Psychiatr. Pol. 2022; 56(5): 1017–1032
PL ISSN 0033-2674 (PRINT), ISSN 2391-5854 (ONLINE)
www.psychiatriapolska.pl
DOI: https://doi.org/10.12740/PP/OnlineFirst/134330

Ketamine – a long way from anesthetic to a prototype antidepressant. Review of potential mechanisms of action

Bartłomiej Pochwat

Department of Neurobiology, Maj Institute of Pharmacology,
Polish Academy of Sciences in Krakow

Summary

Published research studies on the antidepressant activity of ketamine in the last twenty years have significantly changed the way people think about potential new antidepressants and the biological basis of depression. The symptoms of depression may subside for several days after the administration of a dose of ketamine. In contrast, achieving a therapeutic effect with classic antidepressants requires chronic administration. The critical issue for ketamine is understanding the biological basis of its amazing effects. Because one of the main molecular mechanisms of ketamine action is the blockade of NMDA-activated glutamate receptors, a great effort has been directed at understanding the role of the glutamate system in the pathophysiology of depression and the unique antidepressant profile of ketamine.

This review discusses the most relevant glutamate hypotheses explaining the molecular and cellular mechanisms of ketamine action. In the first place, phenomena such as the disinhibition of glutamate release and the inhibition of NMDA receptors stimulated by spontaneously released glutamate are discussed, followed by the relationship between the antidepressant effects of ketamine, glutamate and the functioning of the lateral habenula. The last part of the review discusses the involvement of the individual enantiomers and ketamine metabolites in its antidepressant activity.

Key words: ketamine, NMDA receptors antagonists, depression

Historical background

Phencyclidine or phenylcyclohexyl piperidine (PCP) was first discovered by Victor Maddox, a chemist at the Parke-Davis pharmaceutical company in Detroit, Michigan, in 1956 [1]. Initial preclinical studies revealed a remarkable effect of PCP on the behavior of different animal species. Therefore, the research team and management of the Parke-Davis pharmaceutical company decided to conduct clinical trials to investigate the potential anesthetic activity of the newly discovered drug. From

the very beginning, it was clear that PCP, given at the right dose, was safer than most anesthetics available at that time. Unfortunately, some of its side effects were of grave concern. About 30 percent of patients developed a state of delirium characterized by hallucinations and dreams that could progress to acute agitation. Also, people under the influence of PCP thought they were gliding in space and experienced significant psychomotor disturbances [1, 2]. Because of the pronounced side effects, Parke-Davis continued the research on PCP to obtain safer derivatives of the parent molecule. In August of 1964, another drug (CI-581) known today as ketamine, an analog of PCP, was administered to humans for the first time. The first clinical trials of ketamine proved it to be an effective anesthetic with a shorter duration of action than PCP. Moreover, the risk of delirium was much lower for ketamine. Because the anesthetic effect observed after ketamine administration was associated with a peculiar state of consciousness consisting of a kind of sensory disconnection of patients from this world, this type of anesthesia was called dissociated anesthesia, and the drug inducing it a dissociative anesthetic [1, 2].

Over the past twenty years, ketamine has once again come under the spotlight of pharmacologists and clinicians. This renaissance is mainly due to the work of American psychiatrists from Yale University [3], who in the year 2000 published the results of their studies showing that a single, subanesthetic dose (0.5 mg/kg/100 min) of ketamine administered by intravenous infusion may be effective in the treatment of depression. In the following years, various research groups have demonstrated the antidepressant activity of ketamine in treating affective disorders. The effectiveness of ketamine has been confirmed in drug-resistant depression, depression in people with bipolar disorder, and depressed patients with suicidal thoughts [4–7]. The culmination of research on the antidepressant activity of ketamine was the FDA's registration of its enantiomer (S-ketamine) in the form of a nasal spray to treat drug-resistant depression.

Three interesting surprises came out of the studies just described. First, a single dose of the drug was adequate to provide therapeutic efficacy. The onset of the drug's action appeared quickly, as seen in the rapid improvement in the patients' mood a few hours after the infusion. Third, depending on individual differences, remission lasted from a few days to two weeks [8]. Side effects such as depersonalization, dissociation, or perception disorders appeared in the first several dozen minutes after starting the infusion. However, the intensity was much lower than after the administration of an anesthetic dose [4–7]. Compared to traditional monoaminergic antidepressants, ketamine works faster, longer, and does not require chronic use [9]. Such significant differences in the mode of action of ketamine and monoaminergic antidepressants raise questions about its unique mechanism of action. Understanding the biological processes behind its unique clinical profile will provide an opportunity to develop a new generation of rapid onset antidepressants. Moreover, a comprehensive understanding of the mechanism of ketamine's action may answer the question of whether

it is possible to separate its side effects from its therapeutic effects. Accordingly, the best empirically-based hypotheses explaining the antidepressant properties of ketamine will be discussed in this review article.

