Current Therapeutic Research 91 (2019) 25–32

Contents lists available at ScienceDirect

Current Therapeutic Research

journal homepage: www.elsevier.com/locate/curtheres

The Effect of Glutamatergic Modulators on Extracellular Glutamate: How Does this Information Contribute to the Discovery of Novel Antidepressants?

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ARTICLE INFO

Article history:
Received 19 March 2019
Accepted 29 August 2019

Keywords:
Depression
Glutamate
Glutamate uptake
Ketamine
Kir4.1 channel
Riluzole

ABSTRACT

Background: In the search for new antidepressants, clinical researchers have been using drugs that simultaneously modulate multiple targets. During preclinical and clinical trials, the glutamatergic modulators riluzole and ketamine have received particular attention. Glutamatergic agents have a modulatory effect on synaptic transmission, so they can act on both neurons and astrocytes. In addition to influencing the quantity of glutamate released, these modulators can also affect the expression, localization, and functionality of glutamate-binding sites.

Objective: This review discusses the complexity of the glutamatergic system, the ambiguity of data regarding glutamate levels in patients with depression, as well as the mechanisms of action for riluzole and ketamine, which includes their relation to the physiology of glutamatergic transmission. The principal aim is to contribute to the development of novel glutamatergic antidepressant medications whilst emphasizing the need for innovative approaches that evaluate their effects on extracellular glutamate.

Methods: Literature was obtained via PubMed by searching the term *depression* in combination with each of the following terms: *riluzole*, *ketamine*, and *glutamate*. The search was restricted to full-text articles published in English between 1985 and 2018 relating to both the modulatory mechanisms of glutamatergic-binding proteins and the antidepressant actions of these medicines. Articles about mechanisms associated with synaptic plasticity and antidepressant effects were excluded.

Results: Although experimental data relates glutamatergic signaling to the pathophysiology of major depression and bipolar disorder, the role of glutamate—as well as its extracellular concentration in patients with said disorders—is still unclear. Riluzole's antidepressant action is ascribed to its capacity to reduce glutamate levels in the synaptic cleft, and ketamine's effect has been associated with increased extracellular glutamate levels.

Conclusions: The strategy of using glutamatergic modulators as therapeutic agents requires a better understanding of the role of glutamate in the pathophysiology of depression. Gaining such understanding is a challenge because it entails evaluating different targets as well as the effects of these modulators on the kinetics of glutamate uptake. Essentially, glutamate transport is a dynamic process and, currently, it is still necessary to develop new approaches to assay glutamate in the synaptic cleft. ORCID: 0000-0002-3358-6939.

Introduction

The primary target for 7% of approved drugs is still unknown. Moreover, in the case of up to 18% of such drugs, the mechanism of action (MOA) is not fully understood. It is important to bear in mind that the effects of many drugs are mediated by more than 1 target, so identifying only 1 target may be insufficient for the determination of a drug's MOA.[1] High specificity and affinity for a target have long been used for drug discovery. However, this strategy often proves unsuccessful for complex disorders such as depression. In fact, seeking single-target drugs (so-called magic bullets) for multifactorial disorders—that often relate to subtle dysfunctions—has proved costly and inefficient. Recently, a new

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https://doi.org/10.1016/j.curtheres.2019.100566
0011-393X/

strategy appears to be emerging in relation to the use of drugs that simultaneously modulate multiple targets.[2] With several MOAs, these medicines are called dirty drugs (ie, multitarget drugs), and they are used by clinical researchers engaged in the study of depression.[3]

Riluzole and ketamine are among the multiple-target drugs initially used for other applications, and these are currently described as glutamatergic modulators (for a review, see Pałucha-Poniewiera[3] as well as Zarate and Manji[4]). It is well known that, through different trophic actions, riluzole and ketamine may alter the connectivity among cells.[3,4] That said, such mechanisms associated with synaptic plasticity and antidepressant effects will not be explored in this review. Instead, the focus shall be on glutamatergic binding proteins (receptors and transporters) and their modulatory mechanisms, which are, potentially, related to the antidepressant actions of these medicines. To highlight the challenges in the evaluation of new modulators, both the glutamatergic actions of riluzole and ketamine as well as the complexity of glutamate signaling will be discussed. In essence, to complement current methods of neuroimaging, it shall be argued that it is necessary to create novel technologies for evaluating extracellular glutamate and/or its transport.

