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ORIGINAL ARTICLE

NMDA receptor antagonists ketamine and Ro25-6981 inhibit evoked release of glutamate *in vivo* in the subiculum

TL Stan, A Alvarsson, N Branzell, VC Sousa and P Svenningsson

Preclinical and clinical data have identified ketamine, a non-selective NMDAR (N-methyl-D-aspartate receptor) antagonist, as a promising medication for patients who do not respond to treatment with monoamine-based antidepressants. Moreover, unlike the current monoamine-based antidepressants, ketamine has a long-lasting effect already after a single dose. The mechanisms of ketamine action remain to be fully understood. Using a recently developed microelectrode array (MEA), which allows sub-second measurements of fluctuating glutamate concentrations, we studied here the effects of *in vivo* local application of the ketamine and of the N2B subunit-specific antagonist Ro25-6981 upon evoked glutamate release. Both ligands inhibit glutamate release in subregions of the hippocampus and prefrontal cortex. Likewise, acute systemic ketamine treatment, at an antidepressant dose, caused a reduction in evoked glutamate release in the subiculum. We suggest that the effects of ketamine and Ro25-6981 in the subiculum could involve blockade of presynaptic NMDA receptors containing N2B subunits.

INTRODUCTION

Depression, the predominant form of affective or mood disorders,[1] is one of the leading causes of disease burden worldwide, with a great impact on the health status.[2] Major drawbacks with currently used antidepressants, which mostly target the monoamines, are that therapeutic effect is only manifested after weeks of treatment and that many patients do not respond to them.[3] Therefore, the development of novel fast antidepressants, with alternative mechanisms of action, is a crucial goal in depression research.

L-Glutamate (Glu), the major excitatory neurotransmitter in the mammalian central nervous system, has been shown to be a key neurotransmitter in depressive pathology.[4,5] Clinical studies have found evidence for glutamatergic dysfunction using neuroimaging and in samples of plasma, serum, cerebrospinal fluid and post-mortem brain tissue of depressed patients.[6] In the past decades, pharmacological studies, targeting the ionotropic N-methyl-D-aspartate receptors (NMDARs), particularly the N2 subunits, indicate that NMDARs have a major role in the etiology of depression.[7]

Human studies have found that excitatory neural circuits within the hippocampal–prefrontal cortical system, which regulate stress responsiveness and mood, are over-activated in patients with major depressive disorder.[8] Likewise, studies in rodent models have shown that different types of chronic stress induce depression-like changes on behavioral, morphological (for example, synaptogenesis) and signal transduction parameters[9–13] within the glutamatergic hippocampal–prefrontal cortical circuitry.[14] Importantly, a single, sub-anesthetic dose of the NMDAR antagonists ketamine or Ro25-6981, a N2B subunit-specific antagonist, shows rapid antidepressant effects and also counteracts depressive-like behaviors in chronically stressed rodents.[15] The rapid antidepressive effect of ketamine is accompanied by alterations in postsynaptic glutamatergic signaling and synaptogenesis.[15] However, no studies have examined the region-specific effects of ketamine or Ro25-6981 on local glutamate release using modern methods with high temporal and spatial resolutions.

The fast analytical sensing technology (FAST) enables *in vivo* detection of low levels ($< 1\,\mu\text{M}$) of tonic and depolarization-induced release of glutamate, and its clearance, with a high spatial and temporal resolution ($< 1\,\text{s}$).[16,17] Using FAST, the current study aimed to investigate how local application of the NMDAR antagonists ketamine or Ro25-6981 affects tonic and evoked glutamate release in different brain regions relevant to depression. Furthermore, we measured levels of tonic and evoked glutamate release in the subiculum in a time course of 2 h following an acute and systemic administration of an antidepressant-like dose of ketamine.

