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REVIEW

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Ketamine as a Fast Acting Antidepressant: Current Knowledge and Open Questions

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SUMMARY

Several recent studies have shown that a single intravenous subanesthetic dose of ketamine, a NMDA receptor antagonist, exerts rapid antidepressant effects in patients with treatment refractory mood disorders and reduces suicidal ideation. Those insights have fueled tremendous excitement in the efforts to elucidate the mechanism underlying ketamine's antidepressant properties in animal models of depression, as well as in humans through the use of brain imaging as well as peripheral blood measurements. For example, there is emerging evidence that ketamine's antidepressant properties rely on increasing AMPA signaling and rapidly inducing synaptogenesis. While pilot clinical studies are promising, a number of critical questions still remain unanswered. They relate to the safe and effective use of ketamine in patients with mood disorders regarding the optimal dose range, modality and method of administration for acute and long-term maintenance of effect, and the biomarkers associated with response/nonresponse. In this review article, we first summarize the clinical evidence about the use of ketamine in mood disorders, as well as preclinical and humans studies which investigated the mechanisms of action of ketamine, and predictors of antidepressant response in clinical populations. We then provide a critical overview of the knowledge gaps about the use of ketamine in depression and suggest some future research directions for the investigation of ketamine as a promising tool to develop novel more effective and fast acting antidepressants.

Ketamine Exerts Rapid Antidepressant Effects in Patients with Depression

Ketamine inhibits the *N*-methyl-D-aspartate (NMDA) receptor, reducing the glutamatergic signal transduction via this subtype of glutamate receptors in the brain. The racemic mixture of ketamine has been approved for the induction and maintenance of anesthesia via intramuscular or intravenous administration and has been marketed in the United States and worldwide since the 1970s. The World Health Organization [1] has listed ketamine as a core medicine (a minimum medical need for a basic health care system) both for adults and for children. The package insert for ketamine hydrochloride (HCl) states that an intravenous bolus injection of 1 to 4.5 mg/kg or 1 to 2 mg/kg at a rate of 0.5 mg/kg/min can rapidly induce anesthesia starting within 30 seconds and lasting for 5–10 min [2]. Ketamine has a short systemic half-life and is extensively metabolized in the liver, resulting in a high first pass effect, which makes the drug unsuitable for oral delivery.

Efficacy in Treatment-Resistant Depression/ Bipolar Depression

Converging lines of evidence suggests that major depression is associated with abnormalities in glutamatergic synaptic transmission [3] resulting in loss of synaptic plasticity in mood and emotion circuits [4,5]. Ketamine inhibits glutamatergic NMDA receptor and a number of small clinical studies and case reports found that slow subanesthetic infusions of ketamine HCl (0.5 mg/kg over 40 min) had same–day antidepressant effects, even in patients who had responded poorly to conventional antidepressant drugs [6–10]. In selected patients, the antidepressant effects of ketamine have been shown to last for 4–7 days after a single-dose application, and the antidepressant response can be maintained for weeks with repeated infusions [11]. Interestingly, the antidepressant effects of a single dose persist significantly longer, that is, approximately 5 days than the half-life of ketamine, which is approximately 3 h. Published single dose and multiple dose studies of ketamine in treatment-resistant depression (TRD) and bipolar depression are summarized in Table 1.

Those studies consistently show that ketamine has a rapid onset of efficacy within hours and that the effect is robust and clinically meaningful in patients with TRD and in patients with bipolar depression. Nearly, two-thirds of all patients respond and a third achieve remission within 1 day. This is particularly meaningful in contrast to the oral antidepressants where the remission rate is <20% [12].

A critique to the earlier studies is that the adverse events profile associated with ketamine (i.e., dissociation and hallucinations)

Table 1 Published human studies of intravenous ketamine HCl in depressive episodes

