Published in final edited form as:

Biol Psychiatry. 2012 June 1; 71(11): 1022–1025. doi:10.1016/j.biopsych.2011.11.006.

¹H -[¹³C]-NMR Spectroscopy Measures of Ketamine's Effect on Amino Acid Neurotransmitter Metabolism

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Abstract

Background—Ketamine has recently gained significant attention owing to its psychotomimetic and more recently discovered rapid antidepressant-like properties. ¹H-[¹³C]-NMR studies were employed to explore potential physiological processes underlying these unique effects.

Methods—[1-¹³C]glucose and [2-¹³C]acetate-NMR *ex vivo* studies were performed on the mPFC and hippocampus of rats acutely treated with 30mg/kg or 80mg/kg ketamine and compared to saline treated animals to determine the effects of ketamine on amino acid neurotransmitter cycling and glial metabolism.

Results—A sub-anesthetic, but not anesthetic, dose of ketamine significantly increased the percentage ¹³C-enrichments of Glutamate, GABA, and Glutamine in the mPFC of rats.

Conclusion—Sub-anesthetic doses of ketamine increase mPFC amino acid neurotransmitter cycling as well as neuronal and glial energy metabolism. These data add to previous reports suggesting increased mPFC levels of glutamate release, following the administration of sub-anesthetic doses of ketamine, are related to the drug's acute effects on cognition, perception and mood.

Keywords

NMDA; glutamate/glutamine cycle; ketamine; magnetic resonance spectroscopy; prefrontal cortex; GABA

INTRODUCTION

Ketamine has increasingly become a major interest to neuropsychiatric researchers owing to its psychotomimetic effects and rapid onset of antidepressant activity (see (1, 2) for recent reviews). Although ketamine has been shown to have effects on several neurotransmitter receptors, the majority of ketamine's pharmacological effects appear to be mediated through the N-methyl-D- aspartate receptor (NMDAR) (3, 4), where it acts as an open channel, non-

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Supplementary material cited in this article is available online.

All other authors report no biomedical financial interests or potential conflicts of interest.

competitive antagonist, binding to the phencyclidine (PCP) site within the ion channel and blocking ion influx (5, 6). However, microdialysis studies suggest ketamine and other NMDAR antagonists are also capable of transiently raising extracellular glutamate levels, suggesting increased release of forebrain glutamate (7, 8).

A transient increase in glutamate release induced by the NMDAR antagonists has been postulated to mediate some of the behavioral effects of the drugs. In support of this claim, agonists of mGluR2 receptors, shown to attenuate presynaptic glutamate release (9), block many of the physiological and behavioral effects of NMDAR antagonists (10-15). Moreover, three recent studies demonstrated the necessity of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) activation following NMDAR antagonist treatments, to generate the antidepressant-like effects in rodent models (16-18).

Ex vivo ^1H - ^{13}C -NMR studies are employed here to examine the effects of ketamine on glutamate release and recycling into glia (glutamate cycling) and neuronal and glial energy metabolism in the medial prefrontal cortex (mPFC) and hippocampus of the rat. ^{13}C -labeled *glucose* is metabolized mainly in the neuronal TCA cycle and labels neuronal glutamate and GABA, which are released and taken up by astrocytes, followed by conversion (and labeling) of glutamine. ^{13}C -labeled *acetate* is metabolized by astrocytes labeling glutamine, which is released and taken up by neurons for synthesis of glutamate and GABA. Together ^{13}C -labeled glucose and acetate studies provide information on glutamate and GABA neurotransmitter cycling as well as neuronal and glial cell metabolism, reflecting neurotransmitter activity (19-21). As increased rates of metabolism in the mPFC and hippocampus, regions believed to mediate several of ketamine's behavioral effects (22-25), have been demonstrated in rodents following sub-anesthetic doses of ketamine (26-29), both regions were examined in this study.

