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Review

Perisynaptic astrocytes as a potential target for novel antidepressant drugs

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ABSTRACT

Emerging evidence suggests that dysfunctions in glutamatergic signaling are associated with the pathophysiology of depression. Several molecules that act on glutamate binding sites, so-called glutamatergic modulators, are rapid-acting antidepressants that stimulate synaptogenesis. Their antidepressant response involves the elevation of both extracellular glutamate and brain-derived neurotrophic factor (BDNF) levels, as well as the postsynaptic activation of the mammalian target of rapamycin complex 1. The mechanisms involved in the antidepressant outcomes of glutamatergic modulators, including ketamine, suggest that astrocytes must be considered a cellular target for developing rapid-acting antidepressants. It is well known that extracellular glutamate levels and glutamate intrasynaptic time-coursing are maintained by perisynaptic astrocytes, where inwardly rectifying potassium channels 4.1 (Kir4.1 channels) regulate both potassium and glutamate uptake. In addition, ketamine reduces membrane expression of Kir4.1 channels, which raises extracellular potassium and glutamate levels, increasing postsynaptic neural activities. Furthermore, inhibition of Kir4.1 channels stimulates BDNF expression in astrocytes, which may enhance synaptic connectivity. In this review, we discuss glutamatergic modulators' actions in regulating extracellular glutamate and BDNF levels, and reinforce the importance of perisynaptic astrocytes for the development of novel antidepressant drugs.

1. Introduction

More than 300 million people in the world suffer from depression; this makes the disease the most significant cause of global disability.[1] Patients with depression exhibit diverse symptoms, including blunted mood, an inability to experience pleasure (anhedonia), cognitive impairments, sleep disturbances, and somatic symptoms.[2,3] It is known that depression is closely associated with a reduction in monoamines in the brain (monoamine hypothesis).[3] Based on the monoamine hypothesis, numerous agents that enhance the activities of the noradrenergic and serotoninergic systems are widely used. These drugs include selective serotonin reuptake inhibitors (e.g., fluoxetine, sertraline and paroxetine) and serotonin-noradrenaline reuptake inhibitors (e.g., milnacipran, duloxetine, and venlafaxine), as well as noradrenergic and specific serotoninergic antidepressants (e.g., mirtazapine).[3,4] Nonetheless, there are still unmet clinical needs for the treatment of depressive disorders, including 1) delayed onset of antidepressant action with currently available drugs, 2) inadequate efficacy for severe depression and the existence of a treatment-resistant population and 3) various adverse reactions such as gastrointestinal side effects, serotonin syndrome and malignant syndrome. Therefore, the development of new drugs that overcome these unmet clinical needs is urgently required.

The intricacy of the mechanisms associated with depression biology continues to challenge clinical researchers. Data obtained from patient studies,[5–8] and animal models,[9,10] points to a glutamatergic imbalance in the pathophysiology of depression. Even though the hypothesis that associates the glutamatergic system with depression was proposed three decades ago,[11] the role of glutamate in this illness is not still completely understood.[12] The complexity of glutamatergic signaling and methodological limitations regarding glutamate evaluation in patients can explain this difficulty.[13] Evidence suggests that the rapid-acting effects of glutamatergic modulators are brought about through functionally

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interconnected mechanisms that reestablish the connectivity and activity of specific brain areas.[14,15]

Glutamatergic modulators mainly act in the tripartite synaptic zones that include axons, dendrites, and astrocyte processes.[13] In these highly complex areas, perisynaptic astrocytes play an essential role in maintaining extracellular glutamate homeostasis. This activity is critical because glutamatergic transmission requires fine adjustment to maintain physiological conditions, and avoid excitotoxicity. Glutamate is removed from the synaptic cleft by astrocytes via excitatory amino acid transporters (EAATs), especially EAAT-1 and EAAT-2, which reuptake 80–90% of the total extracellular glutamate in the brain.[16] Glutamate uptake limits the temporal and spatial extent of glutamatergic transmission, which allows local modulation of signaling in the synaptic cleft.[17] However, astrocytes may also release a tiny quantity of glutamate that acts as a gliotransmitter to the adjacent neurons, synchronizing their firing, and modulating their excitatory or inhibitory transmission.[16]

An understanding of the temporospatial significance of extracellular glutamate within the synaptic cleft may contribute to the development of novel rapid-acting antidepressants. This review focuses on signaling mechanisms and cellular components of the glutamatergic synapse, and discusses the interplay between neurons and perisynaptic astrocytes. The major aim is to propose extracellular glutamate as a pivotal element in the biology of depression, reinforcing the role of perisynaptic astrocytes as an additional target for developing novel antidepressant drugs.