First author (years)	Study design	Subjects		Ketamine HCl administration	Other medications	Key outcome measures	Results/conclusions
		Diagnosis	N				
Berman (2000)	Randomized, double-blind, placebo-controlled	Unipolar or bipolar depression	9	0 or 0.5 mg/kg over 40 min i.v. (single dose)	Subjects were drug free	Change from baseline HDRS score	Significant improvements in depressive symptoms were observed within 72 h after ketamine but not placebo infusion
Zarate (2006)	Randomized, double-blind, placebo-controlled, crossover	Unipolar TRD	18	0 or 0.5 mg/kg over 40 min i.v. (single dose)	Subjects were drug free	Response[a], using HDRS score	Of subjects treated with ketamine, 71% responded after 1 day, and 35% maintained a response for $\geq$1 week
Mathew (2010)	Randomized, placebo-controlled continuation	Unipolar TRD	26	0.5 mg/kg over 40 min i.v. (open-label, single dose)	2 h before ketamine: Randomized, lamotrigine or placebo 72 h after ketamine: Responders randomized, riluzole or placebo	Response[a] and time to relapse, using MADRS scores	Responses were observed in 65% of subjects at 24 h and 54% of subjects at 72 h Lamotrigine did not attenuate the mild, transient side effects of ketamine, and did not enhance its antidepressant effects. Riluzole did not prevent relapse in the first month after ketamine treatment
Ibrahim (2012)	Randomized, double-blind, placebo-controlled	Unipolar TRD	42	0.5 mg/kg over 40 min i.v. (open-label, single dose)	Randomized, placebo or riluzole, starting after ketamine infusion	Response[a] and time to relapse, using MADRS scores	At 4–6 h after the ketamine infusion, 62% of subjects (26 of 42) had responded. The average time to relapse was approximately 17.2 days in the ketamine-riluzole group and 9.8 days in the ketamine-placebo group; the difference was not statistically significant
Diazgranados (2010)	Randomized, double-blind, placebo-controlled, crossover, add-on	Bipolar TRD	18	0 or 0.5 mg/kg over 40 min i.v (single dose)	Subjects were stable on lithium or valproate	Response[a], using MADRS scores	Depressive symptoms improved within 40 min and remained significantly improved through Day 3 In the ketamine group, response rates were 44% after 1 day and 71% of subjects overall
Zarate (2012)	Randomized, placebo-controlled, double-blind, crossover, add-on	Bipolar TRD	15	0 or 0.5 mg/kg over 40 min i.v. (single dose)	Subjects were stable on lithium or valproate	Response[a], using MADRS scores	Depressive symptoms improved within 40 min and remained significantly improved through Day 3
Murrough (2012)	Multiple Dose, open-label, three times weekly over 12 days	TRD	24	0.5 mg/kg over 40 min i.v. (open-label)	Subjects were drug free	Response using MADRS Scores	70.8% of subjects were responders; response was sustained for the duration of the study. Median time to relapse in responders was 18 days

HDRS, Hamilton Depression Rating Scale; MADRS, Montgomery–Asberg Depression Rating Scale; TRD, treatment-resistant depression. [a]Response defined as change of 50% or more from baseline.

may have caused some unblinding of the subjects and the clinicians. However, in a recently completed study, a single dose of ketamine was compared with IV midazolam, a short acting benzodiazepine, which was used as an active placebo. Preliminary results suggest that this might be a viable strategy to provide a more adequate protection of the blinding mechanism; the authors validated the effective blinding of the active placebo using adequacy of blinding questionnaire [13]. In summary; ample evidence from independent well conducted randomized, controlled, single dose studies of ketamine exists, showing a robust and rapid onset of antidepressant response.

A number of key unknowns include: effective dose range, drug exposure profile requirements and efficacy with less invasive routes of administration. Oral route of administration, though, is less likely to be effective due to the poor bioavailability [14].

The key question after the single-dose studies is how to maintain the response. Two strategies have been tested thus far.

Ketamine – Riluzole

Riluzole is a glutamate modulator approved for the treatment of amyotrophic lateral sclerosis. There are pilot data on antidepressant efficacy of riluzole; furthermore, riluzole was shown to increase AMPA-mediated transmission and possibly promote synaptic plasticity. Two studies have been published to assess whether riluzole may be able to maintain the antidepressant effect of ketamine. In one study, subjects who initially responded to a single dose of ketamine were randomized to riluzole or placebo to maintain the response (n = 14) [15]. In the second study, all subjects who received ketamine then received riluzole or placebo (n = 42) [16]. The two studies did not show any significant benefit of riluzole in maintaining the response. In the absence of convincing data on an antidepressant effect of riluzole and on the time required to develop such a possible antidepressant effect, that is, the antidepressant effect of ketamine may have diminished before any onset of maintenance efficacy of riluzole, final conclusions on a possible role of this compound in maintaining the ketamine benefit cannot be made. Additional studies are needed with agents with a glutamatergic mechanism to test this hypothesis further.