The Glutamatergic System

The cellular structures of the glutamatergic synapse are complex and include axons, dendrites, and glial processes. This intricate network of both neurons and glial cells—separated by narrow and winding spaces—is known as neuropil. The glutamatergic system has several particularities that explain its highly complex functions, including large differences in the spatial distribution of glutamate, which ranges from nanomolar (synaptic cleft) to millimolar (intracellularly) (for review, see Murphy-Royal et al[5]), the absence of its extracellular inactivation (for review, see Danbolt[6]), and the similarity between its binding affinity for its receptors and transporters.[7] Another characteristic of this neurotransmitter system is its heavy dependence on the activity of transporters, which can be modulated at different levels.[6]

Glutamatergic transmission requires fine adjustment to be maintained under physiological conditions. Such fine-tuning is challenged by the absence of an extracellular metabolism that deactivates glutamate. Consequently, the clearance of glutamate is essential to avoid the excitotoxicity that results from the overstimulation of their receptors. This neurotransmitter is removed from the synaptic cleft by astrocytes via excitatory amino acid transporters (EAATs).[6] The glutamate uptake limits the temporal and spatial extent of glutamatergic transmission, which allows local modulation of signaling in the synaptic cleft.[8] This activity is a high-affinity process with a massive capacity for transport and, also, as the driving force, it uses the electrochemical sodium and potassium gradients across the plasma membranes. Among the 5 subtypes of EAATs, EAAT2 (glutamate transporter 1, GLT-1) is the major isoform responsible for cerebral glutamate uptake.[6]

Regarding glutamate receptors, although the ionotropics include N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate receptors, the metabotropics are divided into 3 groups and 8 subtypes.[5,6] AMPA receptors are composed of the subunits GluR1, GluR2, GluR3, and GluR4, which are mainly responsible for the excitatory transmission in the central nervous system. The expression of these subunits seems to be an important mechanism for regulating postsynaptic responsiveness. The NMDA receptor is a tetra-heteromeric complex that is typically formed through an assembly of 2 GluN1 and 2 GluN2 (ie, GluN2A, GluN2B, GluN2C, and GluN2D) subunits. The composition of GluN2A shows quicker kinetics in relation to structures with GluN2B subunits.[9,10] Concerning GluN2B-containing NMDA receptors, another difference is their cellular location; that is, they are predominantly extrasynaptic. In comparison with GluN2A-containing receptors, the presence of GluN2B subunits in NMDA endows it with a higher sensitivity to agonists as well as a decreased sensitivity to magnesium-mediated blocks (for a review, see Miller et al[11]).

Glutamate uptake is a dynamic process with different levels of complexity and modulation.[6,12] Glutamate transporters are subject to various types of controls and regulations, such as transcription and translation,[13,14] cell surface expression, stabilization,[15–18] and internalization.[19–22] This transport may be influenced by astrocyte cytoarchitecture,[23] by EAATs' surface diffusion,[24] and by presynaptic neuronal activity.[8] For instance, as has been shown, the expression[12] and functionality[8] of astrocytic EAAT can be rapidly and transiently modulated by neurons. The fact that neuronal activity is known to depolarize astrocytes[25]—which may reduce the glutamate uptake[8]—is another example of the modulatory cross-talk. This depolarization can remain restricted to microdomains because of the properties of the astrocyte membrane. It can then result in areas of astrocyte surface with distinct uptake activity.[26,27] The slowing of this transport results in increased glutamate permanence in the extracellular space, which prolongs the duration of postsynaptic currents by GluN2A-containing NMDA receptors.[8]

The importance of cell membrane polarization for glutamate uptake is well known[28] because the depolarization of astrocytes may also reduce glutamate uptake.[8] Indeed, astrocytic transporters are much more effective at negative resting potentials[29] and, also, they depend on channels identified as inwardly rectifying potassium channels 4.1 (Kir4.1).[30–33] In these channels, the current flow is increased when the astrocyte membrane shows a more negative potential. Further, Kir4.1 channels are responsible for the strongly negative resting potential that is essential to glutamate uptake.[31] The reduction of the Kir4.1 channel activity evokes astrocyte depolarization, which impairs the driving force of the glutamate transporter. Consequently, both the concentration and the permanence of glutamate in the synaptic cleft can increase and potentiate the excitatory action of this neurotransmitter.[29,34]