2. Glutamate hypothesis in the biology of depression

Depression is known to develop by lesions of various brain areas and is associated with atrophy of specific brain structures such as the medial prefrontal cortex (mPFC), limbic regions (e.g., amygdala and hippocampus), and basal ganglia.[18–23] The decreased volume is attributed to a reduction in the number of neurons, glial cells, dendritic spines, and synaptic connectivity.[18–22] In the 1990s, researchers investigating depression considered that the pathophysiology of this illness was related to increased extracellular glutamate or decreased levels of brain-derived neurotrophic factor (BDNF). Based on the first hypothesis, the action of an antidepressant drug was considered to be associated with a reduction in extracellular glutamate,[11,24,25] and in the second, an increase in BDNF levels.[26,27] Of note, other studies have also shown that brain BDNF levels may be increased by glutamatergic activation,[28–30] suggesting an interaction between BDNF and glutamate in synaptic signaling. In addition, evidence of the regulatory action of glutamate-induced BDNF on neuronal connectivity was first demonstrated in the prefrontal cortex of primates.[31] The role of these cellular mechanisms in depression has been investigated by researchers over the last few years, and many studies have reported evidence that links glutamatergic activity, BDNF expression, and synaptic connectivity.[32] Several studies have reinforced the functional interplay between BDNF and synaptic connectivity in depression biology. Also, stimulation of the BDNF–tyrosine kinase B receptor (TrkB) signaling pathway has been considered as a potential antidepressant strategy.[14] Recently, abnormalities in BDNF-TrkB signaling were related to the onset of depression in a postoperative model. In this study, ketamine-antidepressant effects were related to this signaling pathway's up-regulation.[33] Another study has recently reported a rapid and sustained antidepressant-like effect elicited by DNA methyltransferases inhibitors in the prefrontal cortex. This effect involved the BDNF-TrkB pathway's disinhibition and mTORC1 activation.[34] Differently of the BDNF role in the biology of depression, glutamate function in this disease's pathophysiology has been reviewed recently, mainly due to the discovery of novel mechanisms associated with ketamine.[12,15]

The mechanisms and cellular processes underlying ketamine exposure are complex and varied. Microdialysis studies have shown that subanesthetic doses of ketamine cause a rapid and transient elevation of extracellular glutamate in the mPFC[35–37] and evoke antidepressant effects.[38,39] Ketamine blocks the tonic firing of GABAergic interneurons, which may indicate the glutamate burst results from the disinhibition of glutamatergic terminals.[40] The increase in glutamate levels in the mPFC evoked by ketamine is rapidly followed by an enhancement in synaptic connectivity in this cortical area.[41,42] It has been shown that ketamine rapidly increases levels of the activated form of the mammalian target of rapamycin complex 1 (mTORC1) and induces expression of glutamate receptors and structural synaptic proteins. These effects are in agreement with ketamine's stimulatory action on synaptic connectivity and activity in the mPFC.[43,44] These ketamine stimulatory effects are blocked by rapamycin, a selective mTORC1 inhibitor.[45] Additionally, the synaptogenic effect mediated by mTORC1 signaling is associated with BDNF release, which is also required for the actions of ketamine.[14]

Over the last two decades, data obtained from studies with ketamine have contributed to clarifying some mechanisms related to the biology of depression. It has been hypothesized that transient N-methyl-D-aspartate (NMDA) receptor inhibition of GABAergic interneurons induces glutamate release. The glutamate released acts postsynaptically on α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors, activating neurons in the prefrontal cortex. This brief activation results in BDNF release, stimulation of the mTORC1 signaling factor, increased synthesis of synaptic proteins, and, finally, an enlargement of connectivity.[14] Evidence suggests that extracellular glutamate, postsynaptic receptor activation, BDNF expression, mTORC1 signaling, and synaptogenesis, are events functionally interconnected and potentially related to the positive antidepressant outcomes (Fig. 1). Thus, the convergent hypothesis for the actions of rapid-acting antidepressants is that these agents may restore the depression-induced loss of synaptic connectivities in the prefrontal cortex and limbic regions.[15]

Ketamine acts on other receptors and ion channels beyond NMDA, which suggests that its actions may interfere with multiple neurotransmitter systems. Other NMDA antagonists, or those non-related to NMDA, are also currently being investigated regarding their potential antidepressant effects and have mechanisms that resemble ketamine. Data from preclinical and clinical studies have shown that this convergence of events underlying the antidepressant response can be evoked by several antagonists and agonists of distinct neurotransmitter systems. Thus, based on its rapid and robust antidepressant effect, researchers have also been investigating ketamine as a model for developing novel drugs for depression.[46,47] However, clarifying its mechanism of action has been a challenge, since as a glutamatergic modulator, ketamine may directly or indirectly affect the functionality of diverse glutamate binding sites.[13] Currently, different approaches are been used in an attempt to elucidate the mechanisms related to ketamine's antidepressant effect. Data from these studies may also support comparisons with the activity of other glutamatergic modulators.