Multiple Doses of Ketamine

An open-label study tested whether repeated dose ketamine administration could sustain the single-dose response, starting with 10 subjects [6] and later adding 14 more, for a total of 24 subjects with TRD [11]. Subjects underwent a washout of antidepressant medication, followed by up to six intravenous infusions of ketamine HCl (0.5 mg/kg over 40 min), administered three times weekly over a 12–day period. At the end of the study, the overall response rate was 71%. Among responders, median time to relapse after the last ketamine infusion was 18 days. Patients responding to the 1st ketamine administration (n = 17) all kept responding to the consecutive treatments, whereas those not responding to the 1st treatment and completing the 6 IV session study (n = 4) consistently failed to respond. The multiple dose study suggests that the initial antidepressant response can be maintained with multiple doses. However, there is significant variability in the duration of response confirming previous evidence observed in single dose studies [10,15,16]: the median time of relapse was 18 days (range: 4 to >83 days), and 25th and 75th percentile were 11 and 27 days, respectively [11]; interestingly, four of the 17 responders to ketamine were relapse free at the end of the study (i.e., day 83). Thus, the data suggest that there seem to be a subset of subjects who are able to sustain response for several weeks to months after the last dose of ketamine, possibly implying that individualized ketamine treatment frequencies might be required to maintain the clinical benefit over longer periods. However, in the studies which showed the highest interindividual variability in duration of response ketamine was administered open-label [11,15,16], so longer term placebo-controlled studies are needed to test this approach further. Finally, preclinical data suggest similarities in mechanism of action of lithium and ketamine via Glycogen Synthase Kinase-3beta (GSK-3) inhibition [17]. The antidepressant effects of ketamine were absent in GSK-3 knocked-in mice, suggesting that the inhibition of this pathway might be a key downstream effect of NMDA blockade underlying ketamine's antidepressant properties [17]; however, the fact that administration of lithium, which is a potent GSK-3 inhibitor, does not induce rapid antidepressant response might also indicate that ketamine-induced GSK-3 inhibition is a simple epiphenomenon not particularly relevant for ketamine's antidepressant effects. Further studies are needed to clarify the role of GSK-3 inhibition in the mechanisms of action of ketamine as an antidepressant.

In studies with bipolar disorder, subjects were initially treated with lithium or valproate for 4 weeks prior to dosing with ketamine [8]. The relapse rates were similar in subjects on lithium or valproate or those in the TRD study with ketamine monotherapy.

Additional studies are needed combining ketamine doses with oral antidepressant, perhaps similar to combined ECT and pharmacotherapy to test whether the acute response to ketamine can be maintained.

Ketamine Safety Profile

Ketamine has been widely and continuously used throughout the world as its introduction in 1963 as an anesthetic and is considered to have a very good medical safety profile [18,19]. Due to its use as an anesthetic, which is typically a onetime use, there is limited data on chronic/long-term use. Ketamine has a wide therapeutic range and patients have recovered fully after receiving 10 times the normal dosage [20]. Induction of anesthesia is usually achieved by an intravenous infusion of 1 to 4.5 mg/kg or 1 to 2 mg/kg at a rate of 0.5 mg/kg/min, which is significantly higher than the dosages used in the publications cited above.

Adverse Events Associated with Short-Term use of Ketamine at Subanesthetic Doses

In a series of studies in 469 healthy volunteers, single-dose subanesthetic dosages of ketamine were associated with short-lasting, dose-dependent psychosis/dissociative effects and symptoms were reversible upon cessation of drug administration [20]. Adverse mental status events were documented in 2% of these subjects and were deemed related to ketamine administration that led to discontinuation. These events included three medically stable

subjects who became unresponsive to verbal stimuli for a few minutes. Six subjects reported distress related to the mental effects of ketamine, resulting in discontinuation of the infusion. The distress was described using terms such as "no control," "very unpleasant," "weird," and "too high". These adverse events of sedation/dissociation require monitoring/observation of subjects during the period of drug administration and up to 1 h following the completion of administration. Follow-up data (up to 6 months) found no evidence of ketamine abuse or psychiatric problems related to single-dose ketamine exposure. There was also no evidence of sensitization in subjects who had multiple exposures [20]. In the published ketamine studies in patients with unipolar and bipolar depression, no serious adverse events occurred; ketamine appears to have a similar safety profile in patients with mood disorders as in healthy volunteers: the most common adverse events reported from depression studies include perceptual disturbances, drowsiness, confusion, elevations in blood pressure and pulse and dizziness, which usually resolved shortly after the end of the infusion [8–10,13].

Adverse Events Associated with Chronic use of Ketamine

Much of the literature on chronic use of ketamine is derived from reports in ketamine abusers rather than systematically conducted clinical studies. Frequent ketamine users (greater than five times a week) exhibit impairments in both short- and long-term memory. However, memory impairments may be reversible, as they were not found in a group of 30 ex-ketamine users who had been abstinent for at least a year. Furthermore, infrequent or recreational ketamine use does not appear to be associated with long-term cognitive impairment. In addition, dosages reported by street users are generally much higher than the dosages intended for use in treating TRD [21].