Ketamine and glutamate

N-methyl-D-aspartic acid receptors (NMDAR), the primary molecular target of ketamine, were discovered in the 1980s by a group of British researchers [10] who showed that ketamine and PCP inhibit the stimulation of rat spinal neurons by blocking ionotropic glutamate (the receptor effector mechanism is associated with the influx of ions inside the cell) NMDA receptors. In the years following this discovery, several studies [11] have explained this mechanism in more detail, describing ketamine as a use-dependent antagonist of the NMDA receptor. This means that in order to block the NMDA receptor, ketamine must encounter it in the open state. Under physiological conditions, NMDA receptors are activated in a rather complex manner. The NMDA receptors are activated only when two molecules of co-agonist (glycine or serine) and two molecules of the endogenous agonist – glutamate (the main excitatory neurotransmitter in the central nervous system) are attached to the receptor, followed by the removal of magnesium ions inside the molecular channel by depolarization. As a result, calcium and sodium ions enter the neuron through the receptor channel, while potassium ions flow out. Subsequently, calcium, as a secondary transmitter that has penetrated inside the nerve cell, activates intracellular signaling pathways, which changes the functional state, and often the morphology and structure of the nerve cell [12].

It is worth mentioning that NMDA receptors are composed of subunits within which there is structural differentiation. This is of great importance in the context of studying the mechanism of action of ketamine and the search for new antidepressants. The NMDA receptor's basic structural unit is its protein subunits; a single receptor is tetrameric. This means that it consists of four subunits. Each receptor contains two obligatorily GluN1 subunits (binding site for a co-agonist) and GluN2A, GluN2B, GluN2C, or GluN2D subunits present in different configurations (GluN2 subunits are the binding sites for glutamate). NMDA receptors consisting of GluN3 subunits that are glycine-dependent have been discovered in certain brain regions. It should be emphasized that the composition of the subunits determines the kinetic and electrophysiological properties of individual receptors, how they are regulated, and the intracellular processes that are triggered as a result of receptor activation [12].

Although it has been known since 1983 that ketamine is an NMDA receptor antagonist whose biological effects are associated with the functional modification of the glutamatergic system [10], it took a few more years to associate the blockade of the receptor with antidepressant activity. At the beginning of the 1990s, a group of US pharmacologists (from Phil Skolnick's laboratory) [13] published a series of research papers, the results of which indicated the antidepressant activity of NMDA receptor

antagonists such as MK-801 or AP-7 in rodents. The same group [14, 15] proved that other compounds with antidepressant properties could regulate the expression of NMDA receptors in the rat brain. At this point, it is necessary to emphasize the participation of Polish researchers in the research on the antidepressant activity of NMDA receptor antagonists and the role of NMDA receptors in depression. Two Polish pharmacologists, Piotr Popik and Gabriel Nowak, formed the then core of Skolnick's research group, significantly contributing to the initiation of interest in NMDA receptor antagonists in treating depression.

Although the Skolnick group's research began in the early 1990s, the first clinical trial with ketamine took place in 2000. However, the earliest comprehensive hypothesis that explained the unique mechanism of ketamine's antidepressant action was not formulated until 2010 and is still being refined to date.

Disinhibition of glutamate release by blocking NMDA receptors on GABA interneurons as the central hypothesis explaining the mechanism of ketamine's antidepressant action

Understanding the mechanism of ketamine's antidepressant action is inextricably linked to providing a precise molecular target for the drug. It could involve identifying the NMDA receptor subtype, whose blockade determines its antidepressant activity, and explaining the biological mechanisms activated due to the drug attaching to its molecular target. These mechanisms must explain the quick onset of action and the long-term therapeutic effect. The most complex and integral proposition explaining the mechanism of ketamine's antidepressant action is the so-called hypothesis of mTOR kinase activation by the disinhibition of glutamate release. As previously mentioned, this hypothesis was first published in 2010 by a group of American pharmacologists led by Ronald Duman and is still being developed to date [16]. However, the research results were published several years earlier and constitute a fundamental premise for this hypothesis. In 1997, an article was published in which the authors showed that subanesthetic doses of ketamine (10–30 mg/kg body weight) increased glutamate release in the prefrontal cortex of rats. Interestingly, this phenomenon was not observed with higher and anesthetic doses of ketamine [17]. By publishing their first paper in 2010 to explain the antidepressant activity of ketamine, Duman made mTOR kinase activation the focal point of the hypothesis [16]. However, not much was known about how ketamine activates this pathway. From the very beginning, it was puzzling that mTOR activation was only observed after the administration of lower doses of ketamine [16], which somewhat correlated with previously reported results of studies on glutamate release [17]. In the following years, in his review publications Duman [18, 19] postulated that the disinhibition of glutamate release in the prefrontal cortex of rats may be of key importance for the mechanism of ketamine's antidepressant action and the related activation of mTOR. Thus, two issues remain to be clarified,

how a compound that blocks the glutamatergic system can increase glutamate release, and how the increased glutamate release leads to mTOR activation. The fact that the increased release of glutamate by ketamine in the prefrontal cortex of rats could be caused by the blockade of NMDA receptors located on inhibitory GABA (gamma-aminobutyric acid) interneurons innervating glutamate pyramidal cells in this region was an unproven theoretical postulate. The consequence of this blockade is the inhibition of GABA interneurons and the increased activity of pyramidal cells innervated by these interneurons culminating in the increased release of glutamate from cortical pyramidal cells (the so-called glutamate burst) (Figure 1) [18, 19]. The studies from Duman's group published in 2020 [20, 21] confirmed that ketamine blocks NMDA receptors on GABA interneurons in rodents. These studies indicate that the key to the antidepressant effect is the blockade of NMDA receptors containing the GluN2B subunit. In other words, according to this hypothesis, the blockade of interneuronal NMDA receptors and the subsequent increased release of glutamate by pyramidal cells are mechanisms that initiate a series of other molecular and cellular events that constitute the biological substrate for the antidepressant activity of ketamine. In the next step, the glutamate released from the presynaptic terminals binds to postsynaptic AMPA glutamate receptors, which activate the voltage gated calcium channels (VGCC). Calcium ions entering the nerve cell by this route increase the release of