We specifically chose to examine a subanesthetic dose of 30mg/kg because similar doses have been shown to have large effects on glutamate efflux (7) and neuronal metabolism. An anesthetic dose of 80 mg/kg was chosen since it was previously shown not to have significant antidepressant-like effects on behavior (17).

MATERIALS AND METHODS

Animal Preparation

All experiments were conducted under protocols approved by the Yale IACUC. Male Sprague-Dawley rats (~180-200g) were prepared with tail vein catheters under isoflurane anesthesia. Animals (5-6 rats/group) were allowed to recover from anesthesia for at least 30 min prior to receiving intraperitoneal injections of ketamine-HCl (30mg/kg, or 80 mg/kg in 0.9% saline, i.p.) or saline. Ten minutes after injection of ketamine or saline, a solution of $[1-^{13}\text{C}]$ glucose (99 atom%; Cambridge Isotopes, Andover, MA, USA) dissolved in water (0.75 M per 200 g body wt.) was infused for 8 min (20) or sodium $[2-^{13}\text{C}]$ acetate (99 atom %; Cambridge Isotopes) dissolved in water (2 M, pH 7) was infused for 15 minutes through the catheter. *Ex vivo* ^1H - ^{13}C -NMR analysis of cortical and hippocampal extract and blood sample were performed as previously described (21, 30). See supplementary information for more details on methods.

Statistical Analysis

Metabolite levels were compared among groups (control, ketamine 30mg/kg, ketamine 80mg/kg) using analysis of variance (ANOVA). Significant group effects were followed by Dunnett's multiple comparisons procedure where each treatment is compared to control. * ($p < 0.05$) significance ANOVA, # ($_{\text{adj}}P = 0.05$) Dunnett test.

RESULTS

Effects of Ketamine on Behavior

Ketamine (30mg/kg) produced stereotyped progressive behavioral responses including back and forth head movements and ataxia, followed by a period of hyperactivity. Within two minutes of injection, ketamine (80mg/kg) resulted in immobility, but not loss of tail pinch reflex for the majority of animals tested.

Concentration and ^{13}C Enrichment of Plasma Glucose and Acetate

No significant differences in plasma glucose concentrations or percentage of ^{13}C enrichments were seen between the saline and ketamine treated animals at the end of the 8 min glucose infusions (saline: (mean $\pm$ SD) 13.08 $\pm$ 0.98 mmol/L and 47.7 $\pm$ 1.5%; versus ketamine 30mg/kg: 14.22 $\pm$ 0.53 mmol/L and 46.80 $\pm$ 1.70%; $P>0.4$ and ketamine 80mg/kg: 12.61 $\pm$ 1.67 mmol/L and 48.6 $\pm$ 2.5%; $P>0.5$).

The average concentrations of acetate were similar in plasma samples from all groups (saline: 4.27 $\pm$ 0.91 mmol/L versus ketamine 30mg/kg: 4.11 $\pm$ 0.71 mmol/L and ketamine 80mg/kg: 4.30 $\pm$ 1.10 mmol/L). Similar results were found for the percentage ^{13}C enrichments: (saline: 78.1 $\pm$ 2.9 % versus ketamine 30mg/kg: 79.2 $\pm$ 2.6 % versus ketamine 80mg/kg: 75.4 $\pm$ 4.7 %).

Effects of 30 and 80mg Ketamine on Amino Acid ^{13}C Labeling from [1- ^{13}C]Glucose and [2- ^{13}C]Acetate in mPFC and Hippocampus

Medial Prefrontal Cortex (mPFC)

Glucose: There was a significant effect of ketamine dose on the percent ^{13}C enrichment following an 8 min [1- ^{13}C]glucose infusion for all three metabolites in the mPFC region ((Glu-C4 ($p<0.02$), GABA-C2 ($p<0.02$), and Gln-C4 ($p<0.02$)) Figure 1. Post hoc Dunnett test comparing both treatments groups to the saline control revealed that the ^{13}C enrichment for the 30mg/kg group was significantly higher than the saline treated group (Glu-C4 ($\text{adj}p<0.03$), GABA-C2 ($\text{adj}p<0.04$), and Gln-C4 ($\text{adj}p<0.01$)), but there was no significant difference between the saline and 80mg/kg groups for any of the metabolites (all $\text{adj}p \geq 0.2$).

