MK-801 and ketamine were also active in wild-type mice and produced a significant reduction in immobility time when compared to controls injected with saline. The results of this study suggest that GPR39 is probably required to produce a response with monoaminergic-based antidepressants.

Zinc is not only a functional antagonist of the NMDA receptor, but it is also important in maintaining homoeostasis between glutamate and GABA via the GPR39 receptor. A preclinical study showed that zinc deficiency causes GPR39 downregulation, which was found to be correlated with depressive-like activity in zinc-deficient rodents. GPR39 downregulation was also discovered in frontal cortex and hippocampus of suicide victims, suggesting involvement in suicidal behaviour. Administration of monoaminergic antidepressants significantly increased GPR39 protein in frontal cortex of mice, coincident with its involvement in the antidepressant response.[65]

Bdnf. Patients who have MDD have low BDNF levels. A direct relationship with BDNF levels and antidepressant effect is suspected, as BDNF levels are not normalized until 2–3 weeks after initiation of traditional antidepressants, a time course that corresponds to therapeutic effectiveness. BDNF levels are increased in just 4 h following ketamine injection, again corresponding to clinical antidepressant effect.

Evidence from KO mice. Ketamine's rapid-onset antidepressant effect is not present in eEF2 kinase knockout mice, BDNF knockout mice or AMPAR subunit GluA2 knockout mice (Fig. 2).[66] Decreased numbers of NMDA receptors, PSD-95 (post-synaptic density protein 95; synapse-associated protein 90, also termed SAP-90,), mTOR (a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis and transcription), p70S6 kinase (a serine/threonine kinase that acts downstream of PIP3 and phosphoinositide-dependent kinase-1 in the PI3 kinase pathway) and eIF4B (a translation initiation factor) have been demonstrated in patients with MDD. These findings implicate the role of these proteins in the pathophysiology of MDD and represent possible targets for treatment discovery strategies.[67]

GABA and AMPA receptors. Ketamine binds to and inhibits NMDA receptors located on GABAergic interneurons. Normal stimulation of these GABAergic interneurons by glutamate results in inhibition of glutamate release in the prefrontal cortex. The blockade of these GABAergic interneurons by ketamine results in disinhibition of glutamate signalling and produces a transient, rapid release of glutamate.[68] The increase in glutamate release activates AMPA receptors, which causes depolarization via Na^+ influx. This results in release of BDNF from synaptic vesicle stores and activates the mTORC1 pathway. The end result is increased protein translation, synaptogenesis, increased AMPA receptor trafficking and increased levels of BDNF. BDNF activates TrkB (Tropomyosin receptor kinase) receptors, which activates ERK (extracellular signal-regulated kinases) signalling which further increases mTORC1 signalling.[68]

mTOR. Rapamycin co-administered with ketamine blocks the rapid antidepressant effects of ketamine, suggesting that the mTOR pathway is involved in the antidepressant action of ketamine. Activation of the mTOR pathway is associated with increased protein translation, increased synaptogenesis, increased spine formation of dendritic cells, increased synaptic proteins, increased levels of BDNF, increased AMPA trafficking and

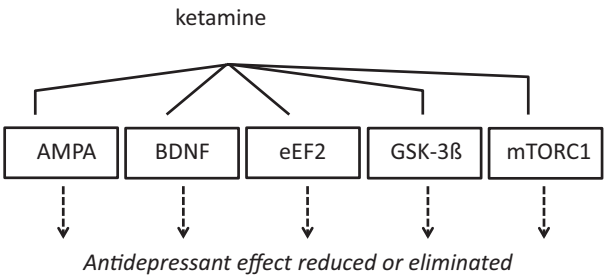

Fig. 2. Non- or indirect NMDA receptor pathways implicated in ketamine's antidepressant action. In addition to the NMDA receptor, several other receptors/pathways have been implicated in ketamine's antidepressant action, because interference with the pathways (using antagonists, receptor mutation or gene knockout) reduces or eliminates ketamine's effect. Specific targets include the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) subtype of glutamate receptor, BDNF (brain-derived neurotrophic factor), eEF2 (eukaryotic elongation factor 2), GSK-3β (glycogen synthase kinase 3, a regulatory kinase recently shown to be involved in the action of Lithium) and mTORC1 (mechanistic target of rapamycin complex 1). Prior to the discovery of ketamine's efficacy, none of these were widely considered to be prime targets for antidepressant pharmacotherapy.

enhanced synaptic activity. Increased levels of synaptic proteins such as PSD-95 and synapsin I are observed within 2 h post-ketamine injection. Increased levels of cytoskeletal Arc protein are increased within 1 h after ketamine injection.

eEF2. NMDA receptors are activated following spontaneous glutamate release, which results in the activation (phosphorylation) of eEF2 by eEF2 kinase, which inhibits BDNF translation. Ketamine binding to NMDA receptors results in deactivation of eEF2 kinase and subsequent increase in BDNF translation.