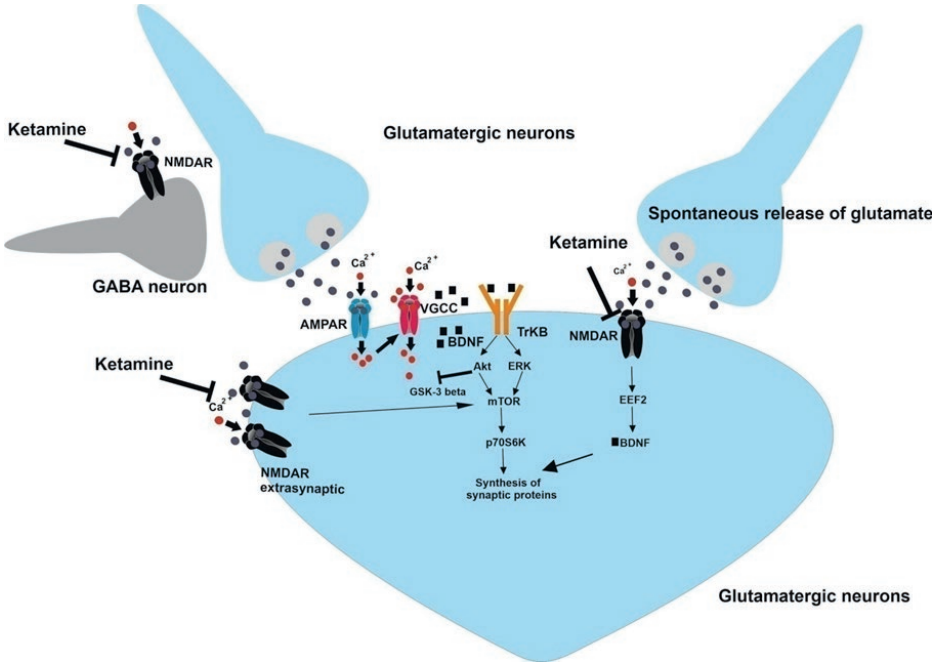

Figure 1. **Graphical abstract. Mechanism of action of ketamine**

BDNF (brain-derived neurotrophic factor) from postsynaptic terminals. In the case of ketamine, BDNF, released outside the neuron, binds to TrkB receptors (tropomyosin receptor kinase B) and activates them. The TrkB receptor through phosphorylation activates intracellular protein kinases such as Akt and ERK (extracellular regulated kinase) and inhibits the GSK-3 kinase pathway. Active Akt and ERK kinases lead to activation of the mTOR kinase leading to the stimulation of the mTOR effector kinases such as p70SK and 4EBP1.

The final molecular effect of the increased activity of the Akt/ERK/mTOR pathway is the local translation of synaptic proteins (such as PSD-95 (postsynaptic density protein-95), synapsin I, or the GluA1 subunit of the AMPA receptor) in dendritic spines. The increased synthesis of synaptic proteins is accompanied by an increased density of dendritic spines in the pyramidal cells of the rat prefrontal cortex [18, 19, 22].

Two important points are worth noting. First, that increased BDNF expression was also noted after chronic administration of classical antidepressants [18]. However, no convincing hypothesis has been formulated to explain how classical antidepressants could lead to the release of BDNF outside the neuronal cell. Second, the key role of BDNF in the antidepressant mechanism of ketamine was also confirmed in other studies by Duman's group [23] using genetically modified mice. These mice carried a variant BDNF Val66Met allele leading to impaired intracellular mechanisms for the expression of the mature form of BDNF and its transport to dendritic terminals. These mice are resistant to the antidepressant effects of ketamine.

In 2011, Duman's group [24] demonstrated that a single dose of ketamine reversed the behavioral, molecular and anatomical effects of chronic unpredictable stress in rats manifested by anhedonia (observed in the sucrose preference test) and the reduced expression of synaptic proteins and reduced dendritic spine density in the prefrontal cortex. The therapeutic effect at all the above-mentioned levels lasted for at least seven days. Recapitulating, in line with the hypothesis of glutamate release disinhibition, activation of kinases such as Akt, ERK, mTOR by BDNF is associated with the onset of the antidepressant effect of the drug, while the persistence of the antidepressant effect is correlated with increased synaptic protein synthesis and rearrangement of synaptic connections associated with alterations in the density of dendritic spines in the PFC. Moreover, pharmacological blockade of the critical components of this molecular cascade, such as mTOR, ERK, Akt kinases, or AMPA receptors, inhibited the antidepressant activity of ketamine [16, 24].

This hypothesis is undoubtedly the most comprehensive theoretical proposal explaining the mechanism of the antidepressant action of ketamine. However, not all research groups have been able to confirm all the findings described above. Moreover, the disinhibition of glutamate release does not explain why other NMDA receptor antagonists do not produce such rapid and long-lasting antidepressant effects. Therefore, alternative hypotheses have emerged that are also worth discussing. Competitiveness

does not presuppose contradictions. Often there are hypotheses whose contents interpenetrate or complement each other.