3. Novel antidepressants demonstrate functionally interrelated mechanisms

Studies have shown that the NMDA receptor subtype b (NR2b) selective antagonist Ro 25-6981 produces a rapid antidepressant action[48] that is blocked by the mTORC1 signaling antagonist rapamycin (Fig. 1).[43] Indeed, Ro 25-6981 produced a transient

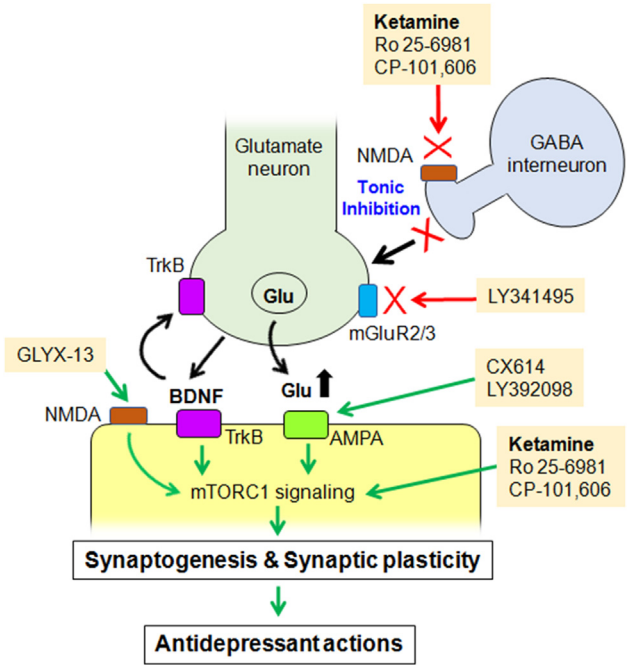

Fig. 1. A schematic diagram illustrates the potential targets of rapid-acting antidepressants (RAADs). The action of RAADs seems to be related to convergent mechanisms that restore synaptic connectivities in specific brain regions. These functionally interconnected mechanisms involve glutamate (Glu) release, postsynaptic receptor activation, brain-derived neurotrophic factor (BDNF) expression, and the mammalian target of rapamycin complex 1 (mTORC1) signaling. RAADs mechanisms of action may involve modulation of diverse receptors that include metabotropic glutamate receptor subtype 2 and 3 (mGluR2/3); N-methyl-D-aspartate receptor (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor (AMPA), and tyrosine kinase B receptor (TrkB). Glutamatergic modulators known to be RAADs are shown, including ketamine; Ro 25-6981 and CP-101,606 (NMDA receptor subtype b (NR2b) selective antagonists); GLYX-13 (NMDA receptor agonist); LY341495 (presynaptic mGluR2/3 receptors antagonist); and CX614 and LY392098 (AMPA receptor potentiators). Studies have demonstrated that RAADs increase glutamate release and activate postsynaptic neurons, enhancing synaptic connectivity in the medial prefrontal cortex.

activation of mTORC1 signaling in synaptoneurosomes from the prefrontal cortex, and increased levels of several synaptic proteins.[43] The antidepressant effect of Ro 25-6981 and CP-101,606 (another NR2b selective antagonist) has been shown to be due to their stimulatory action on prefrontal cortex connectivity (Fig. 1).[49] Furthermore, a magnetic resonance spectroscopy (MRS) study in rats has shown that CP-101,606 (also known as traxoprodil) may activate the mPFC.[50]

A molecule that also exerts a rapid antidepressant effect, but acts through a distinct mechanism, is GLYX-13 (also known as rapastinel) (Fig. 1). GLYX-13 is an NMDA receptor glycine site partial agonist and was shown to increase BDNF and synaptic connectivity in the prefrontal cortex. All of these rapastinel effects were dependent on postsynaptic glutamate-mediated activation.[51,52] A GLYX-13 action on glutamate release has not reported, but its antidepressant effects are inhibited by AMPA receptor blockade.[43,53]

Another pharmacological strategy in studies on depression has focused on metabotropic glutamate receptors (mGluRs). There are eight subtypes of metabotropic receptors that are divided into three major groups with group II, being the most studied in depression. Group II includes the mGluR2 and mGluR3 subtypes, which are autoreceptors expressed presynaptically.[54] Studies have shown that antagonists of mGluR2/3 receptors increase the release of glutamate,[35,55] while other studies have demonstrated that these antagonists produce antidepressant effects.[56,57] It has been shown that the antagonist LY341495 increases extracellular glutamate in the mPFC by blocking presynaptic mGluR2/3 receptors that control glutamate release (Fig. 1).[55] It has also been shown that LY341495 increases mTORC1 signaling in the mPFC[45,53] and has a rapid antidepressant action.[58] This antagonist also increases levels of the synaptic proteins GluR1, PSD95, and synapsin I in the mPFC, suggesting that mGluR2/3 antagonists may increase synaptic connectivity.[53]