Other. Administration of ketamine resulted in the upregulation of PSD95, GluR1 and synapsin within 2 h for up to 72 h and resulted in the upregulation of Arc within 1 h for up to 6 h. Arc is a cytoskeletal protein linked to induction of early- and late-phase long-term potentiation.

Possibly multiplicative. It is possible that ketamine has numerous antidepressive mechanisms of action due to the fact that it binds to many different receptors in the brain. For example, ketamine is an antagonist at the nicotinic acetylcholine receptor subtypes α7 (IC50 = 20 μM) and α4β2 (IC50 = 50 μM).[69] Ketamine's affinity for the D2 receptor is the same as its affinity for the NMDA receptor,[70] and it also has affinity for the 5HT2 receptor.

Another possible antidepressive mechanism of action for ketamine is the inhibition of glycogen synthase kinase-3β.[16] GSK-3β is believed to play a role in lithium's mechanism of action in bipolar disorder. GSK-3β's function is blocked by phosphorylation, and treatment with ketamine induces phosphorylation of this protein. Mice with a knock-in of GSK-3β do not show an antidepressant response to ketamine.[16]

Combinations. When ketamine and AMPA are administered together at doses that are ineffective when administered individually, a significant antidepressant effect is observed in an animal model of depression. A greater level of antidepressant effect was not observed when the animals were given both AMPA and ketamine at a higher dose, suggesting that AMPA has a dose-dependent antidepressant effect and a possible ceiling effect. Western blot analysis revealed a significant increase in BDNF, synapsin and p-mTOR in the rats that were administered ketamine alone, AMPA alone or the combination.

A new series of indazole dual neurokinin 1 receptor antagonists and 5-HT transporter inhibitors have recently been reported to have robust in vivo efficacy in a preclinical depression model.[71]

Antagonism at the GluN2B Receptor. NMDA receptors are hetero-multimeric complexes that are comprised of two GluN1 and two GluN2 subunits. In vivo depletion of GluN2B imitates ketamine's effect on depressive behaviours and excitatory synaptic transmission.[57] When glutamate binds to the GluN2B subtype, which is abundant in cortical neurons, it suppresses mTOR signalling and decreases protein synthesis, resulting in impaired communication between the neurons in the prefrontal cortex, which can potentially lead to depression-like effects. Genetically deleting GluN2B subtype in mice produces an antidepressive effect similar to ketamine. The effects of ketamine are reduced in mice without this subtype. The data suggest that ketamine acts directly on principal cortical neurons to antagonize GluN2B, effectively decreasing the suppression of mTOR. This results in increased protein synthesis, an increase in the number of excitatory inputs and more normalized behaviour.

Ambient glutamate currents are absent in neurons where GluN2B was genetically replaced with GluN2A, demonstrating that the GluN2B subtype may be more sensitive to glutamate. Glutamate is highly regulated by excitatory amino acid transporters (EAATs). Therefore, focusing on agents that can enhance EAAT function and permit a decrease in ambient glutamate might help regulate protein synthesis and depressive symptoms.

WHAT IS NEW AND CONCLUSION

Antidepressant pharmacotherapy has focused primarily on enhancing neuronal serotonin and/or norepinephrine reuptake. Unfortunately, these traditional antidepressant treatments are effective in only approximately one-third of patients with MDD, and there is an average delay of about 2 weeks until there are signs of clinical efficacy. Due to the high risk of suicide associated with MDD patients, there is a need to find treatments that act more quickly, yet are also safe and effective. The recent surprising results of a rapid-onset antidepressant effect of ketamine suggest a promising approach. The mechanism of ketamine's antidepressant action might be via NMDA receptors, but this has not been established definitively. The search for other pathways for ketamine's action has identified potential receptor and other targets, such as HT_{2C} receptors and GluN2B-containing NMDA receptors, certain transporters, such as EAATs glutamate transporters, and others summarized in this review. Because some of these pathways are only in the early stage of research, there are no conclusive studies yet on how effective they will be, or in what populations they will work best. However, the groundwork has been laid, and current research carried out so far can be used to potentially develop new drug classes that may have more rapid-acting onset than the current delayed-onset regimens, without the adverse effects associated with ketamine. It could also be a game-changer for the understanding of the aetiology and time course of MDD.

CONFLICT OF INTEREST

The authors report no conflict of interest that involved or influenced the content of this manuscript.

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