MATERIALS AND METHODS

A ceramic-based microelectrode array (MEA), S2 type (Figure 1; Quanteon, Nicholasville, KY, USA), was used. The MEA contained four platinum (Pt) recording sites ($15 \times 333\,\mu\text{m}$ each) arranged in pairs (100 μm between the pairs, each pair being 30 μm apart). One of the pairs functioned as recording sites and the other pair functioned as reference (sentinel) sites (Figure 1a). To be able to measure glutamate release, the MEAs were selectively coated (Figure 1a) as described before.[17,18] Briefly, the recording sites were first coated with L-glutamate oxidase (Yamasa Corporation, Tokyo, Japan), bovine serum albumin (Sigma-Aldrich, Stockholm, Sweden) and glutaraldehyde (Glut; Sigma-Aldrich), whereas the sentinel sites were only coated with bovine serum albumin and Glut. Later on, the MEA assembly was inserted into a solution of 5 mM methaphenylen diamine dihydrochloride (Fisher Scientific, Göteborg, Sweden) in degassed 0.05 M phosphate-buffered saline (pH 7.4). An electric potential of +0.5 V was applied between a Ag/AgCl reference electrode (Pronexus Analytical, Stockholm, Sweden) and the MEA platinum (Pt) sites for 22–24 min, thus
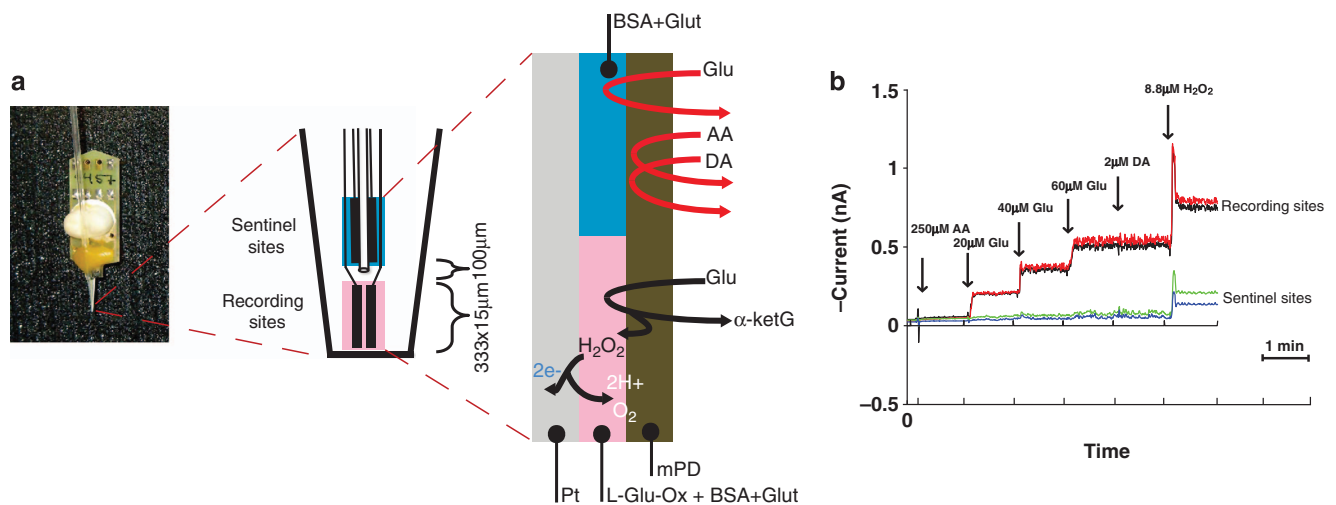
Figure 1. Illustrations of the recording method. (**a**) Close-up of a microelectrode array (MEA) tip with an aligned micropipette and a schematic illustration of the selective coatings. The glass micropipette served to locally deliver depolarizing solution. One of the platinum (Pt) pairs was used as recording sites and the other pair as reference (sentinel sites). The MEA recording sites were coated with a mixture of L-glutamate oxidase (L-Glu-Ox), bovine serum albumin (BSA) and glutaraldehyde (Glut), whereas the sentinel sites were only coated with BSA and Glut. All MEA sites were coated with a protective layer of meta-phenylenediamine dihydrochloride (mPD) to block electroactive interferents commonly found in the brain, such as ascorbic acid (AA) and dopamine (DA). Glu-Ox caused enzymatic breakdown of glutamate (Glu) into α-ketoglutarate (α-ketG) and peroxide (H_2O_2). Thus, the sentinel sites responded only to molecules not repelled by mPD, except Glu, and were used for self-referencing. Responses recorded at the sentinel sites were subtracted from those of the recording sites with the resulting signal representing Glu measurements. (**b**) *In vitro* calibration of a MEA. This test provided information about the MEA responses towards electroactive interferents such as AA and DA, added for final buffer concentration of 250 μM (AA) and 2 μM (DA). Three consecutive additions of Glu changed the buffer concentration of glutamate to 20, 40 and 60 μM, respectively, and were performed to calculate the MEA selectivity and sensitivity. A final addition of peroxide (H_2O_2; 8.8 μM final buffer concentration) was used to evaluate whether all the recording sites responded similarly.

creating an exclusion layer of methaphenylen diamine dihydrochloride over the MEA sites.