A significant effect of treatment group was also seen following an 8 min [1- ^{13}C]glucose infusion in the mPFC for ^{13}C concentrations at Glu-C4 ($p<0.01$), GABA-C2 ($p<0.02$), and Gln-C4 ($p<0.01$). Post hoc test again revealed that the 30mg/kg groups were significantly higher than the saline treated animals (Glu-C4 ($\text{adj}p<0.01$), GABA-C4 ($\text{adj}p<0.05$) and Gln-C4 ($\text{adj}p<0.01$)) while there was no statistically significant difference between saline and 80mg/kg (data not shown).

Acetate: Similarly, following a 15 min [2- ^{13}C]acetate infusion, a significant effect of group was found on percent mPFC ^{13}C enrichment of Glu-C4 ($p=0.05$) and Gln-C4 ($p<0.01$), and there was a strong, but non-significant trend for GABA-C2 ($p=0.07$) in the mPFC. Post hoc test revealed that the ^{13}C enrichment for the 30mg/kg group was significantly higher than the saline treated group (Glu-C4 ($\text{adj}p<0.05$), GABA-C2 ($\text{adj}p<0.05$), and Gln-C4 ($\text{adj}p<0.01$)), but there was no significant difference between the saline and 80mg/kg groups for any of the metabolites ($\text{adj}p \geq 0.5$) Figure 1.

A significant effect of treatment group was also seen following a 15 min [2- ^{13}C]acetate infusion in the mPFC for ^{13}C concentrations at Glu-C4 ($p<0.04$) and Gln-C4 ($p<0.02$). Post hoc test revealed that the 30mg/kg ketamine groups were significantly higher than the saline treated animals for both (Glu-C4 ($\text{adj}p<0.04$) and Gln-C4 ($\text{adj}p<0.02$)). There was a non-

significant trend for increased ^{13}C GABA-C2 concentrations ($\text{adj}p < 0.09$) in the 30mg/kg group, while there was no difference between saline and 80mg/kg (data not shown).

Hippocampus—Although there was a consistent numerical tendency for ^{13}C enrichment and ^{13}C concentrations of Glu-C4, GABA-C2, or Gln-C4 to be increased in the 30mg/kg ketamine treated group under both $[1-^{13}\text{C}]$ glucose and $[2-^{13}\text{C}]$ acetate infusions, there were no significant effects of ketamine dose on the percent ^{13}C enrichment or ^{13}C concentrations for any of the three metabolites in the hippocampus (see Figure S2).

Effects of 30 and 80mg ketamine on Levels of Amino Acids and Metabolites

Interestingly, there was a significant effect of ketamine treatment on total concentrations ($\mu\text{mol/g}$) of Glu under the $[1-^{13}\text{C}]$ glucose infusion conditions in both the mPFC ($p = 0.01$) and hippocampus ($p = 0.01$). In both regions the post hoc test revealed the effect was mainly accounted for by a lower Glu content in the 80mg/kg group compared to the saline control animals (PFC ($\text{adj}p < 0.03$); hippocampus ($\text{adj}p < 0.01$)). There were no significant treatment effects evident in any of the other metabolite concentrations under either infusion condition. Based on earlier reports suggesting a potential change in the relative Gln/Glu ratio we also examined the effects of ketamine treatment on this measure. However, we only saw a significant effect of ketamine treatment in the mPFC following the $[2-^{13}\text{C}]$ acetate infusion ($p = 0.05$). The post hoc test demonstrated that only the 80mg/kg group was statistically increased compared to the saline control animals ($\text{adj}p < 0.04$).