In the search for novel antidepressants, AMPA stimulators were also considered as candidates based on evidence that glutamate release by ketamine is necessary for postsynaptic AMPA receptor activation that evokes the antidepressant response. This is in line with a study that showed that postsynaptic glutamatergic blockage with AMPA receptor antagonists abolishes ketamine antidepressant effect, the induction of mTORC1 signaling, and the synthesis of synaptic proteins.[43] A study showed that the ampakine CX614 (positive AMPA modulator) markedly increased the expression of BDNF.[30] Furthermore, both a stimulatory action on BDNF expression[59] and an antidepressant effect was also observed after exposure to another AMPA receptor potentiator, LY392098 (Fig. 1).[60]

It has been suggested that the actions of AMPA receptor potentiating drugs and mGluR2/3 antagonists are closely linked to synaptic levels of glutamate. The efficacy of both depends on either potentiating postsynaptic actions of glutamate at AMPA receptors or blocking presynaptic glutamate actions at mGluR2/3 receptors.[14] A transitory increase in extracellular glutamate following administration of diverse types of antidepressant agents has been reported by several authors.[12] However, this transient increase in glutamate levels does not seem to be exclusively associated with an antidepressant action on a glutamatergic receptor since it is also observed after scopolamine exposure.[14]

Placebo-controlled crossover studies have demonstrated that the muscarinic cholinergic receptor antagonist scopolamine produces a rapid antidepressant response in depressed patients.[61,62] It has been shown that scopolamine is a potent stimulator of mTORC1 signaling and that it also increases the number and function of new dendritic spines in the mPFC. This cholinergic antagonist also produced an antidepressant effect that was blocked by inhibition of mTORC1 signaling or by blockade of AMPA receptors.[63] Furthermore, it was also shown that scopolamine rapidly increased extracellular glutamate levels in the mPFC, as determined by microdialysis.[63] Other studies have also demonstrated that scopolamine increases glutamate release and postsynaptic activation, as well as BDNF levels and synaptic connectivity in the mPFC. These scopolamine effects have been shown to depend on postsynaptic glutamate activation.[39,64,65]

4. Extracellular glutamate as a biomarker of depression

Because evidence has associated glutamatergic signaling with the pathophysiology of depression, many researchers use glutamate levels in diverse brain areas as a biomarker of this disease. Indeed, clinical studies on glutamatergic metabolites (glutamate + glutamine) have shown that glutamatergic dysfunction is related to depression. The levels of these metabolites were evaluated in depressed patients through MRS studies; however, these investigations reported contradictions regarding glutamate levels in different brain areas.[6–8,66–68] A divergence regarding glutamate levels has also been reported in the plasma and cerebrospinal fluid of depressed patients.[69–74] It should also be noted that antidepressant approaches, such as pharmacological and electroconvulsive therapy, have been shown to increase these metabolites in the mPFC of depressed patients.[7,8,35,74,75] Recently, a meta-analysis of MRS studies systematically reviewed reported reduced levels of glutamatergic metabolites in the mPFC of patients with depression.[76] In this meta-analysis, the authors noted that the

findings assessing glutamate levels in depression were inconsistent. These inconsistencies could be potentially due to the studies themselves sample sources, and to the heterogeneity of depressive disease.[76] Thus, despite technological advances and sophisticated approaches to evaluate extracellular glutamate in cerebral areas of interest, these attempts remain a notable challenge.[13]

Moreover, extracellular glutamate increase seems to evoke different responses in distinct brain regions. A recent study compared the somatosensory and frontal cortices and showed differences in glutamate uptake between these regions that may influence NMDA receptor activation.[77] Authors have reported that in the frontal cortex, glutamate was removed faster from the extracellular space in response to high-frequency stimulations, and that this response was not influenced by the amplitude, but rather by the frequency, of the stimuli. Contrary to the somatosensory cortex, glutamate uptake increased with higher frequency synaptic stimulation in the frontal cortex, which could be related to persistent neuronal activity, a feature of frontal cortex networks.[77]

A significant effort is also being made to understand the meaning of extracellular glutamate in the synaptic region, where rapid-acting antidepressants act. However, this understanding is still more difficult at the glutamatergic synapse, where glutamate binding sites may be rapidly altered in response to glutamate levels.[78,79] A critical example concerning the importance of glutamate and its location at the synapse relates to the consequences of ketamine-mediated glutamate release.[37] A glutamate action on synaptic NMDA receptors may increase synaptic growth and strength.[43] In contrast, activation of the extrasynaptic NMDA receptor is related to a reduction in synaptic connectivity.[80] Thus, the time-course of extracellular glutamate seems to be essential because it may determine which glutamatergic receptors are activated.

Glutamate leakage from the synaptic cleft is an improbable event because uptake mediated by astrocytes has a high capacity for transport.[81] However, this possibility needs to be taken into account since in the case of glutamate escape from the synaptic cleft, it may activate extrasynaptic NMDA receptors and evoke undesirable effects (e.g., excitotoxicity).[80] This detrimental event could be due to pathophysiological conditions that impair the activity of the uptake system (e.g., energetic failure) or that exceed the transport capacity (e.g., excessive glutamate).[81] The glutamate uptake system depends on the activity of its transporters, which can be modulated at different levels.[13] It has been already shown that several molecules considered as potential antidepressant agents,[82] as well as some commercial antidepressants, produced a direct or indirect modulatory effect on glutamate transporters.[83] This effect on EAAT activity indicates the importance of evaluating mechanisms that may affect the homeostasis of extracellular glutamate.