Assembly of the MEA and micropipette

A single barrel glass pipette (1 mm o.d., 0.58 mm i.d., A-M Systems, Carlsborg, WA, USA) without filament was pulled (P1000 micropipette puller, Sutter Instruments, Novato, CA, USA) to obtain a glass micropipette (tip inner diameter of 10–15 μm). The micropipette was positioned, under microscopic magnification, at 50–100 μm distance from the MEA recording sites (Figure 1a).

Calibration of the MEA for glutamate measures

Before the *in vivo* experiment, each MEA assembly was calibrated *in vitro* at constant applied potential of +0.7 V versus an Ag/AgCl reference electrode for parameters including slope (electrode sensitivity), linearity (R^2) and selectivity (Figure 1b) as previously described.[16,19] During calibration, the MEA was placed into a beaker containing a continuously stirred phosphate-buffered saline (pH 7.4 at 37 °C) and challenged to final concentrations of 250 μM ascorbic acid (Sigma-Aldrich), 20, 40 and 60 μM Glu (Sigma-Aldrich), 2 μM dopamine (Sigma-Aldrich) and 8.8 μM H_2O_2 (Sigma-Aldrich). H_2O_2 became oxidized with the loss of two electrons in the presence of the constant applied potential of +0.7 V. The generated current was amplified and digitized using the FAST-16 MKII system. An MEA was selected for further *in vivo* experiments if the selectivity (Glu: ascorbic acid) was 20:1.[16,19]

Animals

Adult male C57Bl/6 J mice (Charles River, Erkrath, Germany), 12 weeks of age, were used ($n = 6$–14 animals per group). Mice were group-housed in Type III Macrolon cages in humidity and temperature controlled conditions. The mice were kept in a 12-h light–dark cycle (lights on at 0700 hours) with *ad libitum* food and water. All efforts were made to reduce the number of animals used and to minimize their suffering. All experiments were carried out in agreement with the European Council Directive (86/609/EEC) and the experimental procedures were approved by the local Animal Ethics Committee (Stockholms Norra Djurförsöksetiska Nämnd) approval number N24/12.

The animals were anesthetized with isoflurane (Baxter Medical, Kista, Sweden), 3% for induction and 1.0–1.5% for maintenance. The animals were placed in a stereotaxic frame (David Kopf Instruments) fitted with a Cunningham Mouse Adaptor (Stoelting, Wood Dale, IL, USA). During the whole experiment, the body temperature of the animal was maintained constant at 37 °C via a heating pad (World Precision Instruments, Stevenage, UK).

Pharmacological agents

An isotonic depolarizing solution (70 mM KCl, 79 mM NaCl and 2.5 mM $CaCl_2$, pH 7.4), was freshly prepared on day of experiment, filtered and used as the vehicle to dilute the drugs applied locally with micropipette. The non-selective NMDAR antagonist, S-ketamine (Pfizer), was diluted to a final concentration of 100 μM, which was previously shown[20] to have a physiological NMDAR blockade effect. The N2B subunit-selective NMDAR antagonist, Ro25-6981 hydrochloride (Tocris, Ellisville, USA), was diluted to a final concentration of 100 μM. For the acute systemic treatment experiment, animals received a single intraperitoneal (i.p.) injection (total volume 10 ml kg^{-1}) of 0.9% saline (vehicle) or S-ketamine delivered at the dose of 15 ml kg^{-1} and diluted in saline. The doses of NMDAR antagonists used were based on previous studies.[12,20–22]

