Ketamine: An Update on Cellular and Subcellular Mechanisms with Implications for Clinical Practice

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Background: Ketamine is one of the oldest hypnotic agents used to provide an anesthetic agent with analgesic properties and minimal suppressive effects on respiration. The ability of ketamine in modulating glutamatergic (N-methyl D-aspartate) pain receptors has made this anesthetic drug a new option for the management of patients with chronic pain syndromes. Further preclinical and clinical findings suggest ketamine may have wide ranging effects on both cognition and development. Recent advances have revealed an unprecedented role for ketamine in the acute management of depression.

Objectives: The purpose of this review is to integrate a number of basic science, preclinical, and clinical studies with the goal of providing insight into the possible signaling events underlying ketamine's biological effects in pain management, depression, cognition and memory, and neurodevelopment.

Study Design: Narrative literature review.

Setting: Health science library.

Methods: A comprehensive literature search was performed for the following medical subject headings and keywords (ketamine, anesthesia, pain, analgesia, depression, NMDA receptors) on PubMed, Google Scholar, and Medline from 1966 to the present time. The search was then limited to those in the English language. The full text of the relevant articles were printed and reviewed by all authors.

Results: We provided a comprehensive review of the literature that explored the pharmacologic aspects of ketamine from its conception as an anesthetic to its evolution as a drug used for treatment of depression and pain. To address the patient response variability observed in clinical studies, we have provided possible patient-specific factors that could contribute to outcome variability.

Limitations: Like any review, this study was limited by publication bias and missing information on negative studies which were denied publication.

Conclusions: Ketamine, an old anesthetic agent with analgesic properties, is currently being considered for treating patients with chronic pain and depression. The complex pharmacological characteristics of ketamine make this medication a multifaceted therapeutic option in these cases.

Key Words: Ketamine, anesthetics, pain, depression, pharmacology

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Since the synthesis and discovery of its fast-acting analgesic properties in the 1960s, compound CI581, later known as ketamine, was quickly adopted for clinical and military use (1). In 1970, wounded soldiers in the Vietnam conflict were the first to receive ketamine in a non-experimental, field hospital setting. It became a popular analgesic as soldiers could quickly and easily administer ketamine to each other without the need of medical attention for assistance (2). It rapidly took the place of the current pharmacologic interventions for pain since the prominent drugs at the time, phencyclidine and opiates, carried with them serious psychotomimetic effects and addiction liabilities. Furthermore, opioid tolerance posed major clinical impedance toward managing chronic pain.

Ketamine, a derivative of phencyclidine, exerts its therapeutic effects by reversibly blocking the activity of N-methyl-D-aspartate receptors (NRs). NR hyperactivity is the underlying mechanism of sensitization to noxious stimuli (3,4) and opioid unresponsiveness (5). Given the promising therapeutic potential of ketamine during an early period of burgeoning interest and need for pain management due to military conflict, the primary use of ketamine has been its application to anesthesia and pain management. Consequently, the historically accepted purpose and treatment modality of ketamine has been to block NRs to elicit analgesia. Today's clinical uses of ketamine have scarcely wavered from this perspective and it is mostly used for anesthesia and for perioperative analgesia. However, new research has identified numerous cellular and molecular mechanisms that highlight the potential for clinical diversification of ketamine administration.

A review of the contemporary research has provided compelling evidence for a versatile range of biological and physiological changes that result from ketamine exposure (6). In addition to the well-established analgesic and general anesthetic effects, several preclinical and clinical lines of evidence suggest ketamine may also exhibit a fast-acting anti-depressant property and reduced suicidality within hours after administration (7-9). In addition to their involvement with depression, NRs have essential roles in synaptic long-term potentiation (LTP) and long-term depression (LTD). These processes are believed to be critical components of Hebbian learning paradigms and, therefore, information integration and storage. Hence, it is not surprising that ketamine is also implicated in modulating learning and cognition (10,11).

In addition, NRs are also dynamic in their pharmacology and physiological function. NRs are a subclass of the ionotropic glutamate receptor (iGluR) family which mediates the majority of excitatory glutameric synaptic transmission in the central nervous system. The principal iGluRs at central neuronal synapses are AMPA receptors and NRs. NRs assemble as heterotetramers composed of 2 obligate glycine-sensitive GluN1 subunits and 2 glutamate-sensitive GluN2(A – D) or glycinergic GluN3(A-B) subunits. In addition to this diversity, the cytoplasmic tail region of GluN1, which is critical for protein interactions and post-translation modifications, is organized into several cassettes that are present in various combinations depending on pre-mRNA splicing events. A short region in the extracellular region (exon 5) is also a site of splicing. These events lead to 8 distinct functional GluN1 isoforms. Therefore, many combinations of NR subunits can be achieved, each with potentially distinct cellular and subcellular expression, functional, and pharmacological profiles. NRs allow Na+/Ca^2+ influx into the cell which, in addition to mediating the slow component of excitatory postsynaptic currents, regulates many signal transduction pathways important for cell survival or apoptosis, learning, and memory (12).

A consequence of the NR-mediated rise of intracellular Ca^2+ is the downstream regulation of gene expression. In particular, the change in brain-derived neurotrophic factor (BDNF) expression as a consequence of ketamine treatment has been of interest. Early preclinical evidence has identified lower BDNF levels in animal models of depression that can be elevated by antidepressant therapies (13). Like other antidepressant treatments (14), ketamine administration has been correlated with an increase in plasma BDNF levels in patients with treatment-resistant depression (15,16). However, because ketamine targets a different system than classical antidepressants (e.g., selective serotonin re-uptake inhibitors [SSRIs] and monoamine oxidase inhibitors [MAOIs]) there is great potential therapeutic value in exploiting the glutamatergic signaling pathways by ketamine to modulate BDNF expression in depressed patients.