Discussion

The findings demonstrate that rodent $^1\text{H}-[^{13}\text{C}]$ -NMR *ex vivo* studies can provide a means of assessing drug effects on amino acid neurotransmitter cycling. Ketamine, at a sub-anesthetic dose, acutely increased mPFC glutamate, glutamine and GABA labeling from both $[1-^{13}\text{C}]$ glucose and $[2-^{13}\text{C}]$ acetate, suggesting Glu/Gln and GABA/Gln cycling, as well as oxidative metabolism are acutely induced by the drug. This is consistent with earlier studies showing the drug to transiently increase glutamate efflux and neurotransmission in the PFC (7).

The absence of an effect on mPFC cycling and metabolism by the higher (80mg/kg) anesthetic dose is consistent with earlier findings showing similar anesthetic doses of ketamine not to have significant effects on glutamate efflux (7) and protein phosphorylation (17). It is possible that the limited NMDA receptor antagonism resulting from the lower dose of ketamine causes a net increase in the neural and metabolic activity of cells in the PFC region, while the higher dose results in a level of NMDA antagonism that raises the threshold to neuronal activity. This suggested inverted U-type relationship between ketamine's effects on energetics and amino acid neurotransmitter cycling is further supported by observations showing even higher levels of ketamine (100mg/kg) to suppress glutamate efflux (7) and brain metabolism (27) below control levels. Homayoun *et al.* had originally proposed that this dose response curve could be related to the selective effects of subanesthetic doses of ketamine on inhibitory GABAergic interneurons causing cortical excitation by disinhibition of pyramidal neurons (8). Our current data showing an increase in GABA cycling may at first appear inconsistent with this hypothesis. However, it should be noted that the ^{13}C enrichment data provides the averaged values of all the GABAergic cells in the region, thus it is quite possible that a subset of GABAergic neurons, such as those directly modulating pyramidal cell firing, could be selectively made quiescent while others provide the signal of increased metabolic activity. In general, cortical GABA synthesis and Gln/GABA cycling does increase with increased activity of glutamatergic principal cells (31), the current data suggests that low dose ketamine retains this relationship, leaving the

nature of the postulated reduction in GABA inhibition (i.e., whether cytoplasmic (tonic) or synaptic (phasic) release of GABA is reduced) to be elucidated.

Of potential importance to future antidepressant drug development efforts, Li et al. (17) found the antidepressant-like and plasticity enhancing effects of ketamine in mPFC to be limited to sub-anesthetic doses. The current findings demonstrating sub-anesthetic, but not anesthetic, doses of ketamine to increase rates of glutamate cycling and neuronal metabolism in the mPFC suggest an initial increase in glutamatergic neurotransmission may be a critical factor in the drug's mechanism of antidepressant action. This hypothesis is further supported by other recent studies demonstrating the necessity of AMPA activation in generating the antidepressant-like response in rodent models (16-18).

The failure to find a significant effect of ketamine at either dose on hippocampal cycling and metabolism could suggest the mPFC is selectively affected by the 30mg/kg dose. However, the fact that there was a non-significant trend for all three metabolites in the hippocampus raises the possibility that we did not have the appropriate statistical power to detect a real, but smaller magnitude effect in this region. Previous ^{14}C -2DG autoradiography studies found a very narrow activation of subiculum lacunosum moleculare (27), which comprises a relatively small volume of the hippocampal formation. Our hippocampal dissection included the entire hippocampal formation, thus it is possible that partial volume averaging obscured sub-regional effects within the hippocampus.