Direct inhibition of NMDA receptors on glutamate neurons

In 2011, a study by a team of American pharmacologists led by Lisa Monteggia was published in the journal *Nature*. It was obvious at first glance that, despite several common features, the findings of this group differ in several important points from the key assumptions of the glutamate disinhibition hypothesis. The central point of this hypothesis is that ketamine blocks NMDA receptors, which are activated by spontaneously released glutamate leading to the so-called miniature excitatory postsynaptic currents (mEPSC) in the mouse hippocampus [25]. When the released glutamate binds unhindered to the NMDA receptor under physiological conditions, the eEF2 kinase (eukaryotic elongation factor 2) (Figure 1) is activated. The eEF2 kinase inactivates the eEF2 factor by phosphorylation, thus inhibiting translation processes (e.g., BDNF translation is inhibited). As a result of ketamine administration, the spontaneously released glutamate cannot activate NMDA receptors, which leads to decreased activity of the eEF2 kinase, which in turn reduces its phosphorylation activity leading to enhanced BDNF translation [25].

As in the case of the glutamate release disinhibition hypothesis, Monteggia et al. [25] demonstrated that the antidepressant activity of ketamine is dependent on the activation of AMPA receptors because blocking these receptors with an appropriate antagonist abolished the antidepressant effect of ketamine. This hypothesis tries to explain the differences in the antidepressant activity of individual NMDA receptor antagonists. Memantine also blocks the NMDA receptor, but unlike ketamine, it does not exhibit long-lasting antidepressant activity in mice [26]. For clarification, one of the physiological blocking agents of the NMDA receptor is the magnesium ion located inside the receptor channel. *In vitro* culture studies of hippocampal cells showed that memantine and ketamine block the NMDA receptor under magnesium-free conditions. On the other hand, under normal physiological concentration of magnesium ions only ketamine can block the receptor and thus activate translation processes that depend on the eEF2 factor [26].

The hypothesis of direct inhibition of NMDA receptors has another interesting variant. Multidisciplinary studies indicate the anatomical-functional differentiation of NMDA receptors within their local neuronal expression giving rise to synaptic (lying in synaptic density) and the so-called extrasynaptic (lying outside the synapse) NMDA receptors. Extrasynaptic receptors are activated by glutamate, which is always present in the extrasynaptic space. When the concentration of extrasynaptic glutamate is too high, extrasynaptic receptors may increase their activity, leading to atrophy and even death of nerve cells due to excitotoxicity [27] (Figure 1). It is suspected that this mechanism is one of the body's possible responses to chronic stress or inflammation

(it may be related to the disruption of glial function, which regulates glutamate concentration outside the cell). Thus, the blockade of these receptors may be an important cellular mechanism that should also be taken into account in the mechanism of action of ketamine. At the molecular level, increased activity of extrasynaptic receptors is associated with the inhibition of mTOR kinase activity. Administration of ketamine reverses this process [28].

Overall, the results obtained by the Monteggia group did not confirm the findings of the Duman group regarding the key role of the mTOR kinase. Administration of rapamycin, which is an mTOR inhibitor, did not block the antidepressant effects of ketamine. However, it should be noted that the experimental models selected by both teams differed in terms of the animal species used, the research methodology, or the route of rapamycin administration [16, 24, 25]. Despite these apparent differences, both hypotheses affirm the participation of BDNF and AMPA receptors in the antidepressant effect of ketamine. While each of these hypotheses draws attention to different cellular functions of this neurotrophin, BDNF is undoubtedly necessary for a complete ketamine-induced antidepressant response. The following question remains open – is it possible to reconcile the mechanism described earlier into one common biological process activated by ketamine? The answer to the fundamental question of whether this type of theoretical unification is possible requires further research and new empirical data.

Finally, it is worth noting that according to these hypotheses, the action of ketamine is related to the activation of cellular processes in the prefrontal cortex (Duman – rats) or the hippocampus (Monteggia – mice) of laboratory animals. Both clinical and preclinical studies indicate that the dysfunction of the PFC and hippocampus are causally related to the development of depression in humans or pro-depressive behavior in animals [29]. However, it should be borne in mind that depression in humans or chronic stress (understood as an animal model of depression) are pathological conditions whose biological effects are not limited only to the dysfunction of the PFC and hippocampus. Therefore, it is also worth asking if ketamine regulates the function of other brain structures involved in the pathological processes leading to depression.

Blockade of NMDA receptors located in the nuclei of the lateral habenula

For some time, the nuclei of the lateral habenula (the structure of the epithalamus) has been of interest to scientists dealing with the biological basis of depression because chronic stress activates this structure in animals [30]. Also, volumetric analysis and positron emission tomography (PET) studies in people with depression indicate that the function of the nuclei of the lateral habenula may be perturbed [31]. From an anatomical and functional point of view, stress increases neuronal glutamate activity in the nuclei of the lateral habenula in laboratory animals, with the consequent inhibition of dopaminergic neurons in the midbrain. This is important because the midbrain do-

paminergic neurons are involved in processing information about reward stimuli [31]. These processes can be significantly impaired in people suffering from depression and animals exposed to various types of chronic stress [31, 32].

In 2018, the journal *Nature* published an article about the relationship between the lateral habenula and the antidepressant activity of ketamine [33]. The authors showed that ketamine inhibited the NMDA-dependent release of glutamate in the nuclei of the lateral habenula (glutamate bursts) in mice. This activity was associated with its antidepressant property. The same researchers [33] also discovered a causal relationship between NMDA-dependent activity of the lateral habenula and calcium currents dependent on low-voltage T-type calcium channels, which could also be used in the future development of new antidepressants. Unfortunately, the first clinical trial with ethosuximide, a drug that blocks this channel, was unsuccessful [34]. Similarly, animal studies have questioned the potential importance of blocking these calcium channels in the treatment of depression [35]. However, it is worth noting that the inhibition of the activity of neurons in the lateral habenula is probably related to the onset of ketamine action but does not necessarily have any impact on its long-term effects. Further research is thus needed to resolve this fully.