In vivo measurements of glutamate release

The MEA assembly was attached to a micromanipulator (Narishige Scientific Instrument Lab, RO-10) on a stereotaxic frame and guided to the regions of interest. The stereotaxic coordinates were defined according to the mouse brain atlas.[23] For the hippocampus (including the CA1 and dentate gyrus (DG)) the anterior–posterior (AP) value in respect to bregma was set to AP: − 1.7 mm and medial-lateral (ML) coordinates according to the midsagittal suture line were set to ML: ± 1.0 mm. The MEA assembly was lowered in increment steps from the surface of the brain at the dorsoventral coordinates CA1: − 1.5 mm; DG: − 2.0 mm. For the subiculum, the coordinates were AP: − 3.1 mm, ML: ± 1.5 mm and dorsoventral : − 1.5 mm. For the prefrontal cortex (including prelimbic (PreL) and infralimbic (IL) regions) the coordinates were AP: +1.54 mm, ML:
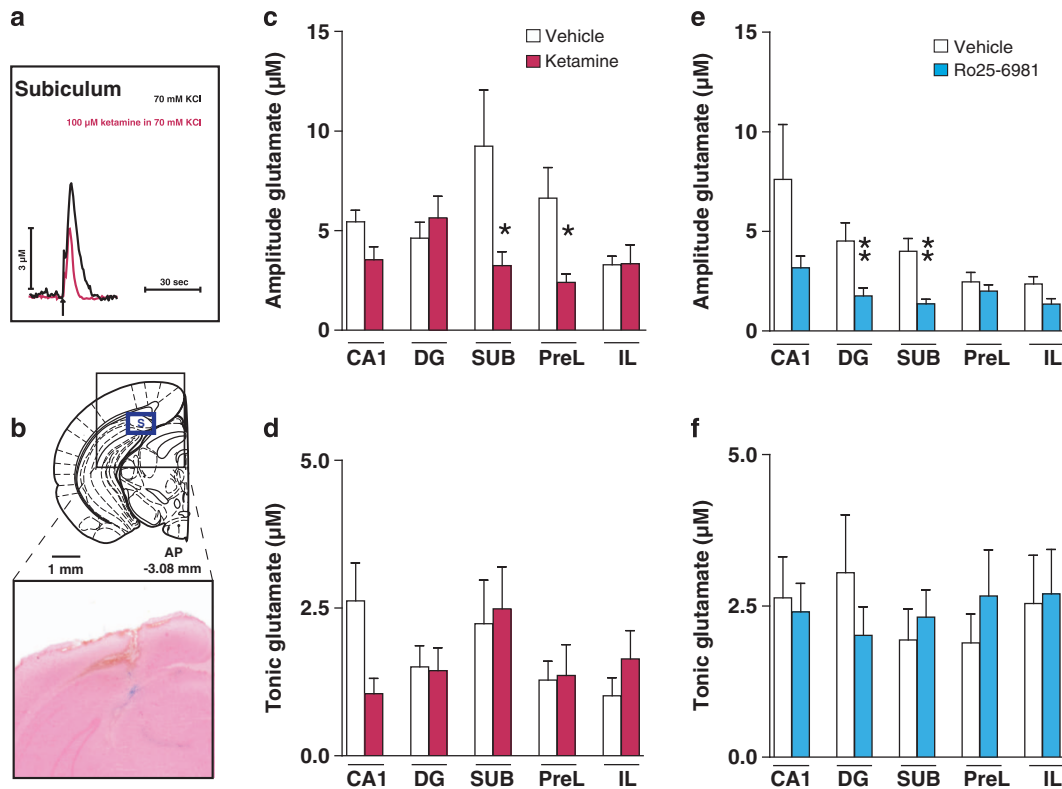
Figure 2. Evoked and tonic glutamate release after local application of ketamine and Ro25-6981. Representative glutamate peaks from the subiculum (**a**). Photomicrograph showing histological verification of the MEA recording site by local methylene blue injection in a 50-μm coronal brain section of the subiculum (indicated on the right hemisphere), after counterstaining with Nuclear Fast Red (**b**). The depolarizing solution (70 mM KCl) was pressure-ejected (↑) in the absence (vehicle) or presence of ketamine or Ro25-6981 (100 μM) for 1-s duration with 1-min interval between each application. The evoked glutamate (**c**, **e**) and the tonic levels (**d**, **f**) are depicted per studied region. Note that local application of ketamine caused a significant decrease in presynaptic glutamate release in the subiculum and prelimbic area of the prefrontal cortex (**c**), whereas local application of Ro25-6981 caused a significant decrease in the subiculum and dentate gyrus (**e**). Tonic extracellular glutamate levels did not change after local application of either ketamine (**d**) or Ro25-6981 (**f**) in any of the regions analyzed. Data represent mean ± s.e.m. from five glutamate peaks from 8–14 animals. $*P < 0.05$; $**P < 0.01$, paired Student's t-test versus vehicle within region. CA1: cornu ammonis 1, DG: dentate gyrus, SUB: subiculum, PreL: prelimbic region of the prefrontal cortex, IL: infralimbic region of the prefrontal cortex.

± 0.3 mm. The PreL dorsoventral value was − 2.1 mm, whereas for IL was − 2.6 mm.