5. Role of perisynaptic astrocytes in extracellular glutamate homeostasis

Dynamic cross-talk between neurons and astrocytes allows fine-tuning of glutamate signaling in the glutamatergic synapses. Beyond establishing the synaptic cleft limits, astrocytes control the glutamate time-course inside it through a robust uptake system (Fig. 2).[78] The glutamate uptake activity is a high-affinity process mediated by EAATs, being mostly carried out by EAAT2 (GLT-1) in the cerebral cortex.[17,81,84] This transport uses the electrochemical gradient of sodium and potassium as the driving force across the astrocytic plasma membranes.[81]

Astrocytes regulate neural activities through potassium spatial buffering, which removes excess extracellular potassium caused by neural firing and transports it to regions with low potassium levels (e.g., microcapillaries).[85–88] This spatial potassium-buffering function is primarily mediated by inwardly rectifying potassium channels 4.1 (Kir4.1 channels), which are specifically expressed in astrocytic membranes. In addition, spatial potassium buffering is closely coupled to extracellular glutamate uptake because a reduction in Kir4.1 channel-mediated potassium conductance depolarizes astrocyte membranes and reduces EAATs-activity.[13,88,89] Thus, reduced activities of Kir4.1 channels (e.g., channel blockade and expressional suppression) may elevate extracellular glutamate levels (Fig. 2). Moreover, recent evidence revealed that blockade or expressional knockdown of Kir4.1 channels facilitated BDNF expression in astrocytes.[90] It is therefore likely that inhibition of Kir4.1 channel activities elevates extracellular potassium, glutamate, and BDNF levels at synapses, which may trigger the events functionally interconnected and potentially related to the positive outcomes of rapid-acting antidepressants.[13,91,92]

Increased neuronal activity elevates the extracellular potassium concentration, which also depolarizes astrocytes[93] and reduces glutamate uptake.[17] Moreover, augmented presynaptic neuron activity may locally reduce glutamate uptake in some specific regions (microdomains) of the astrocyte membrane.[17,78] The consequent slowing of glutamate uptake prolongs the time during which glutamate remains free in the extracellular space. This extension in the glutamate time-course in the synaptic cleft may alter the kinetics of its transport[81] and potentiate the activation of glutamatergic receptors.[17]

In accordance with potential role of astrocytic Kir4.1 channels in the pathogenesis and treatment of depression, it has been shown that expression of astrocytic Kir4.1 was upregulated in the lateral habenula of the animal model of depression, congenitally learned helpless rats.[94] Increased expression of Kir4.1 channels was also reported in parietal cortex from patients with major depressive disorder.[95] In addition, astrocyte-specific gain of function transgenic treatment of Kir4.1 channels in the lateral habenula caused a hyperpolarization of habenula neurons, producing bursting firing pattern and depression-like behavioral phenotypes.[94] By contrast, knockdown or blockade of Kir4.1 channels caused a depolarization of habenula neurons, shifting the bursting firing pattern to tonic firing and then silent (depolarization block) pattern and ameliorate depression-like behaviors. These findings illustrate a crucial role of astrocytic Kir4.1 channels underlying the pathogenesis of depressive disorder.[91,96] In addition, the rapid-acting antidepressant ketamine has been shown to suppress bursting of in the lateral habenula and rapidly relieve depression-like behaviors.[97] Moreover, ketamine modulated astrocytic Kir4.1 channel expression by reducing trafficking cytoplasmic Kir4.1 to plasma membranes, which consequently reduce the Kir4.1 channel activity in astrocytes.[98] Thus, it is likely that astrocytic Kir4.1 channels may be involved in the rapid-acting antidepressant action of ketamine.[96] However, further studies are required to validate this hypothesis, since influences of Kir4.1 channel inhibition on expression of depressive behaviors are not yet fully evaluated, and glutamate transport mechanisms are topographically different among brain regions.[77,94,99,100]

The importance of perisynaptic astrocytes to maintain extracellular glutamate homeostasis is remarkable, achieved by balancing glutamate uptake and release.[16] In addition to the glutamate transporters and Kir4.1 channels, several other astrocytic membrane-proteins act to ensure this fine control, some of which are also found in neurons. Glutamate receptors present on astrocytes also include mGluRs and the three classes of ionotropic receptors (AMPA, NMDA, and kainic acid).[16,101] More than that, astrocytes express receptors and transporters for all the major neurotransmitter systems, being thus targets for antidepressants developed to acts on neuron cells. Indeed, antidepressant therapies