Glutamatergic transmission is also partly regulated by the lateral diffusion of receptors in neurons[35] and astrocytes.[36] Likewise, surface trafficking of the EAAT2 (GLT-1) has an active role in modulating glutamate transmission by providing a sufficient number of transporters to compete with the receptors for released glutamate. Once again, cross-talk in the synaptic cleft seems to exert a modulatory action because the neuronal activity affects the distribution of EAAT2, increasing or reducing their surface diffusion in response to high or low activity, respectively.[24] In terms of shaping synaptic transmission, the physiological role of EAAT2 surface diffusion greatly increases the complexity of glutamate modulation in the synaptic cleft.

Morphologic alterations in astrocytes as well as in the characteristics of their cell membranes are also potential targets for glutamate modulation. It has been shown that the processes of astrocytes have high mobility,[37–39] and also that their movement may, over time, affect astrocytic morphology,[40] which may interfere with the time course of glutamate in the synaptic cleft.[5] Another important movement occurs on the astrocytes' surface, and that is where EAAT2 diffusion varies according to the cell region and the glutamate presence.[24,41] EAATs have an affinity for glutamate that is similar to glutamate receptors, which means the 2 may compete and thereby reduce the receptors' activation.[7,42] Thus, the mobility of these transporters along the astrocytic membrane may readily interfere with the permanence of glutamate in the extracellular space,[5] and as a result, alter the kinetics of its transport.[34]

Essentially, neuron-astrocyte communication may affect glutamate clearance in the synaptic cleft, which is where astrocytes are

actively involved in shaping excitatory transmission. In response to increased presynaptic neuron activity, glutamate uptake can be locally reduced in the microdomains of the astrocyte membrane.[8,24] This indicates that glutamate uptake is a mechanism that dynamically controls the extracellular permanence of glutamate.[8] The activity-dependent slowing of the uptake prolongs the time during which glutamate remains free in the extracellular space, and thus may potentiate the activation of glutamatergic receptors.

The events occurring in the glutamatergic synapse depend on the number of glutamate molecules released, where they are released, and both the characteristics and the density of their binding sites. Together, these conditions may determine the permanence of glutamate in this extracellular space and, thus, modulate its signaling. Glutamatergic modulators—such as ketamine and riluzole—mainly act in these complex zones of neuropils, which is where components from different cells cooperate to ensure the efficiency of synaptic transmission.

Ketamine

Ketamine is a rapid-acting antidepressant drug that modulates glutamate neurotransmission.[43,44] A meta-analysis review of short-term, randomized, acute-phase trials reported an antidepressant effect of ketamine. The effect appeared within hours and lasted, postinfusion, up to 1 week.[45] Furthermore, a magnetic resonance spectroscopy (MRS) study of patients with major depressive disorder described a rapid and robust ketamine-induced increase in glutamatergic compounds (glutamate and glutamine) in the medial prefrontal cortex.[46] More recently, among humans, a ketamine-induced increase in the release of prefrontal glutamate has been shown.[44] According to the dose used, a paradoxical effect of ketamine on glutamate neurotransmission has been described by preclinical studies. Although anesthetic doses block glutamatergic transmission,[47] subanesthetic doses present a stimulatory action.[48,49] Ketamine acts on several pharmacologic targets, which include, among others, receptors (eg, NMDA, AMPA, and opioid) and channels (eg, L-type voltage-dependent calcium, voltage-gated sodium, and hyperpolarization-activated cyclic nucleotide) (for a review, see Li and Vlisides[50]).