These data suggest that it may be possible to administer the drug over a longer time period in an individualized, carefully supervised fashion to sustain the antidepressant effects.

Ketamine's Mechanism of Action: Preclinical Studies

Subanesthetic Ketamine Acutely Increases Glutamate Release

The clinical observations in depression with a compound showing a mechanism of action very different from that of currently available antidepressant drugs have fueled tremendous effort in understanding the mechanism of action of ketamine's antidepressant effects in preclinical models; recent studies have generally tried to link (intra) cellular phenomena to behavioral changes that reflect antidepressant efficacy. Acute administration of subanesthetic doses of ketamine triggers a complex intracellular cascade which ultimately leads to increased synaptogenesis and spine formation which seem to be essential for antidepressant effects to happen (see Duman et al., 2012 for a comprehensive review on the topic [22]).

The first preclinical studies using subanesthetic ketamine as relevant to psychiatric diseases were done from a completely different perspective, that is trying to understand the biology underlying the psychotic-like symptoms triggered by ketamine (see Moghaddam and Krystal, 2012 for an excellent review on the use of ketamine as a model of psychosis [23]). In one of the first reports which investigated the effects of subanesthetic ketamine on the glutamatergic system, Moghaddam et al. [24] showed increased extracellular glutamate in the prefrontal cortex in awake animals, as well as increased dopamine release; increase in glutamate release was observed with low doses of ketamine (i.e., 10, 20, and 30 mg/kg) but not with intermediate (50 mg/kg) or anesthetic doses (200 mg/kg); interestingly, increased dopamine release as well as working memory impairment induced by ketamine were blocked by AMPA antagonist application, suggesting that increased glutamate levels induced by ketamine reflected heightened glutamatergic neurotransmission and not simple metabolic status changes [24]. The effects of subanesthetic doses of ketamine has also been recently investigated *in vivo* through the use of [^{1}H] magnetic resonance spectroscopy ([^{1}H]-MRS): Kim et al. [25] showed increased prefrontal glutamate levels after subchronic (i.e., 7 days) administration of subanesthetic ketamine doses (30 mg/kg) in anesthetized rats, with a corresponding decrease in glutamine/glutamate ratio. Several limiting factors contributed to the uncertain relevance of the findings for depression. For example, the dose used in this experiment was higher than the one used to model antidepressant effects (i.e., 10 mg/kg) and no measurement was performed after a single dose of ketamine. Those issues, as well as the difficulty of interpreting an increase in total glutamate not paralleled by an equivalent increase in glutamine, make the relevance of these findings for depression rather uncertain. The above-described findings contrast with evidence that administration of a low dose of the NMDA antagonist phencyclidine increases glutamine/glutamate ratio in the prefrontal cortex in rats [26]. Interestingly, the acute increase in glutamatergic transmission triggered by ketamine seems to be a relatively transient phenomenon, which might only last for up to 2 h, that is well below the average duration of antidepressant effects observed in humans after a single dose of ketamine, which is 4–7 days [27].

Recently, Chowdhury et al. [28] used ^{1}H-[13C]-Magnetic Resonance Spectroscopy to investigate the effects of subanesthetic (30 mg/kg) and anesthetic (80 mg/kg) doses of ketamine on glutamate release in the prefrontal cortex in awake rats. An acute increase in prefrontal glutamate, glutamine and GABA labeling was observed in the animals treated with subanesthetic but not in those treated with anesthetic ketamine, suggesting that ketamine increases glutamate/glutamine cycle as well as glutamine/GABA cycle. Those effects seemed to be region-specific, as no similar effect was found in the hippocampus. Subanesthetic ketamine was not associated with any changes in the total concentration of glutamate, glutamine or GABA, which seems to be consistent with some negative findings reported in humans using ^{1}H-MRS. Those findings replicate and extend the original findings reported by Moghadamm demonstrating a direct effect of ketamine on glutamate release; however, it is unclear whether they reflect ketamine's pyschotomimetic or antidepressant effects. The biological determinant of increased glutamate release are not entirely clear, but one of the most plausible hypotheses is that blockade of NMDA receptors on tonic GABAergic interneurons induced by ketamine is able to disinhibit glutamatergic pyramidal cells in the

prefrontal cortex [29]. From the published literature, it appears that there is a clear distinction between subanesthetic and anesthetic doses of ketamine on cortical disinhibition, with only subanesthetic doses producing an acute increase in glutamate release. Among subanesthetic doses, there is uncertainty whether increase in glutamate release is only relevant for ketamine-induced psychotic-like symptom or also for its antidepressant effects. Future studies will need to investigate the effect of a lower subanesthetic dose of ketamine (i.e., 10 mg/kg or lower) on glutamate/glutamine cycle to determine whether increase in glutamate release is also relevant to ketamine's antidepressant properties.