Antidepressant mechanism of (S) – and (R)-ketamine and their metabolites

The studies described so far relate to the antidepressant activity of the racemic ketamine mixture, which contains equal amounts of two enantiomers: (S) – and (R)-ketamine. Except that these stereoisomers twist polarized light in opposite directions, their physical properties are the same. The most important point, however, is that they differ in their biological activity. Basic affinity analysis of the NMDA receptor reveals apparent differences. (S)-ketamine has the strongest affinity for the NMDA receptor (K_i = 0.2 μM), while the racemate binds moderately (K_i = 0.54 μM), with (R)-ketamine having the lowest affinity (K_i = 1.2 μM) [36]. Japanese scientists from Kenji Hashimoto's group [37, 38] have shown that (R)-ketamine is more active than (S)-ketamine in several animal models of depression. It also has a lower potential to cause side effects [39].

Data on side effects obtained from animal studies seem to be in line with clinical observations [38] and show that (S)-ketamine has a higher potential to induce psychomimetic effects. From a mechanistic point of view, the antidepressant activity of both enantiomers in animals exposed to chronic stress depends on the activation of the intracellular BDNF/TrkB signaling pathway and AMPA receptors, since pharmacological blockade of TrkB and AMPA receptors completely blocks their antidepressant efficacy [38]. Both enantiomers increase the expression of BDNF, the phosphorylated (active) form of the TrkB receptor and the GluA1 subunit of the AMPA receptor in the prefrontal cortex and dentate gyrus of stressed mice. These changes correlated with the increased density of dendritic spines in these structures following the administration

of both stereoisomers [38]. Reduced expression of the active phosphorylated form of the TrkB receptor and BDNF was also seen in the CA3 field of the hippocampus of stressed mice. More importantly, only the (R)-ketamine enantiomer inhibited these changes [38]. At the moment, it is difficult to determine how this difference translates into the antidepressant-like activity of individual enantiomers.

While discussing the hypothesis of the disinhibition of glutamate release, the very crucial role played by ERK and mTOR kinases in the intracellular signal transduction cascade, essential for the antidepressant activity of the racemic ketamine mixture, was mentioned [16, 24]. Studies on the mechanism of action of the individual enantiomers have shown that their activity is differentially regulated by the phosphorylation of ERK and mTOR kinases. Chronic stress caused reduced phosphorylation of both kinases in the prefrontal cortex and the hippocampus of mice. Interestingly, a single dose of (S)-ketamine normalized phosphorylated mTOR kinase levels to their baseline levels. Additionally, the administration of rapamycin, an mTOR kinase inhibitor, inhibits the antidepressant activity of (S)-ketamine. The pharmacological blockade of the ERK kinase did not alter the activity of (S)-ketamine. However, the (R)-ketamine enantiomer requires the phosphorylation of the ERK kinase to reverse the behavioral and biochemical effects of chronic stress in mice. At the same time, the administration of an ERK kinase inhibitor abolished (R)-ketamine's antidepressant activity, while the blockade of mTOR did not affect it [36].

These results become even more interesting in light of recent clinical trials. In 2019, American psychiatrists [40] administered rapamycin orally to people suffering from drug-resistant depression to determine if it would weaken the antidepressant efficacy of a single dose of ketamine. Surprisingly, oral rapamycin did not reduce the clinical efficacy of ketamine but extended the duration of action for both drugs. The simplest explanation for this phenomenon may be that the biological mechanism of ketamine action is different in rodents compared to humans. This explanation may be too simplistic and bears the hallmarks of premature surrender. In animal studies, rapamycin is most often administered directly into the brain structures [16, 24], which most likely causes the brain concentration to be much higher than that observed after enteral administration. It is uncertain if the concentration of oral rapamycin is sufficient to block mTOR kinase. Thus, the enhanced clinical efficacy of the ketamine and rapamycin combination may be largely dependent on the peripheral anti-inflammatory properties of rapamycin.

Analyzing the situation described above, it cannot be ruled out that the human administration of rapamycin reaches the brain in appropriate concentration and blocks mTOR kinase. However, assuming that in humans, as in mice, the antidepressant effect of (R)-ketamine is not dependent on mTOR kinase activation, rapamycin can only block those mechanisms that are specifically activated by (S)-ketamine. Thus, the synergistic effect of ketamine and rapamycin may be related to the anti-inflammatory activity of rapamycin and the (R)-ketamine enantiomer. As a potential antidepressant

drug, the prospects of (R)-ketamine seem exciting, especially since the first clinical trials conducted on a small group of patients suffering from drug-resistant depression were promising [41].