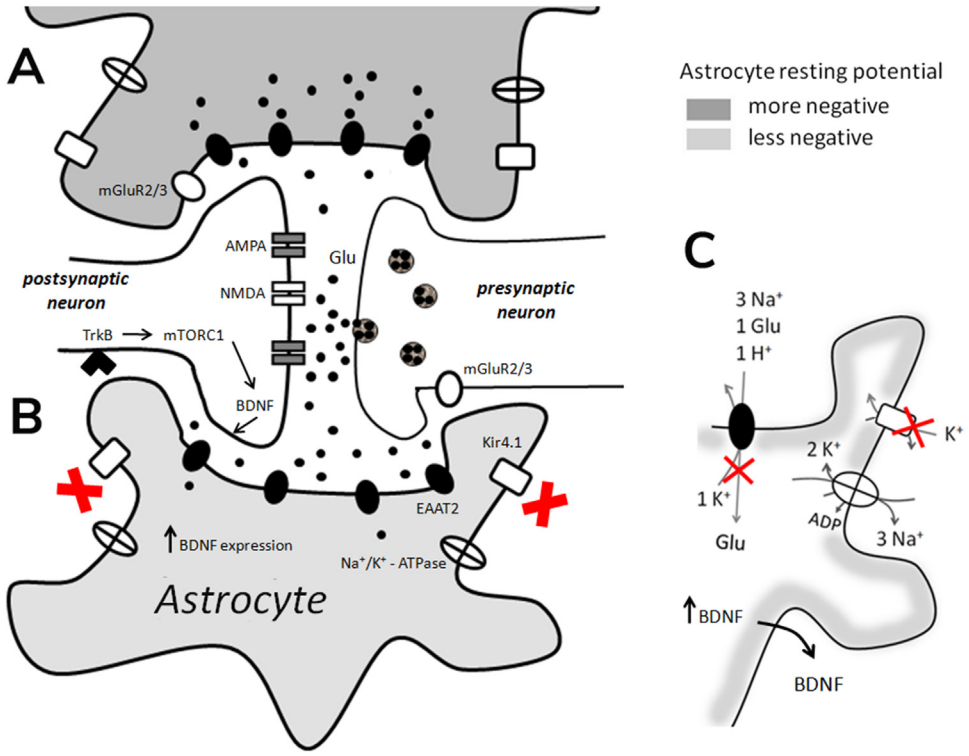

Fig. 2. Representation of the glutamatergic synapse. Astrocytes maintain intrasynaptic homeostasis of extracellular glutamate, determining its time-course inside the cleft. In the cerebral cortex, perisynaptic astrocytes remove glutamate (Glu) released from the presynaptic terminal, mainly through excitatory amino acid transporter 2 (EAAT2). This uptake depends on the electrochemical gradient of Na^+ established by Na^+/K^+-ATPase activity and is much more effective at negative resting potentials. The astrocyte membrane potential is hyperpolarized by the inwardly rectifying potassium channels 4.1 (Kir4.1), and glutamate uptake is closely related to the functionality of these channels. A reduction in Kir4.1 channel activity or expression depolarizes astrocytes, raising extracellular glutamate within the synaptic cleft. The schematic diagrams illustrate the relationship between astrocytes with more negative (A) and less-negative (B) resting potentials, and physiological and reduced glutamate uptake, respectively. Diagram B shows an astrocyte with less-negative resting potential due to inhibition of Kir4.1 channels, which results in higher levels of extracellular glutamate. As a consequence, the probability of postsynaptic glutamatergic receptor activation is increased. Diagram C shows the glutamate transport mechanism with stoichiometric details of EAAT2 and Na^+/K^+-ATPase activities. The effect of Kir4.1 channel blockade on glutamate uptake and BDNF expression is also represented. Other abbreviations in the picture: metabotropic glutamate receptor subtype 2 and 3 (mGluR2/3); N-methyl-D-aspartate receptor (NMDA); α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor (AMPA); tyrosine kinase B receptor (TrkB); brain-derived neurotrophic factor (BDNF); mammalian target of rapamycin complex 1 (mTORC1).

may regulate the expression of several astrocyte-specific proteins beyond stimulating the astrocytic expression of various trophic factors (for review).[102] Besides, authors have suggested that the antidepressant therapies activate astrocytes, which respond modulating several functions related to the control of extracellular glutamate, the release of trophic factors, synaptogenesis, and that result in the reactivation of cortical plasticity. For this reason, perisynaptic astrocytes must be considered when glutamatergic agents are being investigated as rapid-acting antidepressants.