AMPA Receptors Activation is Necessary for Ketamine's Antidepressant Effects

Recent studies show that α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (AMPAR) activation is critical for ketamine's antidepressant effects, which are abolished by pretreatment with the AMPARs antagonist NBQX [30]; this represents one of the most replicated findings on the underlying biology of ketamine's antidepressant effects [31–33]. AMPAR blockade is also able to suppress the ketamine-induced activation of the mammalian target of rapamycin (mTOR) pathway, as well as increased synaptogenesis and synaptic protein increases that are believed to be essential for its antidepressant effects [33].

Ketamine Promotes Synaptic Plasticity

Subanesthetic ketamine (i.e., 3 mg/kg) also determines a rapid increase in the translation of brain-derived neurotrophic factor (BDNF) through the desensitization of eukaryotic elongation factor 2 (eEF2) kinase and dephosphorylation of eEF2 [31] induced by blockade of resting NMDA receptors; increased hippocampal BDNF protein levels were shown 30 min after the administration of ketamine but not 24 h thereafter, while no change was apparent in BDNF mRNA levels at either time point. Ketamine's antidepressant effects were abolished by administration of the protein synthesis inhibitor anisomycin, providing further evidence that increased protein synthesis is necessary for the behavioral effects of ketamine. Blockade of protein translation during neural activity did not abolish the behavioral effects of ketamine, suggesting that ketamine's antidepressant effects are triggered by NMDA blockade specifically during resting spontaneous glutamatergic signaling [31]. Increase in BDNF protein levels was found in the hippocampus but not in nucleus accumbens, possibly suggesting a region specific effect [31]. Behavioral antidepressant effects and increased synaptogenesis induced by ketamine are abolished in mice with the BDNF Val66Met Met/Met genotype, which was previously shown to be associated with lower BDNF release [34]. This provides further evidence that BDNF might be a key player in the mechanism of action of ketamine which might underlie its antidepressant properties.

Autry and colleagues tested the hypothesis whether the activation of the mTOR pathway is also necessary for ketamine's antidepressant effects and whether activation of mTOR pathway is a downstream effect of increased BDNF translation, but they did not observe increased mTOR pathway activation following ketamine administration [31]. This finding contrasts with what had been reported earlier by Li et al. [33], who showed activation of the mTOR complex and key components of translation initiation, p70 ribosomal S6 kinase (p70S6K) and eIF4E-binding after administration of subanesthetic ketamine (10 mg/kg). The activation of the mTOR pathway was rapid, being evident 30 and 60 min after drug administration, but returning to baseline at 2 h. Ketamine also produced an mTOR-dependent increase in synaptic proteins such as PSD95, GluR1, and the presynaptic protein synapsin I; elevation of synaptic proteins was delayed in respect to mTOR activation; it started 2 h post-ketamine and persisted up to 72 h. Besides the elevation of synaptic proteins, ketamine also promoted the formation of spines on the apical dendrites of layer V pyramidal neurons, which translated into increased neurotransmitter induced excitatory post-synaptic currents. This effect was investigated only 24-h post-ketamine but as no other time point was investigated, it cannot be discarded that it might happen even earlier than that; also unknown is how long it persists after a single dose of subanesthetic ketamine.

From the existing evidence, it is apparent that ketamine triggers a complex series of intracellular and extracellular biological processes that ultimately result in increased synaptic plasticity. In the future, it will be extremely important to not only have a better understanding of the temporal dynamics of individual cellular and molecular events triggered by ketamine, but also to investigate their regional specificity, as well as to provide a more thorough characterization of dose-dependent effects and their link to behavioral changes.

Ketamine's Mechanism of Action: Clinical Studies

Because of its unique rapid antidepressant effect and its efficacy even in very refractory patient populations, ketamine represents an extraordinary "tool" compound to investigate the biology of antidepressant response as well as the mechanism underlying the loss of antidepressant effects. This knowledge is key to develop novel antidepressant compounds which act more quickly than existing ones and might provide increased efficacy in patients with TRD. Given the very complex temporal dynamics of ketamine's downstream effects, it is clear that serial measurements of ketamine's pharmacodynamic effects are essential to have a better understanding of ketamine's mechanism of action and a more accurate interpretation of the findings.