The rapid antidepressant action of ketamine seems to be related to its modulation of the glutamatergic system.[51] This hypothesis is consistent with several studies that have reported the involvement of different glutamate receptors in ketamine's MOA.[3] It is well known that ketamine binds to and antagonizes NMDA receptors, so its MOA has been related to this glutamate receptor. Ketamine is a noncompetitive and voltage-dependent blocker[52] that equally blocks GluN2A- and GluN2B-containing receptors.[53] Moreover, the cellular response triggered by this blocking action indicates that the NMDA receptor is tonically active.[11] However, there is some conflicting evidence as to whether or not the NMDA receptor's blockade activities are a necessary condition for ketamine action[3,54,55]; that is, although such a blockade has been shown to induce antidepressant-like activity when triggered by other NMDA receptor antagonists (for a review, see Paul and Skolnick[56]). Conversely, recent data have indicated that the significant role of the NMDA receptor in ketamine's MOA is related to its GluN2B subunit.[11] Ketamine suppresses the GluN2B function in cortical pyramidal neurons, enhancing the synthesis of the brain-derived neurotrophic factor, the phosphorylated mammalian target of rapamycin, and the AMPA receptor subunit GluR1 (GluA1).[57]

Regarding the AMPA receptor, experimental data support that its activation both maintains synaptic potentiation and is responsible for the rapid and sustained antidepressant effects of ketamine or its metabolites.[3] It has been further shown that the AMPA receptor antagonism abolishes the antidepressant effect of ketamine[58–63] or its metabolites,[54] whereas the agonism of this receptor may potentiate its antidepressant effect.[61] The mGlu receptors also seem to be related to the rapid antidepressant effect mediated by ketamine. Some mGlu receptors' ligands have been shown to have potential antidepressant-like effects,[64,65] and what is more, they even evoke effects that seem to be involved in the MOA of ketamine.[66–68]

The NMDA antagonism mediated by ketamine and its influence on the excitatory synapses of the corticolimbic brain regions can be explained by 2 hypotheses. The first hypothesis is that ketamine, selectively, antagonizes NMDA receptors in cortical inhibitory interneurons, which results in an indirect excitation of the pyramidal neurons, and that is followed by an increase in the glutamatergic synapses of these cells. The second hypothesis proposes a direct antagonism of the NMDA receptors in excitatory pyramidal neurons, which are then tonically activated by extracellular glutamate and, thereby, functionally reinforce the excitatory synapses.[11]

Hence, the modulation of glutamatergic transmission seems to be the pivotal mechanism for the rapid antidepressant action of ketamine.[51] More specifically, through inhibiting NMDA receptors located in inhibitory interneurons, ketamine induces glutamate efflux in the prefrontal cortex, initiating alterations that result in its antidepressant effects.[47] Recently, several studies have reported an increase in the glutamatergic compounds evoked by ketamine in patients with depression,[44,46] which reinforces the importance of glutamatergic modulators for the development of novel rapid-acting antidepressant drugs.[3] However, the absence of a functional biomarker for glutamate is seen as a great barrier for tracing glutamatergic transmission and, consequently, for evaluating the MOA of ketamine and other novel potential glutamate-based antidepressants.[44]

Riluzole

Riluzole is a neuroprotective agent with anticonvulsant properties, and it is the only drug approved for the treatment of amyotrophic lateral sclerosis.[4] Currently, it is considered a glutamatergic modulator and a promising candidate for the treatment of psychiatric disorders.[4] The first glutamatergic activity attributed to riluzole was an anticonvulsant action[69] that was related to the antagonism of the NMDA receptors,[70] which was then followed by an inhibitory effect on glutamate release.[71] After that, the effects of riluzole on the potentiation,[72] membrane localization of the AMPA receptor,[73] and the enhancement of glutamate uptake[74–76] have also been described.

Initially, riluzole's activities were related only to neuronal mechanisms, but a stimulatory effect upon glutamate uptake has been described in astrocytes, which ascribes a novel cellular target to this drug.[75] Moreover, based on its capacity for reducing glutamatergic activity, preclinical and clinical studies have suggested the application of riluzole in the treatment of mood disorders and obsessive-compulsive disorders (for a review, see Zarate et al[77]). Over the years, various mechanisms of action have been attributed to riluzole, and some of them have been related to its antidepressant effects. Among the mentioned mechanisms are the distinct ways of controlling extracellular glutamate as well as the regulation of neurotrophic factors.[4] However, although preclinical data suggested the antidepressant effect of riluzole[78–80] clinical studies produced inconclusive results.[81–87]