Unfortunately, that is not the end of the complication with understanding ketamine's antidepressant mechanism of action. Ketamine, like most xenobiotics, is metabolized in the liver by cytochrome P450. In the first phase, the parent molecules, (R) – and (S)-ketamine, are broken down into norketamines (NK) or dehydronorketamines. In the next phase, NK is metabolized to hydroxynorketamines (HNK) [42]. Because ketamine is an optically active compound, the S and R forms of these metabolites are formed. It turns out that some of these metabolites exhibit antidepressant activity in animal studies. The most interesting so far is (2R,6R)-hydroxynorketamine ((2R,6R)-HNK), which has been shown to be effective in some animal behavioral tests [43–45]. Moreover, its antidepressant effects are devoid of the side effects often associated with the racemate or (S)-ketamine [45]. Like the racemic mixture or individual enantiomers, it elicits a long-lasting antidepressant response with interesting sex differences in animals. The (2R,6R)-HNK metabolite is more potent in female mice than in males. In this research [45], ketamine is metabolized to a greater extent to (2R,6R)-HNK in females than in males. In females, the antidepressant effect is observed at a lower dose, which may be associated with its greater metabolism and higher brain concentration of (2R,6R)-HNK.

The intracellular mechanisms induced by (2R,6R)-HNK appear to be the same for the individual enantiomers and the racemate. The activation of kinases such as mTOR and ERK in the prefrontal cortex and the hippocampus as well as the unblocking of BDNF translation by dephosphorylation of the eEF2 factor in the hippocampus are crucial [45, 46]. The importance of BDNF in the antidepressant activity of (2R,6R)-HNK, has also been documented in studies [46] with transgenic mice carrying the Val66Met polymorphism in which the metabolite does not induce an antidepressant response in behavioral tests. Significant differences in the mechanism of action of (2R,6R)-HNK and ketamine seem obvious with respect to their primary molecular targets. Because (2R,6R)-HNK has a negligible affinity for the NMDA receptor, it is unlikely that its mechanism of action is related to blockade of this receptor [47]. It is known that, as with all forms of ketamine, the activation of AMPA receptors is necessary for the action of this metabolite [45]. However, there is no empirical evidence that this compound binds directly to these receptors. In a recently published article [44], the hypothesis about the possible and necessary involvement of type 2 metabotropic glutamate receptors (mGluR2) in the antidepressant properties of (2R,6R)-HNK was advanced. However, these studies did not show a direct interaction of (2R,6R)-HNK with mGluR2.

It is currently impossible to decide the most important molecular target for (2R,6R)-HNK [44]. From a clinical viewpoint, it is important to know how necessary

this metabolite is for the full antidepressant activity of ketamine and whether it can be used as a standalone antidepressant drug. Currently, there are no published clinical studies on the therapeutic potential of (2R,6R)-HNK. On the other hand, attempts to assess the correlation between the concentration of (2R,6R)-HNK in the blood after ketamine administration and the therapeutic response have produced contradictory results [48]. Therefore clinical studies are necessary to finally resolve this issue, especially since not all research teams have confirmed the activity of (2R,6R)-HNK in animal studies. However, it is worth mentioning that other ketamine metabolites such as (S)-NK and (2S,6S)-HNK (both metabolites have a much higher affinity for the NMDA receptor than (2R,6R)-HNK, and their mechanisms are at least partially NMDA-dependent) have exhibited antidepressant activity in murine models of depression [49, 50].

Recapitulation

For several decades, psychiatrists have had different antidepressants at their disposal to treat individuals suffering from this chronic condition. Unfortunately, not every patient responds adequately properly to the prescribed treatment, thereby requiring different treatment strategies than are available. There seems to be a glimmer of hope for this group of patients using therapy based on ketamine or its (S)-ketamine enantiomer. Unfortunately, from a clinical viewpoint, both of these pharmacotherapeutic options carry the risk of serious side effects, such as psychomimetic effects or the risk of addiction. Clinical and preclinical studies on the antidepressant properties of ketamine have significantly changed how we think about the biological mechanisms of depression and its pharmacological treatment. These two fields of research are complementary and inextricably linked. Despite all the reservations about the use of ketamine as an antidepressant, it should be emphasized that for the first time, it has been possible to systematically and repeatedly prove that the symptoms of depression can be reversed for a limited period while avoiding long-term use of antidepressants.

While some aspects of the molecular mechanism of action of ketamine have been elucidated, some mystery remains to be unraveled. There is no delusion that understanding the mechanisms of action in preclinical studies is sufficient to understand how ketamine works in humans and which metabolites or enantiomers may be effective as new antidepressant drugs. Animal research, however necessary, has limited cognitive value. For complete success, clinical tests are needed to ultimately verify, specify, and direct the work of experimental pharmacologists.

In this review, we have highlighted the most important findings regarding the biological aspects of the mechanism of action of ketamine. The most important does not obviously encompass all that is known about ketamine. Because of space constraints, the potential role of other systems like the opioid system in the antidepressant mechanism of ketamine and the relationship between the antidepressant effects of ketamine

and inflammation have not been discussed. Especially the second issue would require a separate and detailed review.