Because of the complexity of both ketamine and its classic target, NRs, the purpose of this review is to summarize the current evidence for a potential clinical use of ketamine as a versatile therapeutic agent. We will explore molecular mechanisms behind these various physiological effects and propose explanations into the variability of patient responses to ketamine treatment.

KETAMINE IN PAIN MANAGEMENT

First recognized for its anesthetic actions, ketamine has been of interest for managing both acute and chronic pain. However, ketamine's psychotropic symptoms have restricted its use to cases of severe pain. To date, there is no objective method to weigh the negative effects of ketamine (psychotropic) against those of opiates (respiratory depression, addiction, death), non-steroidal anti-inflammatory drugs (gastric and renal complications), gapapentinoids (sedation, imbalance, falls, mood disturbances, suicidal ideation, cognitive impairment, weight gain), local anesthetics (cardiac and central nervous system complications), or other medications used in the treatment of pain. Despite the high prevalence of pain in developed nations, the molecular mechanisms underlying pain pathophysiology are incompletely understood. While opioids successfully treat short-term acute pain, the efficacy of opioid use for managing long-term chronic pain is less clear (17,18). Hence, there is an imminent need for more diverse treatment options in the management of pain. A review of ketamine's primary and secondary targets may offer new insights into ketamine's potential uses in pain management.

Molecular Mechanisms

Research into chronic pain has implicated many diverse physiological processes including loss of descending pathway inhibition of pain signals, immune cell activation in the spinal cord, release of inflammatory cytokines, functional changes in neuronal activity (neuroplasticity), and upregulated NR expression and phosphorylation (19-23). Ketamine's analgesic effects on chronic pain have been shown to modulate several of these pathways. A critical process underlying chronic pain pathogenesis is central sensitization (Fig. 1). In contrast to peripheral sensitization where plastic changes in peripheral neurons result in lower thresholds for neural activity in response to otherwise non-noxious stimuli (allodynia, hyperalgesia), central sensitization involves changes in the functional properties of central neurons such that the experience of pain is no longer coupled with the characteristics of the pain stimulus (presence, intensity, duration, and frequency) (4). Because both central and peripheral sensitization involve changes in synaptic plasticity in central and peripheral neurons, respectively, NRs are strongly implicated in these processes given their essential role in plasticity (22,24). Consistent with this, central sensitization is associated with augmented NR-dependent long-term potentiation within the spinal cord central pain pathways (24-26). Ketamine blockade of NR current may, therefore, attenuate the induction of synaptic plasticity and prevent functional changes in central neurons associated with the maintenance of chronic pain states.

Synaptic activity is tightly coupled with regulating downstream genomic targets that are critical for plasticity (27,28). Increased synaptic activity has been shown to increase BDNF levels (29). BDNF is known to reciprocally increase NR levels and, thus, is a critical factor in maintaining long-term plasticity. In pathologic states, BDNF is a contributor to the development neuropathic pain likely through modulating both spinal and supraspinal neural activity (30,31). One effect of ketamine infusion in animal models has been augmentation of BDNF protein translation (32). This increased BDNF expression, in conjunction with NR antagonism, may underlie the mechanisms for the moderate efficacy of ketamine in managing chronic pain (33).

A major challenge to pain management is the tolerance to opioids that develops over time necessitating the development of alternative treatment options. There has been evidence to suggest bidirectional functional coupling between NR activity and opioid receptors. In particular, δ-opioid activation in rat dorsal horn neurons enhances NR currents in these neurons (34). Conversely, this increased NR activity in central neurons in vitro has an inhibitory effect on opioid receptors (35). The ability of ketamine to modulate opioid receptor-mediated analgesia has been reportedly demonstrated in human cohorts (36). While opioid-induced hyperalgesia is antagonized by ketamine co-administration in humans, definitive evidence for the opioid receptor-dependent potentiation of NR activity in a human sample is lacking given ketamine's range of off-target effects and no definitive electrophysiological evidence (37). Nevertheless, this NR/opioid receptor interplay is speculated to underlie the development of opioid tolerance in human populations and may offer insight into the observed benefit of opioid and ketamine co-administration in treating certain pain (38,39). Thus, ketamine inhibition of NRs may prevent the NR-dependent attenuation of the opioid receptor which reduces or delays opioid tolerance (40-42).

In addition to ketamine's action on NRs, it is prudent to consider other relevant off-target sites of ketamine action in order to move toward a comprehensive understanding of ketamine's analgesic effect.

Substance P receptors are found in central and peripheral nervous systems and are critical for nociception by sensing substance P release primarily from afferent neuron C-fibers into the spinal cord (43,44). Early work found these receptors may be upregulated in a chronic pain rodent model, while loss of substance P receptors reduces the animal's pain sensitivity (45,46). Thus, these receptors are strongly implicated in the normal and pathological pain responses. A study in a recombinant system expressing substance P receptors found that ketamine inhibits these receptors by reducing their affinity for substance P (47). Ketamine reduces substance P receptor currents by 6.6 ± 2.0%, 19.3 ± 6.1%, and 37 ± 7.8% at concentrations of 10 μM, 100 μM, and 1 mM, respectively, indicating that ketamine's analgesic effects are in part the result of direct inhibition of substance P receptors.