Although ketamine has been studied as challenge agent to model psychotic-like symptoms in healthy volunteers for over two decades [23] (see Moghaddam and Krystal, 2012 for a review on the topic [23]), it is unclear whether findings in healthy subjects could be meaningful for ketamine's antidepressant properties rather than simply reflect its dissociative and psychotic-like effects. Typically ketamine is used as a challenge agent for psychosis as a bolus infusion (typically 0.23–0.26 mg/kg over 1–2 min), followed by a lower maintenance dose on constant infusion (usually 0.40–0.60 mg/kg over 1 h) [35]; this infusion schema is different from the way ketamine has been administered in depressed patients, where typically 0.5 mg/kg are given over 40 min with a constant infusion rate. In the absence of a direct comparison of the pharmacokinetic and pharmacodynamic effects of these two

infusion protocols, it is unclear whether the challenge protocol is at all informative for depression. Nevertheless, as psychotic-like effects usually dissipate within 1 h from the beginning of the administration of ketamine, next day findings in healthy volunteers might still hold some value and shed some light on ketamine's antidepressant properties.

Because of those important caveats, understanding the biology of ketamine's antidepressant effects needs to rely on studies performed in depressed patients, where an assessment of clinical response can also be performed. For those reasons, herein, we will emphasize studies carried out in patients with depression and only briefly touch upon healthy volunteers studies.

Studies in Depressed Patients

A few studies investigated whether ketamine increases peripheral BDNF levels in patients and whether changes in BDNF correlate with antidepressant improvement. Previous preclinical experiments have implicated BDNF increase as an early effect triggered by subanesthetic ketamine; studies in ketamine abusers also show an increase in BDNF over normative values associated with repeated ketamine use [36]; an early report in 23 patients with TRD which investigated several time points up to 230 min post-infusion failed to show an increase in BDNF [37]. A subsequent study by Duncan et al. [38], which represents an extension of the previous study [37], showed a modest increase in BDNF levels 230 min after ketamine infusion. No correlation between BDNF changes and clinical response was observed. However, none of those studies did factor the Val66Met single nucleotide polymorphism (SNP) into the analysis, so it is not known whether there was any interaction between genotype and response status on BDNF levels. Future studies are warranted to investigate later time points beyond 230 min, as well as the effects of repeated ketamine dosing.

To our knowledge, there is only a single study that looked at amino acid neurotransmitter changes after ketamine administration in patients with depression [39]. This study did not detect any changes in glutamate, glutamine or GABA in the occipital cortex 3 and 48 h post-infusion of 0.5 mg/kg of ketamine. However, the lack of investigation of other brain regions which might be more relevant for depression pathophysiology and for the mechanism of action of ketamine, associated with the lack of investigation at earlier time points, make the interpretation of those negative findings challenging. Studies in healthy subjects (reviewed below) showed a very transient effect of ketamine on amino acid neurotransmitters, which seem to return to baseline levels within 1 h from drug administration [40,41]. This temporal dynamic is consistent with evidence from preclinical studies which show that the glutamate surge is rapid and goes back to baseline within 2 h [24]. However, questions remain whether [^{1}H]-MRS at 3T or 4T is an appropriate method to investigate acute changes induced by ketamine, as glutamate MRS signal reflects only minimally neurotransmission, while the vast majority of it is represented by glutamate intracellular neuronal content which also supports basal metabolic activity. Glutamine or Gln/Glu changes might be more sensitive measures to detect acute drug effects, but their accurate quantifications remain highly challenging at medium field strengths. Future studies at ultra-high field strengths or using [13C]-MRS -which allows quantifying the glutamate/gln cycle- will be very informative.

A recent study used a very innovative method to investigate brain plasticity in depressed patients who received subanesthetic ketamine [42]: previous studies conducted in the Tononi's laboratory showed that both motor and somatosensory evoked potentials are amenable to changes following interventions that promote or decrease brain plasticity, such as repetitive transcranial magnetic stimulation or arm immobilization, respectively [43,44]. As ketamine is thought to exert its antidepressant effects through an increase in brain plasticity, Cornwell et al. tested the hypothesis that ketamine neuroplastic changes could be detectable using evoked potentials as well. They measured somatosensory evoked fields (SEFs) changes using magnetoencephalography recordings and showed that ketamine responders displayed increased SEFs 6–7 h post-ketamine, while this effect was not present in nonresponders. However, SEFs measurements were not acquired at a later time point in a sizable group of patients, so it remains unknown whether maintenance of response is dependent on sustaining the cortical plasticity enhancement [42].