References

1. Domino EF. *History and pharmacology of PCP and PCP-related analogs*. J. Psychedelic Drugs. 1980; 12(3–4): 223–227.
2. Domino EF. *Taming the ketamine tiger*. 1965. Anesthesiology. 2010; 113(3): 678–684.
3. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS et al. *Antidepressant effects of ketamine in depressed patients*. Biol Psychiatry. 2000; 47(4): 351–354.
4. Diazgranados N, Ibrahim L, Brutsche NE, Newberg A, Kronstein P, Khalife S et al. *A randomized add-on trial of an N-methyl-D-aspartate antagonist in treatment-resistant bipolar depression*. Arch. Gen. Psychiatry. 2010; 67(8): 793–802.
5. DiazGranados N, Ibrahim LA, Brutsche NE, Ameli R, Henter ID, Luckenbaugh DA et al. *Rapid resolution of suicidal ideation after a single infusion of an N-methyl-D-aspartate antagonist in patients with treatment-resistant major depressive disorder*. J. Clin. Psychiatry. 2010; 71(12): 1605–1611.
6. Price RB, Nock MK, Charney DS, Mathew SJ. *Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression*. Biol. Psychiatry. 2009;.66(5): 522–526.
7. Zarate CA Jr., Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA et al. *A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression*. Arch. Gen. Psychiatry. 2006; 63(8): 856–864.
8. Pochwat B, Nowak G, Szewczyk B. *An update on NMDA antagonists in depression*. Expert Rev. Neurother. 2019; 19(11): 1055–1067.
9. Rosenblat JD, McIntyre RS, Alves GS, Fountoulakis KN, Carvalho AF. *Beyond Monoamines-Novel Targets for Treatment-Resistant Depression: A Comprehensive Review*. Curr. Neuropharmacol. 2015; 13(5): 636-55.
10. Anis NA, Berry SC, Burton NR, Lodge D. *The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate*. Br. J. Pharmacol. 1983; 79(2): 565–575.
11. Orser BA, Pennefather PS, MacDonald JF. *Multiple mechanisms of ketamine blockade of Nmethyl-D-aspartate receptors*. Anesthesiology. 1997; 86(4): 903–917.
12. Paoletti P, Bellone C, Zhou Q. *NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease*. Nat. Rev. Neurosci. 2013; 14(6): 383–400.
13. Trullas R, Skolnick P. *Functional antagonists at the NMDA receptor complex exhibit antidepressant actions*. Eur. J. Pharmacol. 1990; 185(1): 1–10.
14. Nowak G, Paul IA, Popik P, Young A, Skolnick P. *Ca2+ antagonists effect an antidepressant-like adaptation of the NMDA receptor complex*. Eur. J. Pharmacol. 1993; 247(1): 101–102.
15. Paul IA, Nowak G, Layer RT, Popik P, Skolnick P. *Adaptation of the N-methyl-D-aspartate receptor complex following chronic antidepressant treatments*. J. Pharmacol. Exp. Ther. 1994; 269(1): 95–102.

16. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M et al. *mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists*. Science. 2010; 329(5994): 959–964.
17. Moghaddam B, Adams B, Verma A, Daly D. *Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex*. J. Neurosci. 1997; 17(8): 2921–2927.
18. Duman RS, Li N, Liu RJ, Duric V, Aghajanian G. *Signaling pathways underlying the rapid antidepressant actions of ketamine*. Neuropharmacology. 2012; 62(1): 35–41.
19. Duman RS, Voleti B. *Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents*. Trends Neurosci. 2012; 35(1): 47–56.
20. Gerhard DM, Pothula S, Liu RJ, Wu M, Li XY, Girgenti MJ et al. *GABA interneurons are the cellular trigger for ketamine's rapid antidepressant actions*. J. Clin. Invest. 2020; 130(3): 1336–1349.
21. Pothula S, Kato T, Liu RJ, Wu M, Gerhard D, Shinohara R et al. *Cell-type specific modulation of NMDA receptors triggers antidepressant actions*. Mol. Psychiatry. 2020. Doi: 10.1038/s41380-020-0796-3.
22. Duman RS, Shinohara R, Fogaca MV, Hare B. *Neurobiology of rapid-acting antidepressants: convergent effects on GluA1-synaptic function*. Mol. Psychiatry. 2019; 24(12): 1816–1832.
23. Liu RJ, Lee FS, Li XY, Bambico F, Duman RS, Aghajanian GK. *Brain-derived neurotrophic factor Val66Met allele impairs basal and ketamine-stimulated synaptogenesis in prefrontal cortex*. Biol. Psychiatry. 2012; 71(11): 996–1005.
24. Li N, Liu RJ, Dwyer JM, Banasr M, Lee B, Son H et al. *Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure*. Biol. Psychiatry. 2011; 69(8): 754–761.
25. Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF et al. *NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses*. Nature. 2011; 475(7354): 91–95.
26. Gideons ES, Kavalali ET, Monteggia LM. *Mechanisms underlying differential effectiveness of memantine and ketamine in rapid antidepressant responses*. Proc. Natl. Acad. Sci. USA. 2014; 111(23): 8649–8654.
27. Parsons MP, Raymond LA. *Extrasynaptic NMDA receptor involvement in central nervous system disorders*. Neuron. 2014; 82(2): 279–293.
28. Zanos P, Gould TD. *Mechanisms of ketamine action as an antidepressant*. Mol. Psychiatry. 2018; 23(4): 801-811.
29. Duman RS, Aghajanian GK, Sanacora G, Krystal JH. *Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants*. Nat. Med. 2016; 22(3): 238–249.
30. Li B, Piriz J, Mirrione M, Chung C, Proulx CD, Schulz D et al. *Synaptic potentiation onto habenula neurons in the learned helplessness model of depression*. Nature. 2011; 470(7335): 535–539.
31. Browne CA, Hammack R, Lucki I. *Dysregulation of the Lateral Habenula in Major Depressive Disorder*. Front. Synaptic Neurosci. 2018; 10:46.
32. Gold PW, Kadriu B. *A Major Role for the Lateral Habenula in Depressive Illness: Physiologic and Molecular Mechanisms*. Front. Psychiatry. 2019; 10:320.