Presynaptic NRs have also been identified to modulate the vesicular release of substance P, thereby providing additional cellular level evidence for ketamine activity through the substance P pathways (48). This effect in tangent with direct inhibition of both NRs and substance P receptors may significantly reduce substance P receptor-mediated nociception.

Multiple neuronal circuits are implicated in pain physiology. Impaired dopaminergic signaling is hypothesized to contribute to pain and analgesia (49). Ketamine has been shown in rodent models to potently stimulate D2 dopamine receptors (50,51). In addition, ketamine administration resulted in higher levels of dopamine (52). Thus, ketamine-dependent potentiation of dopamine signaling may contribute to ketamine's analgesia, but additional research will be needed to quantify the clinical significance of these findings.

Early evidence has implicated muscarinic acetylcholine receptors (mAChR) in pain processes (53). Interestingly, mAChR agonists have been shown to increase pain sensitivity thresholds (54). Functional data in recombinant systems show that ketamine inhibits mAChRs (55). Although ketamine has a 10 – 20 fold lower affinity for muscarinic receptors relative to NRs, ketamine's analgesic action may involve direct action on acetylcholine receptors (56). Preclinical *in vivo* experiments have demonstrated increased mAChR expression after ketamine administration (57). Thus, part of ketamine's analgesic action may involve altered expression of the mAChR. Furthermore, a functional interaction between mAChRs and NRs via G-protein and intracellular Ca^2+ signaling has been established. Stimulation of mAChRs can either potentiate or depress NR activity in CA1 and CA3 regions of the hippocampus, respectively (58,59). These region-specific modulations of NR activity can have profound impacts on signal integration and plasticity and, thus, central sensitization.

Serotonergic signaling from the rostral ventromedial medulla in the brainstem facilitated hypersensitivity to pain after mechanical injury (60-62) suggesting serotonergic pathways appear to have a pro-nociception role. Using a mouse infraorbital nerve chronic constriction model for trigeminal sensitization with genetically encoded fluorescent Ca^2+ indicators, this descending serotonergic pathway from the rostral ventromedial medulla facilitated TRPV1-dependent neuropathic (63). Interestingly, one of ketamine's secondary sites of action is the inhibition of serotonin receptor 1 and 2 (64). Therefore, inhibition of these pro-nociceptive pathways may contribute to ketamine's analgesic properties, but remains to be studied in further detail.

Other Clinical Considerations

Ketamine is administered as a racemic mixture of S(+) and R(-) enantiomers which is rapidly metabolized into norketamine and 6-hydroxy-norketamine enantiomers. Norketamine metabolism is extremely slow and more stable (65,66). Despite its rapid production and slow elimination, the effect of norketamine on physiological processes is less well understood. Preclinical *in vitro* and *in vivo* animal models have identified that norketamine also inhibits NRs in central neurons (67). Though a complete characterization of ketamine metabolites is lacking, inclusion of enzyme inhibitors with treatment may increase ketamine lifetime, drug safety, and therapeutic efficacy (68). In addition, patient renal and hepatic health should be taken into consideration when determining individual doses.

Ketamine impacts the physiological processes of multiple cell types at various levels with an implication in clinical practice. The ability for neurons to communicate (synaptic transmission) is tightly regulated by glial activity by controlling glutamate transport, ATP release, glial-to-neuron gap junction communication, regulation of cerebral blood flow, glucose transport, structural support, and cell volume regulation (69,70). Therefore, central processes such as central sensitization require glial activity (71,72). The glial requirement in central pain processes may involve the release of BDNF to control neural plasticity (73) and the release of proinflammatory cytokines (74,75). Pharmacological modulation of glia cells has also been shown to modulate responses in pain models (76-78). Given the role for glia activity

in these processes, the inclusion of glial inhibitor, L-α-aminoadipate, in tangent with ketamine to treat pain showed additive analgesic actions beyond ketamine or L-α-aminoadipate alone (79). L-α-aminoadipate acts by inhibiting glial enzymes including the glutamate transporter, GLT-1 (80). GLT-1 confers to astrocytes the role of glutamate scavenging in central and peripheral nervous systems. In a spinal nerve ligation model of neuropathic pain, persistent astrocyte activation is observed along with a biphasic increase in GLT-1 expression in astrocytes followed by downregulation (81). The initial GLT-1 upregulation may prevent excess glutamate accumulated from activating NRs and central sensitization (82). The subsequent downregulation phase is expected to reduce glutamate uptake, contribute to pain, and lead to increased pain sensitivity (83). In particular, spinal astrocytes play an important role in pain signaling (84,85) and, within the spinal cord, NRs containing GluN2B and GluN2D subunits mediate the majority of glutamatergic neurotransmission, but the clinical effect of ketamine on these signaling components remains to be fully elucidated (86).

Conclusions

The putative mechanisms by which ketamine elicits analgesia span a diverse array of physiological processes. This diversity inherently provides an advantage over highly specific and targeted pharmacological therapies, which can lead to tolerance over time. In addition, the many targets of ketamine are directly involved in various aspects of pain pathogenesis (e.g., NRs and Substance P receptors), but also are implicated in multiple layers of regulation of these processes (e.g., glial activity and mAChRs). This multimodal mechanism of ketamine-elicited analgesia represents an intrinsic advantage over other therapeutic options. However, more research is warranted to identify and objectively weigh the possible side effects that could result from this target diversity.