Despite riluzole's use as a neuroprotective agent, the multiple mechanisms of action ascribed to it enormously complicate the understanding of its biological effects. It has been shown that this drug acts on a variety of ion channels and elicits varied cellular effects.[4,88] In essence, riluzole inhibits voltage-gated sodium channels and activates or deactivates different potassium channels. Its contrasting effects on potassium channels seem to depend on the channel subtype involved. For instance, it inhibits both delayed

rectifier potassium channels—Kv1.5 and Kv3.1—in a concentration-dependent manner.[88] This inhibitory effect suggests a stimulatory action of riluzole on neuron excitability because A-type potassium channels regulate the duration and frequency of the action potential.[88] Another example is its inhibitory effect on Kv4.3 channels,[89] which could also stimulate neuron activity because the activation of these channels limits the back-propagation of the action potential into dendrites while, at the same time, regulating membrane excitability in the hippocampus.[90]

What Is Known about Glutamate Levels in Depression?

The research on major depression and bipolar disorder related to glutamatergic transmission is becoming more attractive for clinical researchers, especially regarding the search for new methods of diagnosis and treatment (for a review, see Wise et al[91]). In an attempt to find correlations with mood disorders, there is a great deal of interest in measuring the glutamate in specific brain regions. MRS is employed to measure glutamate levels in vivo. Likewise, the discovery of a peripheral marker of cerebral glutamatergic function would be of great relevance—particularly, if it could be used in the diagnosis or treatment of mood disorders.[92] Because there is growing evidence that glutamatergic signaling is involved in the pathophysiology of these disorders, many researchers have not only been describing glutamate levels in diverse brain areas of patients (for a review, see Henter et al[93]), but also peripherally, in their blood samples.[94]

Imaging studies have also been reporting both higher and lower glutamate levels in different brain areas of depressed patients.[95–97] Indeed, glutamate levels have been largely investigated in unipolar depression and bipolar disorder, but the findings are divergent. Whereas glutamate levels are reduced in unipolar depression[92,98] they seem to be increased in bipolar disorder.[99] Nevertheless, ketamine is known to increase glutamate levels,[100] and it seems to be effective in treating both disorders. Regarding bipolar depression, ketamine's effectiveness arouses curiosity because this condition already seems to be previously associated with increased levels of glutamate.[92] This contrariety could be explained by the differential effectiveness of ketamine as it accords to the cerebral area studied because, although it demonstrates antidepressant effects in some cortical areas,[100] it is ineffective in others.[101] However, it is important to consider that, in 2 studies that recruited medication-free patients with bipolar depression, a reduction in glutamate levels was observed.[92,102] Despite both studies not being statistically significant, when considered together, they could suggest that bipolar depression, in the absence of medication, could be associated with a glutamatergic hypofunction, and that additional investigations are necessary to settle this issue. If confirmed, this reduction in glutamate levels would be in accordance with the clinical efficacy of ketamine in both unipolar and bipolar depression.[92]

In a study carried out with medication-free patients, Wyse et al[92] examined whether or not peripheral glutaminase levels would positively correlate with anterior cingulate cortex glutamate levels. The enzyme glutaminase is responsible for the conversion of glutamine to glutamate, and its peripheral levels measured in serum were correlated with cortical glutamate levels obtained through MRS. The authors reported that, irrespective of diagnosis, glutamate was reduced in the depressive state, and the results did not confirm this enzyme as a peripheral biomarker of central glutamate levels. In another investigation, a meta-analysis of 12 association studies concerning peripheral blood glutamate levels and major depressive disorder demonstrated elevated levels of glutamate in depressed patients.[94] However, the sample sizes were relatively small and, in the meta-analysis, the heterogeneity among the outcomes of these studies was high.

Concerning glutamate levels associated with mood disorders, it should be considered that the complexity of glutamatergic dysfunctions goes beyond either increased or decreased glutamate levels.[77] There is not a simple association between glutamate levels and mood disorders, but it seems essential to first consider which region of the brain is evaluated (for a review, see Sanacora et al[97]), and then exclude the effects of previous or concomitant pharmacologic therapies.[92] Although it is known that different brain regions can demonstrate distinct levels of glutamate in mood disorders, it is still poorly understood whether or not the current antidepressants may affect the extracellular levels of this neurotransmitter in some way. For example, it has been shown that selective serotonin reuptake inhibitors block Kir4.1 potassium channels, depolarize astrocytes, and reduce EAAT2 activity.[103] A likely consequence of this blockade would be an increase in glutamate levels in the synaptic cleft.[29,34]

Drug Effects on Extracellular Glutamate: Important Information for Antidepressant Development?