The micropipette was connected to a Picospritzer III (Parker Hannifin, Cleveland, OH, USA) allowing local application of 100–150 nl of depolarizing solution, pressure ejected for one second duration with a 60-s interval between each application. The Glu-Ox-coated sites caused an enzymatic breakdown of elicited glutamate into α-ketoglutarate and H_2O_2 (Figure 1a). The unilateral recordings and the treatment applied were performed in a randomized order—that is, starting in a region with either vehicle (only depolarizing solution), or one of the NMDAR antagonist solutions and proceeding contralaterally with a different, freshly prepared solution (as depicted in Figure 2a). In the set of animals given systemic saline (vehicle) or S-ketamine (15 mg kg^{-1}), unilateral recordings from the subiculum were made using only the above mentioned depolarizing solution to measure evoked and tonic glutamate release.

We performed constant voltage amperometry recordings (final gain pA mV^{-1}) at a frequency of 2 Hz by applying a potential of +0.7 V versus an Ag/AgCl electrode. The Ag/AgCl electrode was implanted into a region remote to the recording sites and the signal was allowed to reach a stable baseline recording.[17,18]

Histological verification

To assess the MEA recording sites, 0.25 μl of saturated methylene blue solution was locally injected. Coronal brain slices (50 μm) were sectioned in a cryostat and counterstained with Nuclear Fast Red (Sigma-Aldrich) solution. The correct MEA position was determined under microscopic inspection (Figure 2b), as previously described.[24]

Data collection

The evoked glutamate was measured as the average amplitude of five consecutive, stable and reproducible glutamate release peaks with an amplitude between 1 and 30 μM in a studied region (Figures 2a, c and e) based on a method previously described.[16,17,25] The evoked peaks were reproducible at the 1-min intervals between depolarizations. Results were analyzed offline with the Quanteon FAST Analysis 4.1 software (Quanteon) in conjunction with the calibration reports.[16,19] The tonic glutamate concentrations (Figures 2d and f) were determined by subtracting the recorded current from the sentinel sites from the current of the recording sites. The resulting signal (pA) was divided by the slope (μM = pA) obtained during calibration.[16,19] The glutamate uptake mechanism, defined as T80, was measured as the time of the glutamate response to decay by 80% from the peak amplitude (Figures 3a and b). For the acute ketamine treatment experiment, the first series of glutamate peaks constituted the control values within each subject. The evoked glutamate (Figure 4a) and the tonic levels (Figure 4b) are depicted as a percentage of the recorded control values before the i.p. administration.

Statistical analysis

Data were analyzed by two-way analysis of variance (treatment x region), followed by *post hoc* analysis (GraphPad Prism 5.0, San Diego, CA, USA). Where an overall treatment effect was observed, a Student's t-test was used for testing significance of drug treatment effects within the region. Data from ketamine and Ro25-6981 were from different cohorts; thus, separate statistical analysis were made for each cohort. All data are presented as means ± s.e.m. and the significance was defined as $P < 0.05$.
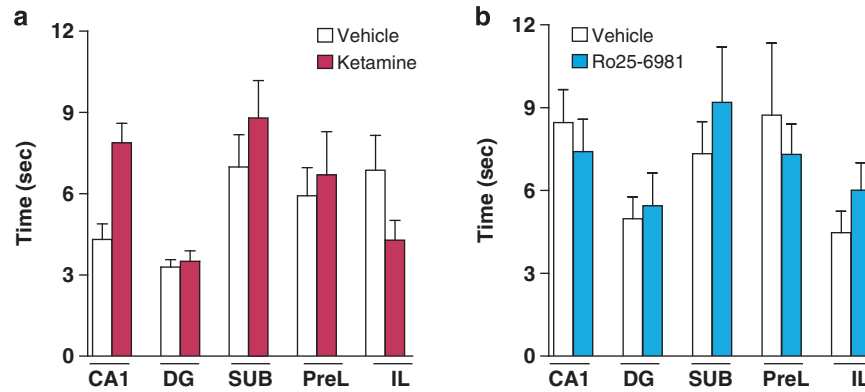
Figure 3. The effects of local application of ketamine and Ro25-6981 on glutamate clearance (T80). The elapsed time from the peak of the evoked glutamate until 80% of the response decayed (T80) was not significantly altered after local application of either ketamine (**a**) or Ro25-6981 (**b**) in any of the regions analyzed as compared with vehicle. Data represent mean ± s.e.m. from five glutamate peaks from 8–14 animals. Vehicle (70 mM KCl); Ketamine (100 μM ketamine in 70 mM KCl); Ro-256981 (100 μM Ro25-6981 in 70 mM KCl). CA1, cornu ammonis 1; DG, dentate gyrus; SUB, subiculum; PreL, prelimbic region of the prefrontal cortex; IL, infralimbic region of the prefrontal cortex.
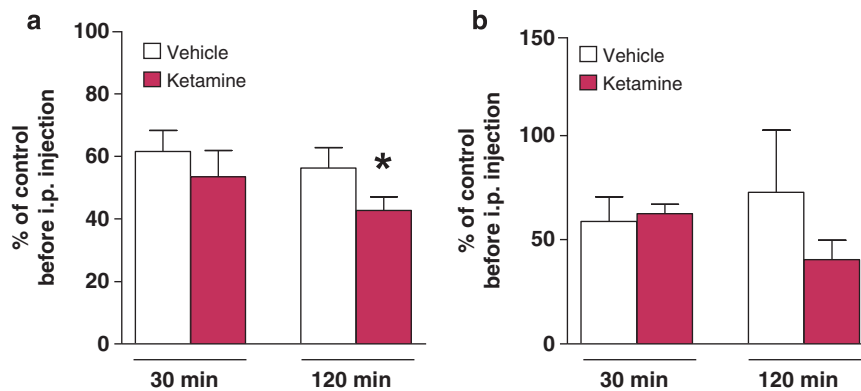