Lastly, a previous study using proton NMR reported a significant rapid increase in anterior cingulate glutamine content in healthy subjects receiving a subanesthetic dose of ketamine (32). We failed to see an increase in the total concentrations of any of the metabolites following treatment with ketamine at 30mg/kg. Of potential interest, we did find a significant effect of ketamine treatment on total Glu concentrations under the $[1-^{13}\text{C}]$ glucose infusion conditions in both the mPFC and hippocampus, but this effect appeared to be mainly explained by the decreased content of Glu in the 80mg/kg group compared to the saline control animals. These findings appear to be more in line with a recent report from Taylor et al. (33) who also failed to see an increase in Glu or Glx (a measure comprised of both Glu + Gln concentrations). Another recent study by Iltis et al. (34) reported an increased Gln/Glu ratio in the PFC of rats shortly following injections of the NMDAR antagonist phencyclidine. We only found evidence of an increased Gln/Glu ratio related to ketamine treatment in the mPFC of $[2-^{13}\text{C}]$ acetate infused rats, where the largest effect appeared to be in the 80mg/kg group. In sum, it is difficult to draw any firm conclusions about ketamine's effects on the total concentration of amino acid neurotransmitter metabolites from this study. Several factors including; species, the route and dose of administration, and timing of NMR spectroscopy measures limit the ability to compare the results across studies. However, this study does provide clear evidence that ketamine, especially at subanesthetic doses, has large effects on Glu, Gln and GABA metabolism that likely represents changes in amino acid neurotransmitter cycling.

In summary, this study demonstrates the ability to use ^1H - ^{13}C -NMR methods *ex vivo* with awake rodents to examine the effects of pharmacological treatments on amino acid neurotransmitter cycling. This approach could be extremely useful in understanding the dose response relationship for NMDAR antagonists as well as in identifying other drugs sharing similar physiological effects. Although the results provide strong evidence that ketamine acutely increases glutamate neurotransmitter cycling, future studies are needed to evaluate whether this effect underlies the perceptual, cognitive and behavioral effects associated with NMDAR antagonists.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institute of Mental Health R01 MH081211 (GS) and 1R01 MH095104 (KLB, GS) and funding from Merck & Co., Inc.

We thank Mr. Brian Pittman for statistical analysis and Xiaoxian Ma for preparation of the animals used in this study.

Dr. Sanacora has received consulting fees from Abbott, AstraZeneca, Bristol-Myers Squibb, Evotec, Eli Lilly & Co., Hoffman La-Roche, Johnson & Johnson, Novartis, and Noven Pharmaceuticals. He has also received additional grant support from AstraZeneca, Bristol-Myers Squibb, Hoffman La-Roche, Merck & Co., and Sunovion Pharmaceuticals. In addition, Dr. Sanacora is a co-inventor on filed patent application by Yale University concerning the use of glutamate modulating drugs in the treatment of psychiatric disorders (PCTWO06108055A1). Dr. Cho was an employee of Merck & Co., Inc., at the time the study was being conducted; he is now employed by Genentech, a member of the Roche Group.

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Abbreviations used

AMPAR	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
Glu	glutamate
Gln	glutamine
GABA	γ -aminobutyric acid
mPFC	medial prefrontal cortex
NMR	Nuclear Magnetic Resonance
NMDAR	N-methyl-D- aspartate receptor
PCP	phencyclidine
TCA	tricarboxylic acid

Figure 1.

Acute effects of ketamine on ¹³C enrichment of amino acids and metabolites in medial prefrontal cortex (mPFC) following [1-¹³C]glucose (8 min) and [2-¹³C]acetate (15 min) infusions. (A) Percent ¹³C enrichment of glutamate (Glu)-C4, GABA-C2, and glutamine (Gln)-C4 following administration of saline or ketamine and [1-¹³C]glucose (mean±SD depicted). (B) Percent ¹³C enrichment of Glu-C4, GABA-C2, and Gln-C4 following administration of saline or ketamine and [2-¹³C]acetate. All ANOVA ($p < 0.05$) except Acetate GABA-C2 which was ($p = 0.07$), * ($_{adj}P = 0.05$) Dunnett test; ns, not significant.