Those findings are consistent with an early study which showed that ketamine potentiated motor evoked potentials and decreased resting motor threshold in healthy volunteers [45]: the authors hypothesized that these phenomena reflected AMPA transmission potentiation, which is one of the strongest candidates to explain the mechanism of action of ketamine in depression (reviewed in [11]).

Future studies will also need to investigate the effects of ketamine on activity/metabolism in the anterior cingulate cortex, amygdala and other brain regions that are part of the visceromotor network and are involved in mood regulation [46] and to establish a time course of effects induced by ketamine in patients with depression. Preliminary results using FDG-PET imaging have been presented recently at international conferences but are not published to date. Finally, resting state functional connectivity measured using Magnetic Resonance Imaging is also a technique of great interest for detecting ketamine's pharmacodynamic effects related to its antidepressant activity. Recent studies showed abnormal functional connectivity of the dorsomedial PFC in depression [47] and that traditional antidepressant might reverse this abnormality [48].

Studies in Healthy Subjects

The evidence of glutamatergic metabolite changes detectable through ^{1}H-MRS in healthy subjects following ketamine administration is conflicting; however, there is also significant methodological heterogeneity across studies. Rowland et al. [40] used a loading dose + low dose maintenance protocol and showed ACC glutamine (gln) elevation only during the loading phase but not at the beginning of maintenance. Those results are consistent with a recent study which showed ACC glutamate increase 35 min after the start of ketamine infusion of ketamine in 13 healthy subjects [41]; gln yielded acceptable quality results only in a minority of subjects (i.e., n = 3 and n = 4 pre- and postketamine, respectively). This study used a slightly different infusion protocol (i.e., the Clements 250 model), which is supposed to

yield more stable levels of ketamine during maintenance infusion [49]. In the only study in healthy subjects which investigated an infusion protocol consistent with how ketamine is administered in depressed patients (i.e., 0.5 mg/kg over 40 min), no changes in either Glx or glutamate was detected during and after ketamine infusion in the ACC [50].

A recent study used resting state functional connectivity (fc) to investigate ketamine's effect on large-scale networks 24 h after a single IV administration [51]; this time point was chosen as previous clinical studies detected a maximal antidepressant response exactly after 1 day [10]; ketamine decreased fc between the posterior cingulate cortex and the "dorsal nexus" [47], the medial prefrontal cortex, as well as the pregenual ACC (pgACC), all areas which have been previously implicated in the pathophysiology of depression (reviewed in [46])and which belong to the default mode network (DMN). As the DMN is associated with self-referential processes and ruminations [52] and shows increased fc in subjects with depression, a decrease in fc after ketamine might reflect restoration of a normative pattern which might be associated with antidepressant response. Future studies in clinical populations will be able to test this hypothesis and investigate whether decreased fc of the dorsal nexus correlates with the magnitude of clinical improvement to ketamine.

Prediction of Response

After the replication study of ketamine's antidepressant effects performed at the National Institute of Mental Health (NIMH) [10], a series of studies investigated whether looking at pre-treatment baseline measures (i.e., brain activity and metabolites, sleep electroencephalography (EEG) measures, genetic polymorphisms) in depressed patients one could identify the subjects more likely to show antidepressant response to a single intravenous infusion of ketamine.

In the first study of its kind, Salvadore et al. [53] used magnetoencephalography recordings in 11 unmedicated patients with TRD while viewing emotionally charged faces to investigate whether baselineACC activity would predict response to ketamine, similar to other conventional antidepressants [54]. Patients with depression showed an abnormal habituation pattern compared with healthy control subjects; pgACC activity to fearful faces was positively correlated with the magnitude of antidepressant response 230 min after ketamine, while an inverse correlation was found for right amygdala activity [53]. Overall, responders to ketamine showed an activity pattern that more closely resembled normative responses in healthy subjects as compared to nonresponders. In a subsequent study, we investigated whether pgACC activity predicted response to ketamine also during a nonemotional task, such as the spatial working memory task n-back [55]. Previous studies had indeed shown that the pgACC displays either enhanced or decreased activity in relationship to the emotional demands of the tasks [56] and that patients with MDD show pgACC hyperactivity during working memory tasks compared with control subjects [57]; patients more likely to respond to ketamine displayed decreased pg ACC activity and lower pgACC connectivity as compared to nonresponders; those findings might indicate that ketamine responders show functional integrity of the cortico-limbic mood regulating circuitry, while nonresponders show abnormal activation of mood regulating circuitry even in the absence of emotionally arousing stimuli.