Figure 4. Evoked and tonic glutamate release in the subiculum after sub-anesthetic injection of ketamine. The depolarizing solution (70 mM KCl) was pressure ejected for 1-s duration with 1-min interval between each application. The evoked glutamate (**a**) and the tonic levels (**b**) are depicted as a percentage of control before the intraperitoneal (i.p.) injection of either S-ketamine (15 mg kg^{-1}) or saline. Note that the sub-anesthetic dose of S-ketamine caused a significant decrease in presynaptic glutamate release in the subiculum 120 min after the administration but not after 30 min (**a**). Tonic extracellular glutamate levels did not significantly change at any time post injection. Data represent mean ± s.e.m. from five glutamate peaks from six to nine animals. *$P < 0.05$, unpaired Student's t-test versus vehicle within region.

RESULTS

Ketamine reduces the evoked glutamate release in the subiculum and in the prelimbic area of the prefrontal cortex

Studies of ketamine's effect on evoked glutamate release in hippocampal and prefrontal cortical areas (Figure 2c) showed a significant overall treatment effect ($F_{(1,111)} = 6.420$; $P < 0.05$) and a significant treatment x region interaction ($F_{(4,111)} = 2.504$; $P < 0.05$). Pairwise analysis revealed that local ketamine application significantly reduced evoked glutamate release in the subiculum ($P < 0.05$) and in the prelimbic cortical area ($P < 0.05$). Ketamine did not significantly change tonic glutamate levels (Figure 2d) or glutamate clearance (Figure 3a) in any region.

Ro25-6981 reduces the evoked glutamate release in the subiculum and in the DG

To increase our understanding of the potential mechanisms by which NMDAR antagonists with antidepressant properties regulate glutamate release, we tested the effects of Ro25-6981, a N2B subunit-selective antagonist, in a separate sets of animals. In response to Ro25-6981, there were significant overall treatment ($F_{(1,81)} = 7.851$; $P < 0.01$) and region ($F_{(4,81)} = 4.498$; $P < 0.05$) effects on evoked glutamate release (Figures 2e and f). Pairwise analysis revealed that Ro25-6981 significantly reduced evoked glutamate release in the subiculum ($P < 0.01$) and DG ($P < 0.01$). Tonic

glutamate levels (Figure 2d) and evoked glutamate clearance (Figure 3b) were not significantly altered after local application of Ro25-6981 in the studied regions.

Acute, systemic ketamine treatment reduces the evoked glutamate release in the subiculum

The significant reduction in evoked glutamate release by local application of either ketamine or Ro25-6981 in the subiculum suggests that the effect of ketamine is N2B subunit-dependent in this region. Indeed, hippocampal axonal projections to the subiculum possess NMDARs containing the N2B subunit.[26] We extended the studies in the subiculum by examining whether alterations in evoked glutamate could be observed also upon systemic ketamine administration, using a dose previously shown to have antidepressive effects in vivo.[12] We therefore investigated the effects of an acute i.p. injection of S-ketamine (15 mg kg^{-1}), or saline (vehicle), upon tonic and evoked glutamate release in the subiculum over a period of 2 h (Figure 4a and b). As in the experiments with local drug administration, glutamate release was evoked by depolarization with 70 mM KCl isotonic solution. The first series of five consecutive peaks constituted the reference within each subject. The i.p. injection with saline or ketamine was performed immediately after the first series. The second and third series of FAST measurements were recorded at 30 and 120 min

post injection. The results show that, compared with vehicle, there was a significant decrease in evoked glutamate release in the subiculum 120 min after acute systemic administration of ketamine ($P < 0.05$, Figure 4a). Tonic glutamate levels did not significantly change at 30 or 120 min after ketamine administration (Figure 4b).