Ketamine as a Novel Antidepressant

The majority of antidepressant medications used today modulate various monoaminergic systems (e.g., serotonin and norepinephrine). However, response rate appears to have plateaued around 60% and require significant time to have observable benefits (87). In addition, remission rates after first line treatment remain as low as 28% (88). Because of these challenges, there is a concerted effort to expand treatment options for the clinically depressed.

Implication of Glutamatergic Transmission in Major Depression

The first recognition of the possible involvement of glutamatergic pathways in depression came when the NR partial agonist D-cycloserine was shown to have antidepressant effects (89,90) implicating NRs and glutamatergic synaptic transmission in depression. Importantly, differences in NR subunit expression are observed in several brain loci in patients with depression (91) and that chronic administration of antidepressants itself alters NR expression (92-94). Together, these findings identify NRs as having a role in depression pathophysiology and highlight ketamine as a potential therapeutic.

Several landmark studies identified ketamine as a potent antidepressant. Patients fulfilling DSM-IV criteria for major depression were given either placebo saline or 0.5 mg/mL ketamine infusion. Significant improvement was observed in the ketamine cohort as early as 4 hours and was stable for 72 hours (7) to 4 weeks (95). The most intriguing aspect of these results is the stability of the antidepressant response beyond the lifetime of the drug, indicating that continuous antagonism of NRs by retaining ketamine cannot explain this prolonged effect (96). Therefore, a mechanism of molecular plasticity must exist.

Molecular Mechanisms (Fig. 1)

Research in animal models has been motivated to identify precise mechanisms of action in an effort to maximize therapeutic potential of modulating glutamatergic systems to treat depression (97). It has long been recognized that BDNF is a critical component in depression pathophysiology (98). BDNF has a neuroprotective role by acting on TrkB receptors. These receptors are highly expressed in various regions of the central nervous system (CNS) including the cerebral cortex, hippocampus, thalamus, choroid plexus, and granular layer of the cerebellum, brainstem, retina, and the spinal cord. TrkB is also coupled to several pro-survival pathways: Ras/ERK, PI3K/AKT, and PLC-γ (99). TrkB has been found to be down-regulated in patients with depression which is thought to reduce the efficacy of BDNF action possibly by reduced PI3K/AKT-mediated inhibition of GSK3 which was found to be necessary for ketamine's antidepressant action and reviewed elsewhere (100-102). BDNF levels are also often reduced in depressed patients and responders to antidepressant effects of ketamine also showed increased BDNF serum concentrations (15).
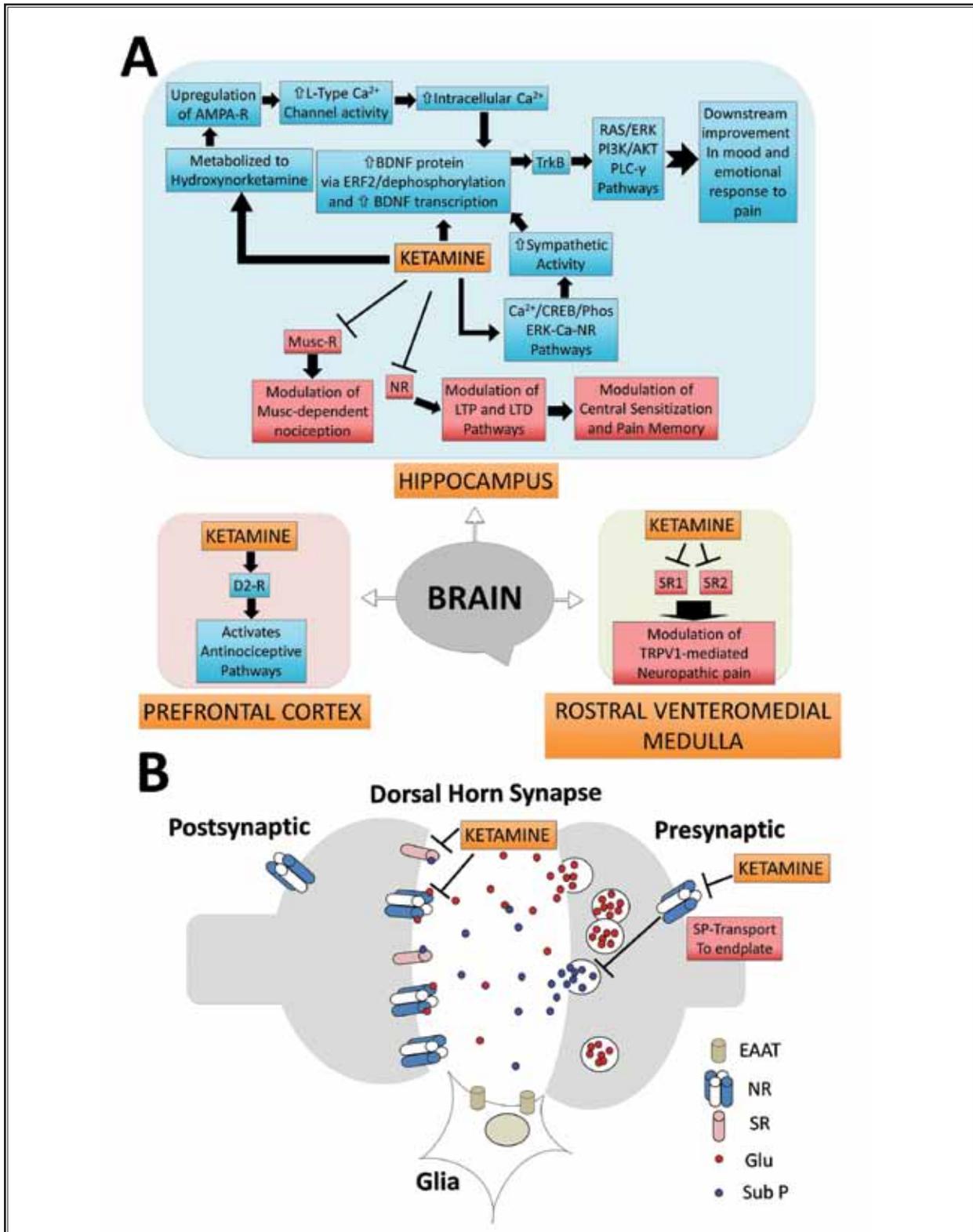
Fig. 1. *Illustration of ketamine's mechanism of action.*