6. Future perspectives

Preclinical and clinical studies on depression have been focusing on pharmacological approaches that modulate, directly or indirectly, glutamate binding sites. A convergent point seems to be that the antidepressant effect of many of these drugs is associated with a transitory increase in extracellular glutamate. Of note, this transient increase in glutamate levels appears to be a key mechanism related to rapid-acting antidepressants, even though the role of this extracellular glutamate in the antidepressant response is still unclear.[12] In the last decade, ketamine has been extensively studied due to its rapid-acting antidepressant effects.[38,39] The multiple targets and varied mechanisms associated with ketamine's action involve glutamatergic receptors and synaptic plasticity. Although ketamine acts as an NMDA antagonist, it is not clearly understood whether its antidepressant effect is due to glutamatergic inhibition or, in contrast, activation (e.g., glutamate release). Because ketamine also evokes a rapid and transient increase in glutamate levels in the mPFC,[35–37] as a consequence, the released glutamate could also activate postsynaptic AMPA receptors. Likewise, the muscarinic cholinergic receptor antagonist scopolamine, which elicits rapid-acting antidepressant effects, also evokes a rapid and transitory increase in glutamate levels in the mPFC.[12] Other antidepressant agents, such as antagonists of mGluR2/3 receptors, increase extracellular glutamate in the mPFC.[55] Transient glutamate activation was also observed with the NR2b selective antagonists Ro 25-6981 and CP-101,606 (traxoprodil), the NMDA receptor glycine site partial agonist GLYX-13 (rapastinel), and the AMPA receptor potentiator LY392098.[12]

The glutamate release and mPFC activation associated with the effect of rapid-acting antidepressants have been shown by image studies.[37,50] Even though it is not still possible to discriminate pre and postsynaptic processes, these results reinforce the evidence that extracellular glutamate has a role in the antidepressant effect. Detection of glutamatergic metabolites in specific cortical regions (e.g., mPFC) may display, even if with some limitations, dynamic synaptic events. However, novel technical advances are necessary to allow the evaluation of extracellular glutamate in more restricted areas, for instance, discriminating intracortical layers. Several studies have been shown evidence of layer-specific characteristics that may contribute to a better understanding of the mechanisms related to rapid-acting antidepressants.

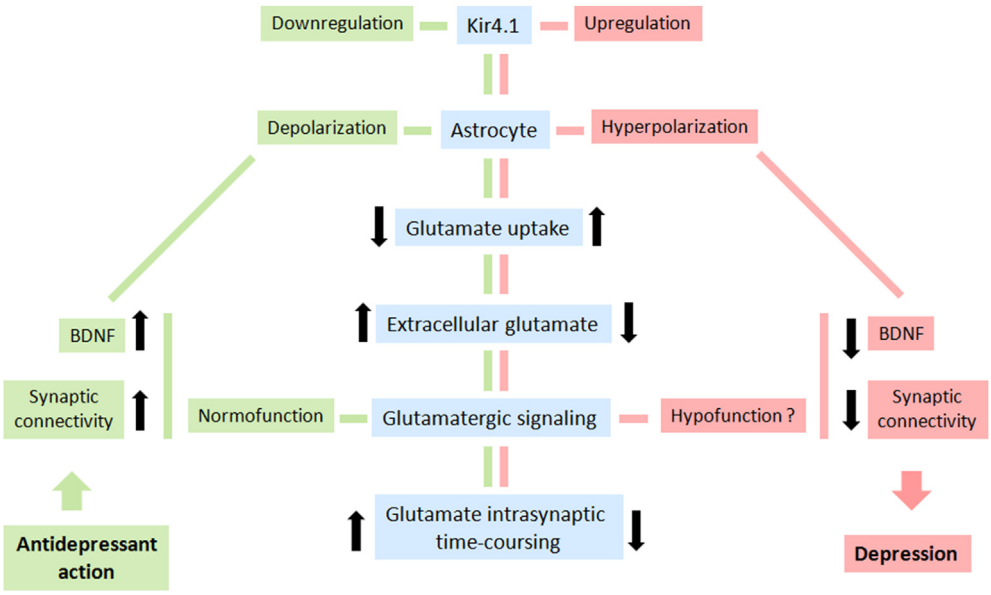

Fig. 3. Events in glutamatergic synapse functionally associated with inwardly rectifying potassium channels 4.1 (Kir4.1). Physiological conditions or positive antidepressant responses are represented by green, while pathological situations related to depression are marked in red. Increased activity of Kir4.1 channels due to upregulation or overexpression may hyperpolarize astrocytes, increasing glutamate uptake, and consequently provoking reduced levels of extracellular glutamate. As proposed, this condition would be lead to a reduction in glutamate intrasynaptic time-coursing and likely glutamatergic hypofunction, with diminished amounts of brain-derived neurotrophic factor (BDNF), and loss of synaptic connectivity. Together, these alterations could be related to a depressive state. Conversely, physiological regulation of Kir4.1 channels or dysfunctions that may reduce their activity (e.g., channel blockade and expressional knockout or down-regulation) may depolarize astrocytes, decrease glutamate uptake, elevate extracellular glutamate levels, and enhance astrocytic BDNF expression. These functionally interconnected events are potentially related to the positive outcomes of rapid-acting antidepressants. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