DISCUSSION

We have found that localized ketamine application significantly reduces evoked glutamate release in the subiculum and in the prelimbic area of the prefrontal cortex. Moreover, the N2B subunit-specific antagonist Ro25-6981 reduces evoked glutamate release in the subiculum and in the DG. Likewise, acute systemic ketamine administration, at a sub-anesthetic and antidepressant dose, reduces the evoked release of glutamate in the subiculum at 120 min post injection. Neither local nor systemic application of NMDAR antagonists altered tonic glutamate levels or glutamate clearance within the studied regions.

Isoflurane anesthesia and neurotransmitter release

The animals used in this study were under isoflurane anesthesia, at a dose range that was previously shown to not affect tonic glutamate levels in vivo when compared with awake levels, using the same recording methods.[27] Furthermore, isoflurane anesthesia has been shown to not affect glutamate uptake[28] or glutamate content in the hippocampus.[29] However, Westphalen et al.[30] have reported that isoflurane decreases Na^+ channel, but not K^+ channel-dependent glutamate exocytosis from synaptosomes in vitro, indicating a possible sensitivity of synaptic vesicle fusion mechanisms to isoflurane. The discrepancy between these observations could potentially arise from the differences between the use of isoflurane solution in vitro[30] versus prolonged in vivo inhalation of isoflurane,[27] as well as the observation methods. It is noteworthy that isoflurane did not affect tonic glutamate levels in vivo[27] neither did it affect in vitro KCl-induced glutamate release from synaptosomes.[30] Therefore, based on these reports,[16–19,27–29] we consider that the isoflurane levels used to maintain anesthesia in the present study were sufficiently light to avoid confounding effects. Nevertheless, it would be relevant in future studies to fully characterize the in vivo effects of ketamine in awake animals.

Differences in reported glutamate levels after ketamine administration

Previous studies that have examined ketamine-induced changes in glutamate release have used microdialysis[31] or in vitro electrophysiology.[10] Microdialysis studies, where a systemic sub-anesthetic dose of ketamine was used, have reported an increased level of extracellular tonic glutamate due to a possible local disinhibition of GABAergic interneurons in the PFC or regions that project to the PFC.[31] However, systemic ketamine administration, at an anesthetic dose, reduced tonic glutamate release.[31] In vitro electrophysiology experiments performed in hippocampal neurons have shown that ketamine inhibits the NMDAR-mediated spontaneous miniature excitatory postsynaptic currents (NMDAR-mEPSCs).[10] Furthermore, according to a proposed model by Kavali and Monteggia[32] a low dose of ketamine would suppress tonic and spontaneous glutamatergic transmission by blocking NMDAR, therefore inhibiting the eEF2 kinase, upregulating BDNF levels and triggering behavioral antidepressant effects. Interestingly, Kavali and Monteggia[32] also suggest that screening for compounds that block the tonic and spontaneous glutamate release itself (acting at the presynaptic level) could prove to be valuable in developing fast-acting antidepressants. Our data are in agreement with Kavali and Monteggia,[32] in that ketamine reduces glutamate release, although in our experiments the evoked component was more affected than the tonic component. Larger sets of animals may be required to find significant changes in tonic glutamate levels using the FAST methodology.