Autry and colleagues (32) demonstrated that homozygous inducible BDNF-knockout mice were insensitive to the antidepressant effects of ketamine as measured by the forced swim test, a test that is highly predictive of monoaminergic antidepressant (tricyclics, SSRI, MAOI, atypical) efficacy. This suggests that BDNF is required for the antidepressant action of ketamine. This same study offered insight into the critical question of whether the ketamine-mediated increase in BDNF protein is due to upregulated transcription of BDNF mRNA or translation of BDNF protein. Single ketamine infusions (3 mg/kg) were given to mice pretreated with intraperitoneal injection of either the protein synthesis inhibitor anisomycin or the transcription RNA polymerase inhibitor actinomycin D. Only anisomycin-treated mice were resistant to the antidepressant effects of ketamine. These results have suggested that the therapeutic increases in BDNF expression associated with ketamine treatment are dependent upon BDNF translation, not transcription. A role for transcription cannot be completely ruled out, however, because the pharmacological inhibitors used have an 80% efficacy. Additionally, other studies have identified changes in BDNF mRNA upon ketamine treatment (103).

Nevertheless, this raises a paradoxical concern: NR-mediated changes in downstream signaling, such as protein translation, are thought to require Ca2+ influx via NR activation to activate these processes. How can blocking NRs with ketamine stimulate BDNF protein synthesis? NR components of miniature excitatory postsynaptic currents were probed in vitro to seek out an answer to this question, showing that ketamine diminished NR spontaneous synaptic activity in a resting neuron (without evoked action potentials) in a dose-dependent manner (1 µM – 50 µM) (32). Picrotoxin, a GABA receptor blocker used to increase synaptic glutamate release and, thus, synaptic activity, did not have any effect on antidepressant behavior when co-applied with ketamine. These results suggest antidepressant effects of ketamine act on resting neurons. Separately, it was found that ketamine results in removal of the inhibitory phosphate on the ribosomal catalytic factor, eEF2, allowing it to function and enhance protein synthesis. Consistent with this, other NR blockers, MK-801 or AP5, augment protein synthesis by enhancing eEF2 dephosphorylation (104). Therefore, it is clear that ketamine enhances BDNF protein translation.

Role of Magnesium in Ketamine Pharmacology

Ketamine acts by blocking NRs in a voltage-dependent manner. The ketamine binding site is located within the pore such that the NR must be open in order for ketamine to gain access to this site. This open-channel blockade results in use-dependent inhibition of NRs. Importantly, ketamine's binding site overlaps with the Mg^{2+} binding site within the pore at the N-site asparagine on the M^2 re-entrant loop within the membrane field (105,106). Therefore, competition with physiological Mg^{2+}, which constitutively blocks NRs at resting membrane potentials (107,108), is expected to impact ketamine pharmacology. However, many preclinical and in vitro experiments have only studied ketamine in Mg^{2+}-free conditions. When ketamine block was studied under physiological Mg^{2+} (1 mM), it revealed that Mg^{2+} enhances ketamine selectivity of GluN2C- and GluN2D containing NRs by shifting the affinity for ketamine to be higher than GluN2A and GluN2B receptors (109). Also, Mg^{2+} competes less effectively with ketamine in GluN2C and GluN2D receptors.

These findings have several implications. Firstly, NR subunits are spatiotemporally regulated in their expression throughout development. Animal models has revealed that only GluN2B and GluN2D subunits are expressed in embryonic rodents. Following birth, GluN2A expression gradually rises and becomes the dominant subunit in adulthood with high immunoreactivity in cortical, olfactory, hippocampal, and cerebellar regions. GluN2B expression peaks between postnatal day 7 to 10 and becomes gradually restricted to similar regions such as GluN2A with the exception of the cerebellar region. GluN2C is not expressed until postnatal day 7 and is localized in the cerebellar and olfactory areas. At all stages, GluN2D is restricted exclusively to the diencephalon and brainstem. This high expression of GluN2D rapidly diminishes into adulthood (110). Therefore, certain central nervous system regions may be more affected by the effects of ketamine than others at any given stage of development. Secondly, brain Mg^{2+} levels are significantly lower in treatment-resistant depressed patients (111). Thus, patient-specific fluctuations in extracellular Mg^{2+} may significantly impact the potency of the administered dose and may, potentially, affect treatment response.