33. Yang Y, Cui Y, Sang K, Dong Y, Ni Z, Ma S et al. *Ketamine blocks bursting in the lateral habenula to rapidly relieve depression*. Nature. 2018; 554(7692): 317–322.

34. Zhang K, Jia G, Xia L, Du J, Gai G, Wang Z et al. *Efficacy of anticonvulsant ethosuximide for major depressive disorder: a randomized, placebo-control clinical trial*. Eur. Arch. Psychiatry Clin. Neurosci. 2020. Doi: 10.1007/s00406-020-01103-4.

35. Tan S, Wang Y, Chen K, Long Z, Zou J. *Ketamine Alleviates Depressive-Like Behaviors via Down-Regulating Inflammatory Cytokines Induced by Chronic Restraint Stress in Mice*. Biol. Pharm. Bull. 2017; 40(8): 1260–1267.

36. Yang C, Ren Q, Qu Y, Zhang JC, Ma M, Dong C et al. *Mechanistic Target of Rapamycin – Independent Antidepressant Effects of (R)-Ketamine in a Social Defeat Stress Model*. Biol. Psychiatry. 2018; 83(1): 18–28.

37. Fukumoto K, Toki H, Iijima M, Hashihayata T, Yamaguchi JI, Hashimoto K et al. *Antidepressant Potential of (R)-Ketamine in Rodent Models: Comparison with (S)-Ketamine*. J. Pharmacol. Exp. Ther. 2017; 361(1): 9–16.

38. Yang C, Shirayama Y, Zhang JC, Ren Q, Yao W, Ma M et al. *R-ketamine: a rapid-onset and sustained antidepressant without psychotomimetic side effects*. Transl. Psychiatry. 2015; 5:e632.

39. Yang C, Qu Y, Abe M, Nozawa D, Chaki S, Hashimoto K. *(R)-Ketamine Shows Greater Potency and Longer Lasting Antidepressant Effects Than Its Metabolite (2R,6R)-Hydroxynorketamine*. Biol. Psychiatry. 2017; 82(5): e43-e4.

40. Abdallah CG, Averill LA, Gueorguieva R, Goktas S, Purohit P, Ranganathan M et al. *Modulation of the antidepressant effects of ketamine by the mTORC1 inhibitor rapamycin*. Neuropsychopharmacology. 2020; 45(6): 990–997.

41. Leal GC, Bandeira ID, Correia-Melo FS, Telles M, Mello RP, Vieira F et al. *Intravenous arketamine for treatment-resistant depression: open-label pilot study*. Eur. Arch. Psychiatry Clin. Neurosci. 2020. Doi: 10.1007/s00406-020-01110-5.

42. Zanos P, Moaddel R, Morris PJ, Riggs LM, Highland JN, Georgiou P et al. *Ketamine and Ketamine Metabolite Pharmacology: Insights into Therapeutic Mechanisms*. Pharmacol. Rev. 2018; 70(3): 621–660.

43. Yao N, Skiteva O, Zhang X, Svenningsson P, Chergui K. *Ketamine and its metabolite (2R,6R)-hydroxynorketamine induce lasting alterations in glutamatergic synaptic plasticity in the mesolimbic circuit.* Mol. Psychiatry. 2018; 23(10): 2066–2077.

44. Zanos P, Highland JN, Stewart BW, Georgiou P, Jenne CE, Lovett J et al. *(2R,6R)-hydroxynorketamine exerts mGlu2 receptor-dependent antidepressant actions*. Proc. Natl. Acad. Sci. USA. 2019. Doi: 10.1073/pnas.1819540116.

45. Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI et al. *NMDAR inhibitionindependent antidepressant actions of ketamine metabolites*. Nature. 2016; 533(7604): 481–486.

46. Fukumoto K, Fogaca MV, Liu RJ, Duman C, Kato T, Li XY et al. *Activity-dependent brain-derived neurotrophic factor signaling is required for the antidepressant actions of (2R,6R)-hydroxynorketamine*. Proc. Natl. Acad. Sci. USA. 2019; 116(1): 297–302.

47. Lumsden EW, Troppoli TA, Myers SJ, Zanos P, Aracava Y, Kehr J et al. *Antidepressant-relevant concentrations of the ketamine metabolite (2R,6R)-hydroxynorketamine do not block NMDA receptor function*. Proc. Natl. Acad. Sci. USA. 2019; 116(11): 5160–5169.

48. Abdallah CG. *(2R,6R)-Hydroxynorketamine (HNK) plasma level predicts poor antidepressant response: is this the end of the HNK pipeline?* Neuropsychopharmacology. 2020; 45(8): 1245-1246.

49. Yang C, Kobayashi S, Nakao K, Dong C, Han M, Qu Y et al. *AMPA Receptor Activation – Independent Antidepressant Actions of Ketamine Metabolite (S)-Norketamine*. Biol. Psychiatry. 2018; 84(8): 591–600.

50. Yokoyama R, Higuchi M, Tanabe W, Tsukada S, Naito M, Yamaguchi T et al. *(S)-norketamine and (2S,6S)-hydroxynorketamine exert potent antidepressant-like effects in a chronic corticosterone-induced mouse model of depression*. Pharmacol. Biochem. Behav. 2020; 191: 172876.

Address: Bartłomiej Pochwat
Maj Institute of Pharmacology, Polish Academy of Sciences in Krakow
Department of Neurobiology
31-343 Kraków, Smętna Street 12
e-mail: pochwat@if-pan.krakow.pl