The discovery of new glutamatergic modulators has attracted the attention of researchers looking for potential alternative drugs, in particular for the treatment of mood disorders. However, considering the complexity of glutamatergic signaling, it is possible to predict that there will be many challenges in the development of these drugs before their application in humans. The repurposing of commercial drugs such as riluzole and ketamine is among the strategies employed in this search for new therapeutic agents for depression. Another approach is the development of bioactive molecules that act as glutamatergic modulators that should be submitted to preclinical evaluations.

Stimulators of EAATs have been suggested as potential antidepressants (for a review, see Lapidus et al[104]), whereas conversely, antagonism of these transporters induces depressive effects.[77] Considering pharmacologic treatment, this could suggest that reducing the glutamate timecourse in the synaptic cleft would be expected in an antidepressant drug. This concept motivated the study of riluzole as an antidepressant agent. The antidepressant action of riluzole has been attributed to its capacity to reduce extracellular glutamate, inhibiting its presynaptic release and enhancing the EAAT-dependent uptake.[75,76,105] That said, many researchers have been trying to understand the extremely rapid and persistent antidepressant effects of ketamine, which, in contrast to riluzole, has been potentially related to increased glutamate levels.[3] Ketamine may act by inhibiting inhibitory interneurons, which are known to modulate glutamatergic hypofunction[100] resulting in an increased glutamate release.[92,100] Therefore, although riluzole and ketamine elicit opposite effects on extracellular glutamate levels, both are considered antidepressant agents.

The identification of a target for a glutamatergic modulator is not a trivial task, yet it is much easier than determining its actual role in glutamatergic signaling. Usually, a glutamatergic agent may act on more than 1 receptor and it may also increase or reduce the glutamate timecourse in the synaptic cleft. The consequences of alterations in glutamate clearance seem to be cerebral-region specific, and this is important because extracellular glutamate is able to modulate both its receptors and its transporters.

The activation of AMPA receptors and the elevated glutamate extracellular concentrations have both been associated with ketamine's effects.[11] Antidepressant actions evoked by low doses of ketamine (subanesthetic) have significantly increased glutamate in the medial prefrontal cortex of rats as well as the anterior cingulate cortex of humans.[100,106] Miller et al[11] proposed that, following ketamine-mediated NMDA receptor antagonism, the activation of AMPA receptors might be necessary for the maintenance of excitatory synapses and the persistence of ketamine's antide-

pressant effect. In contrast to ketamine, the antidepressant effect of riluzole has been associated with lower extracellular glutamate levels. However, riluzole administration at a therapeutically relevant concentration is also associated with its effect on AMPA receptors. In these conditions, riluzole increased AMPA GluR1 and GluR2 distribution on the surface of neurons, which was also accompanied by depolarization of the membrane potential.[73] Concerning the increase in AMPA and NMDA neurotransmission, despite molecular differences, the similar effect of ketamine and riluzole[62,73] has been suggested as a potential common mechanism of antidepressant action that is shared by these medications.[84]

The paradoxical effect of ketamine acting as an antidepressant at low doses and, eventually, evoking anesthesia at higher doses, has been widely questioned. One possible explanation is that GluN2B-containing receptors submitted to tonic activation are more sensitive to low-dose antagonism and, also, are mainly extrasynaptic, which potentially makes them more accessible to exogenous antagonism.[11] At higher concentrations of ketamine, synaptic NMDA receptors might be gradually blocked, leading to dissociative effects. Furthermore, even a potential blockage of other non-NMDA receptors cannot be ruled out.[107–109] The blockage of GluN2B seems to be associated with an antidepressant effect, but not with an increase in extracellular glutamate.[11] Indeed, this relationship is not clear because the potent antidepressant Ro 25-6981—which is a GluN2B antagonist—does not provoke an elevation of extracellular glutamate.[110]