FAST and microdialysis measure different properties of extracellular glutamate

On the basis of previous reports, it is thus surprising to find that ketamine, both when given locally and systemically, as well as locally applied Ro25-6981, reduced evoked glutamate release in some brain areas. The discrepancy between our data and Moghaddam et al.[31] might be because of lower sampling rate of microdialysis on the reported glutamate release as compared with the FAST method.[16,19] It has been extensively described[16,19] that there are discrepancies in the reported resting glutamate levels between the FAST method and microdialysis. The FAST method has a higher temporal (seconds versus minutes) and spatial (μm versus mm) resolution as compared with microdialysis. The FAST method can selectively measure low levels ($< 1\,\mu M$) of tonic and depolarization-induced release of glutamate close to the synapse.[16–19] Furthermore, the source of measured glutamate differs between the FAST and microdialysis methods. Previous studies using the FAST methodology indicated that tonic glutamate source is, at least, 40–50% neuronal and that physiologically evoked glutamate release has a neuronal origin.[16] In contrast, the glutamate overflow sampled (minutes) with microdialysis is not tetrodotoxin-dependent, indicating that glutamate content, as measured by microdialysis, is mainly derived from glia.[16] It can thus be concluded that FAST and microdialysis measure different properties of extracellular glutamate and that it is difficult to translate results between the methods. Although the method used in the current study mainly detects KCl-induced neuronal glutamate exocytosis,[16] we cannot exclude the possibility that a proportion of the detected glutamate originates from Ca^{2+}-dependent astrocytic release.

Presynaptic machinery, target for antidepressants

Studies using isolated hippocampal synaptosomes, where measurements of evoked glutamate release were taken, have indicated that chronic, but not acute, treatment with three classic antidepressants (selective serotonin reuptake inhibitor, tricyclic antidepressant and norepinephrine reuptake inhibitor) reduce the depolarization-evoked glutamate release.[33] It has also been shown that chronic treatment with monoamine-based antidepressants counteract increases in depolarization-evoked glutamate release from frontal cortical synaptosomes from rats exposed to acute footshock.[34] As monoamine-based antidepressants require chronic administration to achieve antidepressive effects, these observations[33] could indicate a link between antidepressive effect and reduced presynaptic glutamate release, which would be consistent with our finding that ketamine (at an antidepressive dose) reduces evoked glutamate release. Interestingly, a recent study[12] revealed that acute ketamine treatment regulates the presynaptic release machinery in the hippocampus by a similar mechanism as chronic antidepressant treatment.[33,34]

Postsynaptic effects of ketamine

Animal studies with ketamine and Ro25-6981 have demonstrated that their fast-acting antidepressant actions involve activation of the mammalian target of rapamycin signaling, as well as other pathways and proteins: mitogen-activated protein kinase, protein kinase B, activity-regulated cytoskeletal-associated protein, post-synaptic proteins (PSD-95) and GluR1.[15] Altogether, these studies suggest that the fast-acting antidepressive effects of ketamine and Ro25-6981 also rely on actions at the postsynaptic site.

The subiculum region, major output of the hippocampus
Interestingly, the effects of local application of either ketamine or Ro25-6981 were consistent in the subiculum. Furthermore, the acute and systemic injection of ketamine, at a dose previously shown[12] to have antidepressive effects, reduced the depolarization-induced release of glutamate in the subiculum 120-min post injection. The subiculum is considered to be the main output of the hippocampus, sending glutamatergic projections to the amygdala, nucleus accumbens, hypothalamus and the prefrontal cortex.[35] The subiculum receives direct axonal projections from the pyramidal neurons of the CA1 area.[36] This input is known to be involved in both short- and long-term plasticity[26,36] and acute exposure to stress disrupts this plasticity.[37] Subicular pyramidal cells in rodents, similar to humans, are classified as bursting-spiking and regular-spiking cells.[26] The bursting-spiking cells outnumber the regular-spiking cells and require the presynaptic Ca^{2+} influx but not an increase in postsynaptic Ca^{2+} for the induction of long-term potentiation, suggesting that long-term potentiation is induced via activation of preNMDARs.[26] The axonal compartments contain preNMDARs[38–40] and CA1- subicular axonal projections possess the preNMDAR including N1 and N2B subunits,[26] providing a rapid control over the action potential-driven, Ca^{2+}-dependent, glutamate release.[38,41,42] Immunohistochemical experiments in rodents showed that CA1 and subicular neurons, but not astrocytes, express N2B subunits.[43] This is relevant, as it disagrees with Mittenauer's proposal[44] that blockade of astrocytic NMDAR by ketamine causes a rapid antidepressant effect.

Thus, based on previous reports,[12,26,38,40,43,45] and our current results, we suggest that *in vivo* local administration of ketamine or Ro25-6981 inhibits the evoked glutamate release, perhaps through a preNMDAR-dependent mechanism. As we observed that acute systemic administration of ketamine, at an antidepressive dose, decreased the evoked glutamate release in the subiculum within 2-h post administration, we suggest that reduced glutamate release in the subiculum may be involved in the fast-acting antidepressive effects of ketamine. Future studies are needed to investigate the effects of ketamine on glutamate release in awake mice while monitoring antidepressant responses.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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