Other Clinical Considerations

While clinical and preclinical studies have suggested that brain-derived neurotrophic factor is essential for appropriate antidepressant response, any given cohort receiving ketamine has responders and non-responders (15,16). No research has been successful in determining the factors that may influence the variability. Several possibilities include a common polymorphism in BDNF, Val66Met (rs6265), which is relatively prevalent (65% Val66Val to 35% Val66Met in the Caucasian population). Mice carrying the Met allele that had reduced hippocampal volumes displayed more depressive behaviors (112). Homozygous mice with the Met allele showed no recovery from depressive behaviors when treated with ketamine (113). Other BDNF polymorphisms (rs11030101 and rs10835210) occur more frequently in depressed patients (114) and influence the efficacy of standard electroconvulsive therapy (115). Taken together, more research is needed to validate a role of individual genetic variations in response to the BDNF modulatory role of ketamine and the correlated clinical outcomes.

In addition to genetic variations, epigenetic variations may also complicate the effects of treatment. BDNF gene expression is spatiotemporally regulated by specific promoter regions that are neural activity-dependent (116,117). These promoters contain or are in proximity to CpG island sites of DNA methylation, which remodel chromatin structure to alter gene expression. While aberrant BDNF gene expression occurs in major depression, dysregulation of DNA methylation has also been implicated in depression and is directly affected by antidepressant treatment (118). It still needs to be investigated whether ketamine specifically influences BDNF epigenetics.

It has recently been shown that the ketamine metabolite, hydroxynorketamine, was essential for mediating ketamine's antidepressant effect in a mouse behavioral model. Importantly, this metabolite was found to be sufficient to elicit an antidepressant response and exhibited minimal adverse side effects (119). However, hydroxynorketamine itself did not inhibit NRs; rather, in vivo electrophysiological evidence showed this metabolite produced a sustained potentiation of synaptic AMPA receptor responses in hippocampal neurons through upregulation of AMPA receptors at synapses which persisted even after the compound was removed. Akin to synaptic long-term potentiation, the increase AMPA receptor density leads to stronger depolarization of the membrane and greater activation of L-type calcium channels to potentiate the intracellular

Ca^2+ signal during synaptic transmission. This stronger signal facilitates the release of BDNF and contributes to a more effective antidepressant response (120).

Conclusions

Ketamine's modulation of glutamatergic signaling extends beyond direct inhibition of NRs and includes regulation of AMPA receptors. This multitiered mechanism can yield a more controllable outcome over other current antidepressant drugs in use. Because several unique pathways with various points of crosstalk are involved in depression, administration of additional agents with ketamine may improve the desired outcome. For example, administration of GSK3 inhibitor, Li+, has been shown to boost ketamine antidepressant efficacy despite WNT signaling pathways not being a direct target of ketamine (102). Further investigation is needed to explore whether such experimental or clinical control over which pathways may be targeted by ketamine can significantly improve the ketamine response or minimize undesired side effects that may arise from the large array of ketamine targets. This represents a possible substantial advantage over SSRIs which have a 20 – 1500 fold selective affinity for serotonin transporters over other targets (121) and over gabapentin which primarily targets the $\alpha2\delta$ subunit of presynaptic voltage-gated calcium channels. This reduces Ca^2+-induced vesicular release of neurotransmitter (e.g., glutamate and substance P) to reduce sensitization, yet because the diversity of neurotransmitter release is controlled by this signaling node, voltage-gated Ca^2+ channels, targeted or controlled therapeutics are more difficult. In contrast, tricyclic antidepressants, which have a broader pharmacological profile much like ketamine, exert their therapeutic action by modulating the amount of norepinephrine and serotonin via inhibition of reuptake and transport proteins. Thus, no long-term molecular plasticity underlies the tricyclic antidepressant mechanisms of action and may lead to lower rates of remission. Together, there is substantial evidence that ketamine offers novel avenues in managing pain while allowing the possibility of exploring targeted therapeutics.

KETAMINE IN COGNITION AND MEMORY

Opposing Roles of Ketamine on Cognition in Healthy and Depressed Individuals

With the emerging therapeutic uses of ketamine, a comprehensive understanding of its actions must be

determined, including the off-target pharmacological sites and the physiological consequences of modulating them. In addition to the known anesthetic, analgesic, and antidepressant modalities of ketamine administration, previous work in healthy participant suggested a negative impact on memory and cognition. Evaluation of healthy participants given ketamine (0.1 mg/kg or 0.5 mg/kg) displayed acutely impaired frontal cortical dysfunction and immediate and delayed recall (122). Given that no long term changes in cognition were found upon receiving anesthetic doses of ketamine (2.5 mg/kg and 5.0 mg/kg) (123), it would seem that the acute effects of ketamine are transient and reversible.

In contrast to healthy volunteers, patients with treatment-resistant depression also demonstrated enhanced neurocognitive function as measured by the Cogstate brief battery, which was accounted by improvement in depressive symptoms (11). From a pharmacological perspective, the molecular substrate of these clinical reports is essential for evaluating the overall therapeutic potential of ketamine.

Molecular Mechanisms

Classically, the neural elements of learning and memory formation have been studied with hippocampal brain slice and primary culture preparations to evaluate the opposing phenomena of long-term potentiation (LTP) and long-term depression (LTD). These are activity-dependent plasticity events whereby the probability of neuronal firing depends on prior neuronal activity. The involvement of glutamatergic receptors, such as NRs, in these processes is undisputed (124). Mechanistically, Ca^2+ influx through NRs stimulates cAMP production by calmodulin-dependent adenylate cyclase to activate protein kinase A and induce its translocation to the nucleus with calmodulin-dependent protein kinase II. These proteins induce expression of immediate early genes involved in synaptic plasticity via the CREB transcription factor (125). Ketamine inhibits expression of these genes presumably via NR blockade consistent with the impaired neurocognition in healthy individuals.