Although riluzole is a potent glutamatergic modulator that acts through different MOAs, a recent double-blind clinical trial did not confirm its antidepressant action.[111] One may attempt to explain this lack of effect by considering that the mechanisms ascribed to riluzole—suggesting a reduction in extracellular glutamate levels—were identified in vitro, which is where its concentrations were known and maintained under control. That said, in the studies with patients, the effective concentrations of riluzole are much more difficult to determine, and this uncertainty must, perhaps, be considered as a possible explanation for its ineffectiveness. Beyond pharmacokinetic studies that show a large variability in riluzole's clearance and its serum concentrations among individuals, food also decreases its absorption.[4]

The structural differences of the 2 drugs notwithstanding, a dose–dependent effect—as observed with ketamine—could also occur with riluzole. It is important to consider that a biphasic effect of riluzole on glutamate uptake, which was riluzole-concentration dependent, has previously been described in cortical astrocytes.[75] Another consideration regarding riluzole doses could be whether or not this result observed in vitro would also be demonstrated in patients with depression. If higher riluzole doses reduce glutamate uptake in patients—as observed in vitro[75]—an increase in the time-course of glutamate in the synaptic cleft as well as the stimulation of the glutamatergic system would be expected. Although this possibility may involve different MOAs, it could suggest similar results for ketamine and riluzole concerning glutamate levels.

Although several studies have associated the neuroprotective effect of riluzole with an extracellular glutamate reduction, evidence of its stimulatory action on glutamatergic transmission has also been reported. For instance, an unexpected effect of chronic riluzole treatment was previously observed to enhance overall glutamate metabolism in rats.[112] This stimulatory effect was considered consistent with, not decreased glutamate release, but rather, increased glutamate release.[112] Likewise, riluzole seemed to rapidly increase glutamate–glutamine cycling in patients with bipolar depression. This effect was more pronounced in the anterior cingulate cortex than in the parieto-occipital cortex. Although this pattern did not reach statistical significance, in terms of glutamatergic activity, it was suggested that these brain regions might respond differently to riluzole treatment.[84]

The nonselective action of riluzole on different ion channels[88,89] may potentially affect glutamatergic transmission in different ways; for example, by modifying the resting potential of astrocytes. This possibility indicates the complexity of the still unknown effects of riluzole. One effect could be the modification of EAATs' kinetics and, consequently, the time-course of glutamate in the synaptic cleft. As a real multitarget drug, riluzole acts on different ion channels and affects several ionic currents.[88,89] Beyond acting on the neuron membrane potential, riluzole could also be, potentially, affecting the polarization of astrocytes. This possibility has not been investigated, but, if this effect is confirmed, it could explain previous results related to the effect of riluzole on glutamate uptake.[75] In addition to explaining riluzole's MOA on the kinetic parameters of EAATs, the confirmation of this effect on astrocyte membrane potential would open a new field for future investigations concerning this drug. Furthermore, this could suggest that higher doses of riluzole would be necessary to evaluate its potential antidepressant action in another double-blind clinical trial.

Conclusions

Glutamatergic synapses are complex zones with glutamate binding proteins whose expression, distribution, and affinity can be rapidly modulated. As a consequence, the number and location of receptors and transporters can be dynamically altered to create microdomains in cell membranes. These areas may present particular characteristics and respond unevenly to glutamate. Therefore, identifying molecules with glutamatergic bioactivity is a challenge because not only should the evaluation of different targets be considered, but also the potential consequences upon the kinetics of glutamate uptake. An innovative technology to measure transporter activity and assess glutamate clearance could, functionally, complement data from MRS imaging. Glutamate uptake is dependent on different functional parameters, such as energetic status and membrane polarization, among others. Thus, functional information could contribute to understanding the events related to variation in glutamate levels in the synaptic cleft, which would help to clarify the participation of this neurotransmitter in patients with depression. Moreover, clinical researchers have not only noted the importance of a functional biomarker for glutamatergic transmission, but also how it would support the diagnoses and identification of potential novel antidepressants. Combined with MRS, this approach could be used for developing the concept of precision medicine for patients with depression in the future.

Acknowledgments

Funding for previous research that allowed the present study was provided by grants from the Brazilian National Research Council. The Brazilian National Research Council had no further role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the manuscript for publication.

Conflicts of interest

The author has indicated that he has no conflicts of interest regarding the content of this article.

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