Recently, a novel layer-specific mechanism, mediated by receptors mGluR2/3, was reported in the dorsolateral prefrontal cortex (dlPFC) of primates.[103] In layer III of the dlPFC, mGluR2/3 receptors are also expressed postsynaptically in the dendritic spines. Moreover, the authors reported that pharmacological stimulation of mGluR2/3 in dlPFC also had an unexpected excitatory effect, which was abolished by the mGluR2/3 antagonist LY341495. Of note, the authors reported that low doses of mGluR2/3 agonists evoked an increase in neuronal firing, while with higher doses, there was an opposite effect. The reduction in neuronal activity with higher doses of agonists was explained by their action on mGluR2/3 receptors located presynaptically, where activation reduces glutamate release, and/or on perisynaptic astrocytes, where mGluR2/3 stimulation increases glutamate uptake.[103]

Another relevant example of region dependence regarding glutamatergic signaling described a differential response to glutamate uptake of layer I astrocytes due to higher frequency synaptic stimulation.[77] The study showed that the frontal cortex seems to maintain extracellular glutamate under greater control by increasing its transport capacity. Moreover, in the frontal cortex, a singular presence of subtype EAAT1 (GLAST-most frequent in the cerebellum) transporter was observed, as well as the expression of EAAT2. Additionally, the authors showed that the increase in glutamate uptake capacity in response to high-frequency stimulations was not attributed to Kir4.1 channels.[77]

Additional evidence concerning astrocyte heterogeneity and essential function of control of extracellular glutamate has been recently shown in the frontal cortex.[100] This study demonstrated astrocyte differences exist not only inter-regionally, but also intra-regionally, since intracortical astrocyte subpopulations were identified. Astrocytes in layer I of the frontal cortex display reduced expression of functional Kir4.1 channels and, being less polarized, have a smaller capacity for transporting extracellular glutamate.[100]

Evidence has already demonstrated the importance of a glutamatergic receptor's location to the effect of its activation.[103] An example is NMDA receptors, where activation on a synaptic position evokes responses different from other extrasynaptic areas.[12] Similar to the mGluR2/3 receptors that may be found on pre and postsynaptic terminals, as well as astrocytes, and where activation of each one results in distinct effects related to glutamate.[103] These examples have been widely studied and are a target of great interest for the development of novel rapid-acting antidepressants. However, further to glutamate receptor location, the permanence of glutamate within the synaptic cleft is another factor that may strongly determine which receptor is activated (Fig. 3).

The influence of Kir4.1 channels on EAATs activity is well known because the efficiency of these transporters depends on astrocyte membrane hyperpolarization.[88,89] Despite the functional significance of Kir4.1 channels regarding extracellular glutamate levels, they have not been considered targets for the development of new glutamatergic antidepressants. However, further significant evidence may support a possible role of Kir4.1 channels as an additional participant among the intricate mechanisms related to depression biology. Studies have shown that serotonin reuptake inhibitors and tricyclic antidepressants reversibly inhibit Kir4.1 channels in a concentration-dependent manner.[91] Moreover, these antidepressants induce BDNF expression in astrocytes according to their relative potencies for Kir4.1 channels.[90] Lastly, based on a great deal of experimental evidence, astrocyte Kir4.1 channels have recently been proposed as a novel target for the treatment of depression.[92,94] A recent postmortem study reported increased expression of Kir4.1 channels in the parietal cortex of depressed patients.[95] Furthermore, it has been shown that ketamine reduces the density of these channels on the astrocyte plasma membrane.[96,98] A likely functional consequence of this effect would be a reduction in astrocyte capacity to uptake extracellular glutamate and an increase in extracellular glutamate level (Fig. 2). This result may strengthen the hypothesis that an increase in glutamate in the synaptic cleft is a critical modulatory event in the rapid-acting antidepressant response. Thus, the inhibition of astrocytic Kir4.1

channels may serve as a novel mechanism of action for future antidepressant agents.

Additional studies on the possible involvement of Kir4.1 channels in depression biology and in antidepressant actions of glutamate modulators are necessary. In addition, further clinical studies are necessary to verify the rapid-acting antidepressant effects of glutamate modulators since no validated controlled studies were performed except for the case of ketamine.

7. Conclusion

Perisynaptic astrocytes modulate extracellular glutamate levels and, indirectly, influence the probability of glutamatergic receptor activation. These astrocytes remove glutamate from the cleft affecting glutamate intrasynaptic time-coursing directly. The astrocyte resting potential is kept hyperpolarized by Kir4.1 channel activity, which is essential for the efficiency of their EAATs. Inhibition of Kir4.1 channels depolarizes astrocyte membranes and reduces glutamate uptake, which may in turn induce glutamatergic postsynaptic activation. Another consequence of Kir4.1 channel inhibition is an increase in BDNF expression that may stimulate the enhancement of synaptic connectivity. Consequently, perisynaptic astrocytes and Kir4.1 channels may be promising targets for developing novel rapid-acting antidepressants.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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