These forms of plasticity are, themselves, highly regulated, and one mechanism that has gained support is metaplasticity (126). This form of regulation takes a neuronal network view of plasticity rather than only considering activity at a single synapse. Since its identification, several forms have been described in the CNS. For example, in the hippocampus, the LTD of inhibitory synapses lowers the threshold of activity for excitatory synapses within the same neuron. Thus, metaplasticity is essential to normal physiology and can function to prime and facilitate the classical Hebbian plasticity normally associated with memory and learning (127,128). Exploration of the effects of ketamine on metaplastic processes is still in its infancy, but preliminary evidence suggests that ketamine facilitates metaplasticity, which contributes to the long-lasting antidepressant effects of ketamine (129,130).

In addition to our evolving view of these macroscopic functional processes underlying memory, the single synapse model of neurotransmission itself has grown in complexity. Until recently, the basic structure of the neuronal synapse involved a presynaptic neuron releasing neurotransmitter into the cleft. These transient fluctuations in neurotransmitter concentration are sensed by receptors on the postsynaptic neuron leading to transmission. This dogma has been challenged in the recent years with the notion that not all components required for synaptic activity are inherently present within neurons.

For example, d-serine was found to be a third endogenous ligand for NRs (in addition to the 2 classic ligands, glutamate and glycine) sharing the glycine binding site on GluN1 subunits. Selective enzymatic depletion of d-serine in hippocampal preparations attenuated NR activity (131). The origin of this endogenous ligand was unclear at the time. Glial cells surrounding synapses are known to contribute to the neurotransmitter pool within synapses and likely have the ability to sense neuronal activity and impact the regulation of synaptic activity. This tripartite model of the synapse has gained support with growing evidence (132). Panatier and colleagues (133) correlated the amount of astrocytic coverage on synapses with the amount of d-serine within these synapses. In the same study, astrocytic coverage of synapses could be positively correlated with degree of induced LTP in slices of supraoptic nuclei preparations. Subsequent work in hippocampal preparations supported this observation and it was additionally found that rises in intracellular Ca^2+ of astrocytes was essential for this astrocytic-dependent potentiation of synaptic activity (134). Introduction of exogenous Ca^2+ chelator (EGTA) into astrocytes blocked this effect. Importantly, there has been clear evidence for NR expression on astrocytes, themselves, as well (135). These results support a role for glial-derived d-serine modulating neuronal activity as opposed to neuronal derived d-serine (136). While these results do not exclude a role of neuronal d-

serine, a tripartite model of synaptic activity may yield more physiologically relevant interpretations of the ketamine effects on memory. Few studies have investigated the effects of ketamine on glia in the context of memory, but preliminary evidence suggests that ketamine may exert a protective effect on astrocytes (137), which may underlie clinically therapeutic actions of ketamine (138). Interestingly, serum levels of d-serine predicted therapeutic response to ketamine treatment in depressed patients in which lower levels were associated with better outcome (139). The exact mechanism underlying this association remains to be elucidated including whether this was glial-derived d-serine given that d-serine is also expressed in glutamatergic neurons (136,140-143).

The role of glia cells in neurological disorders has only recently gained research interest. It is clear that d-serine dysfunction is implicated in various neuropsychiatric disorders (144-146). In a clinical setting, adjuvant d-serine alleviates symptoms of schizophrenia and depression in preclinical (147,148) and clinical studies (149-153). Nonetheless, more research is needed to elucidate the clinical implications of NR-glial interactions pertaining to ketamine administration, cognition, and memory.

While NRs are considered the primary target for ketamine action, the secondary target sites cannot be ignored. One additional target is hyperpolarization-activated cyclic nucleotide-gated channel subunit 1 (HCN1). Ketamine inhibits HCN1-containing channels with a half-maximal concentration range of 8 – 16 µM, which is a clinically relevant concentration (154). HCN channels are voltage-sensitive, permeable to Na+ and K+, and nearly always open at resting membrane potentials to depolarize the membrane and set the final resting membrane potential. They respond to cyclic AMP to further facilitate opening. This hyperpolarization-activated current (Ih) is a critical component to setting the excitability threshold of the cell as well as generating neuronal rhythmicity (155). HCN1 knockout mice display more robust LTP and enhanced learning and memory compared to wild-type mice (156,157). Therefore, ketamine antagonism of HCN1 may partially underlie the improved cognition associated with ketamine treatment in depressed individuals, but further research is needed to corroborate these findings in humans.

Conclusions

While NRs are important components in neuro-cognition, ketamine antagonism of NRs alone does not account for the diversity in observed clinical effects. Our knowledge of the cellular and molecular substrates involved in the functional events which underlie synaptic plasticity and neurocognition continues to grow in complexity. Further research into these processes will only facilitate our understanding of how ketamine modulates neurocognition. In particular, evaluating the effect of ketamine on non-neuronal cell types and their reciprocal interactions with neurons will significantly enhance our ability to evaluate the safety and efficacy of ketamine. Several off-targets (HCN1 channels and glial activity) of ketamine may be implicated in regulating and tuning the magnitude or direction of the response to ketamine treatment. Exploring these and other putative mechanisms can yield insights into the differential neurocognitive effect of ketamine in healthy and depressed individuals.

KETAMINE AND NEURODEVELOPMENT

Conflicting Evidence or Lack of Appropriate Experimental Models?