A recent study suggests that ^{1}H-MRS might also be a valuable technique to investigate predictors of response to ketamine: patients who showed the greatest clinical improvement 230 min after ketamine administration displayed low Glx/Glutamate ratio in the dorsoanterolateral/dorsomedial PFC compared with patients who did not show clinical improvement [58]. As Glx peak is mostly constituted by glutamate and glutamine, Glx/Glu was used in that study as a proxy for glutamine (gln), given the low spectral resolution of gln with 3T MRI fields. Findings in the ventromedial PFC did not reach statistical significance but showed a similar direction. Pre-treatment GABA levels did not correlate with the magnitude of antidepressant improvement in either region. An important caveat that needs to be considered is that for technical feasibility, amino acid neurotransmitters were measured in relatively large voxels (i.e., 18 and 30 mL, respectively), yielding suboptimal spatial resolution which did not allow making any inference in regards to the role of specific anatomical regions in predicting antidepressant response to ketamine.

Besides imaging, some preliminary studies have started to investigate genetic predictors of antidepressant response to ketamine. An obvious candidate which was tested by Laje et al. [59] is the BDNF Val66Met SNP rs6265, which was previously shown to influence some downstream effects of ketamine, as well as its antidepressant-like activity [34]. MDD patients carrying the Met allele showed a lower percentage of improvement (24%) after ketamine as compared to Val/Val homozygous subjects (41%). Those findings are very promising, yet in need of independent replication.

Recent evidence also suggests that pre-treatment sleep EEG might be able to differentiate between ketamine responders and nonresponders. The baseline delta sleep ratio (i.e., the ratio of slow wave activity [SWA] during the first non-REM sleep episode over the second non-REM sleep episode of the night) in particular was inversely correlated with the magnitude of clinical improvement 1 day after ketamine administration. This evidence suggests that subjects with a more disrupted pattern of SWA are the ones who display better clinical improvement after ketamine. According to the synaptic homeostasis hypothesis, SWA might reflect brain plasticity [60]: this might suggest that responders to ketamine are characterized by lower brain plasticity, which might then be increased by ketamine [42].

Conclusions

Several independent pilot studies demonstrate that Ketamine is capable of inducing a robust antidepressant effects in patients with TRD, which were previously refractory to standard treatment with oral antidepressants as well as ECT therapy. The antidepressant effect after a single intravenous infusion of subanesthetic dosages of Ketamine has an onset within hours and is sustained for an average of 4–7 days; evidence reported in the literature from studies with single and multiple doses of ketamine also shows that some subjects might be able to sustain the response for weeks to months. Besides accumulating clinical efficacy data that have been generated in a relatively short-time frame, several academic centers are investigating ketamine's

mechanism of action which underlies its antidepressant properties both in preclinical and in clinical experiments and have generated important knowledge which might benefit the development of novel fast acting antidepressants which modulate the glutamatergic system. This research could greatly benefit from technical advances in brain imaging methods (e.g., resting state fMRI, ultra-high field MRI, etc.) and in fluid biomarkers measurements (e.g., metabolomics, proteomics, etc.).

However, several fundamental issues still need to be addressed regarding the use of ketamine in depression, such as the identification of an effective dosing strategy to maintain the antidepressant effects. From a mechanistic point of view, while several studies are undergoing to understand the biological underpinnings of antidepressant response, relatively few studies are investigating the biological mechanisms of depressive relapse (i.e., why subjects cease to respond to ketamine); this is an equally important area of research that could lead to identify methods to sustain the antidepressant effects of ketamine.

Our literature review revealed that after the study conducted at the NIMH in 2006 in TRD subjects [10], an impressive number of clinical trials investigating the use of ketamine in depression have been initiated and published. Several more are currently under way. Fifty years from its initial discovery as an anesthetic agent, this NMDA antagonist might now find new and promising uses in an area of high unmet medical need.

Disclosure

Giacomo Salvadore and Jaskaran Singh are full-time employees and shareholders of Janssen Pharmaceuticals INC. Janssen Research and Development began Phase 2 studies with intravenous formulations of ketamine and esketamine in the US and Europe this year to systematically investigate the use of this NMDA receptor antagonist for the therapy of TRD.

Conflict of Interest

Giacomo Salvadore and Jaskaran Singh are both employees of Janssen Pharmaceuticals, who supported this manuscript and is currently investigating ketamine and esketamine in TRD.

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