Ikonomidou and colleagues (158) were the first to raise concerns over the use of NR antagonists, such as ketamine, in pediatric populations. Administering MK-801 to rat pups yielded time- and dose-dependent increased rates of neuronal apoptosis. These finding have been further studied in a primate model in which 20 mg/kg induction and 20 – 50 mg/kg/hour maintenance doses of ketamine for anesthesia were assessed in time (159). This study found no adverse effect with up to 3 hours of ketamine therapy, but found adverse effects with excess of 9 hours. With focus on the clinical significance of ketamine in neurodevelopment and neurotoxicity, subsequent preclinical studies noted their most concerning finding was the persistent, dose-dependent behavioral and memory deficits that remained into adulthood resulting from early ketamine use in rat and primate models (160,161). It is important to note that these animals were treated with doses of 20 – 75 mg/kg of ketamine, far exceeding human doses, and that doses less than 50 mg/kg in rats were not associated with hippocampal apoptosis.

Molecular Mechanisms

The controversy surrounding the safety of anesthetic exposure to children underscores the need for more definitive research. Neurodevelopmental concerns, such as a decreased synaptogenesis, were observed in

rodent models upon neonatal ketamine exposure at 5 – 25 mg/kg (162). This observation is contradictory to the observed stimulatory effect of ketamine on synaptogenesis (163,164) and warrants further investigation.

The processes of LTP and LTD are associated with dendritic spine enlargement and shrinkage, respectively (165,166). Ketamine rapidly and reversibly abolishes LTP and LTD in vivo (167-169). While NR blockade is hypothesized to underlie the synaptogenic effects of ketamine by simulation of mammalian target of rapamycin (mTOR), it is unlikely that a blockade of classical NRs to disrupt LTP and LTD can fully explain the observed abnormalities upon developmental ketamine exposure. Thus, a developmental model of ketamine's effects is needed. During development, the relative abundance of GluN2A/B protein shifts to favor GluN2B in juvenile synapses and GluN2A at mature synapses. However, the sensitivity of GluN2A/B receptors to ketamine is largely similar. Thus, alternative mechanisms may determine the differential effects of ketamine in young versus mature animals.

In addition to the developmental switch of GluN2A/B-containing receptors, the nonclassical GluN3 gene family also displays developmental expression patterns with known roles in development. GluN3A and GluN3B do not respond to glutamate and NMDA like their GluN2 counterparts, but respond exclusively to glycine. Thus, these NRs are considered excitatory glycinergic receptors. GluN3A is normally expressed early in development in both rodents and humans while GluN3B is expressed through adulthood (170,171). Interestingly, mice lacking GluN3A display higher dendritic spine density (172) whereas transgenic over-expression has the opposite effect (173). The normal developmental down-regulation of GluN3A is needed for appropriate neurocognitive performance in animal models. Despite the role of GluN3A in synaptogenesis, NRs containing GluN3 subunits appear insensitive to ketamine blockade (174). How then might the GluN3 family be implicated in the synaptic effects of ketamine?

Classical GluN2 and non-classical GluN3 NRs regulate opposing roles in synaptogenesis. Both are tightly coupled with actin pathways involved in synaptic remodeling. In neonatal models, GluN2B and GluN3A NRs will dominate synapses in the CNS based on known developmental expression patterns. GluN2B is coupled directly with Tiam1 in hippocampal neurons (175). Tiam1 is a GTP exchange factor that is activated upon local influx of Ca^2+ through NRs. It was found that Tiam1 can lead to the stimulation of PI3K/AKT pathways and downstream activation of mTOR associated with increased synaptogenesis. On the other hand, GluN3A has an inhibitory role on synaptogenesis (176) and couples with the GTPase, Rheb, which is also linked to mTOR activation. Thus, the inhibitory effect of GluN3A on synaptogenesis could be due to sequestering free Rheb to prevent accumulation of active mTOR (177). It is reasonable then to hypothesize, as GluN3A is down-regulated with development, the inhibitory action on mTOR activation is relieved and ketamine exerts higher synaptogenesis. Consistent with this, patients with depression display aberrant overexpression of GluN3A mRNA transcripts (178). Therefore, blockade of classical NRs with ketamine may stimulate synaptogenesis by providing a compensatory simulation of mTOR to counteract the effects of GluN3A overexpression, which has been hypothesized to increased Rheb sequestration and attenuate mTOR activation. This mTOR stimulation of synaptogenesis is believed to underlie one facet of the antidepressant effects of NR blockade (179).

Conclusions

The historic concern about NR antagonists and impaired neurodevelopment, despite differences in design of animal and human studies, warrants a more rigorous investigation into the specific mechanisms which underlie ketamine-induced structural changes in the synapse and brain. Interestingly, many of the same mechanisms involved in neurocognition, LTP and LTD, are associated with synaptic spine enlargement and pruning, respectively. However, recent evidence has argued that while these changes involved NR activity, conformational changes in the channel alone induced by agonist binding without current influx may mediate these structural changes and have an impact on downstream signaling (180,181). Thus, NRs have novel metabotropic functions. Coupled with the evidence that the GluN3-containing NRs and ketamine interactions may also act independently of the current through these channels, these data argue for completely novel paradigms of ketamine-mediated physiological changes through NRs other than the direct pore blockade model. Investigations into the biophysical interactions between ketamine and NRs will yield new insights into ketamine-induced changes in central neurons. Understanding these mechanisms will facilitate a deeper understanding of the impact developmental changes and patient-specific differences in these proteins will have on the effect of ketamine in adults and children.

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