Ketamine induces delirium-like behavior and interferes with endosomal tau trafficking

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Abstract

Background: Ketamine is an intravenous anesthetic. However, whether ketamine can induce neurotoxicity and neurobehavioral deficits remains largely unknown. Delirium is a syndrome of acute brain dysfunction associated with anesthesia and surgery in patients, and tau protein may contribute to postoperative delirium. Finally, ketamine may affect the function of the endosome, the key organelle for tau release from neurons. Therefore, we set out to determine the effects of ketamine on delirium-like behavior in mice and on tau trafficking in cultured cells.

Methods: We used the buried food test, open field test, and Y-maze test in adult mice to assess the presence of delirium-like behavior in mice. We quantified tau amounts in the serum of mice.

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We used cell fraction methods to determine the effects of ketamine on tau intracellular transfer, extracellular release, and endosome trafficking in cultured cells.

Results: Ketamine induced delirium-like behavior in mice and increased tau amounts in serum of mice. The ketamine treatments also led to increased accumulation of endosomes, as evidenced by increased endosomal markers Rab5 and Rab7. Moreover, ketamine inhibited endosome maturation, demonstrated by decreased membrane-bound but increased cytoplasm amounts of Rab5 and Rab7. Consequently, ketamine increased tau in the endosomes of cultured cells and the cell culture medium.

Conclusion: These data suggest that ketamine may interfere with intracellular tau trafficking and induce delirium-like behavior, promoting future research regarding the potential neurotoxicity of anesthetics.

Introduction

Ketamine is a phencyclidine analog and a non-competitive antagonist of the N-methyl-D-aspartic acid (NMDA) receptor[1]. It is a commonly used clinical anesthetic and may also treat depression[2,3]. Finally, ketamine is a recreational drug owing to its ability to induce visual hallucinations in subanesthetic doses[2,3]. Ketamine induces cognitive impairment[4] and may also be associated with delirium[2]. However, whether ketamine can induce delirium-like behavior in rodents and the underlying mechanism remain unknown.

Delirium is a syndrome of acute brain dysfunction potentially associated with anesthesia, surgery, pain, disease, or medication, manifesting with impaired consciousness, disorganized thinking, lack of purpose, and inability to focus[5]. Previous studies have demonstrated an association between tau and postoperative delirium in patients[6–8].

Tau is expressed mainly in neurons and has a role in stabilizing the cytoskeleton, maintaining anchoring of membrane material, and participating in axonal transport[9]. Tauopathy is a hallmark of Alzheimer's disease neuropathogenesis[10] and contributes to cognitive impairment. However, it is unknown whether ketamine can induce tauopathy, especially tau trafficking. A recent study showed that anesthetic sevoflurane could induce tau trafficking from neurons to microglia via accumulation in exosomal fraction[11]. However, the pathways and mechanisms responsible for tau trafficking inside cells before its transition to the extracellular space remain largely unknown. We, therefore, used ketamine as a clinically relevant tool to determine intracellular tau trafficking in the present study.

Endosomes are vesicle organelles that originate from the trans-Golgi network (TGN) and have extensive bidirectional transport relationships with the TGN, cell membrane, and lysosome, which are responsible for sorting, transport, and degradation of intracellular cargo[12,13]. Endosomes are classified into three main categories—early endosomes (EEs), late endosomes (LEs), and recycling endosomes—based on the order of endocytic cargo transport to various endosomal vesicles[14]. The three types of endosomes are defined mainly

based on their electron microscopic morphology, the cargo transported within the lumen, and surface markers[15]. Surface markers of EEs are EE antigen 1 (EEA1) and Rab5. EEs can endo-emerge to form intraluminal vesicles and gradually transform into multivesicular bodies (i.e., LEs)[16]. Maturation of EEs is demonstrated by increased binding of Rab5 on the endosome membrane[17,18]. LEs are mainly responsible for degradation, and their markers are a cluster of differentiation 63 (CD63) and Rab7. Similarly, LE maturation is demonstrated by increased binding of Rab7 on the endosome membrane[13].

In this study, we investigated the effects of ketamine on delirium-like behavior and changes in tau amounts in mouse serum. Mechanistically, we assessed the effects of ketamine on the number and maturation of EEs and LEs, intracellular transport, and extracellular release of tau in the human neuroblastoma cell line (known as SH-SY5Y). The hypothesis of the present study was that ketamine causes delirium-like behavior and increases endosomal uptake and extracellular release of tau.

Methods

Ethical Approval and Consent to participate

All experimental procedures involving mice were approved by the Standing Committee on Animals at Massachusetts General Hospital, Boston, MA (protocol number: 2006N000219) and conformed to National Institutes of Health (Bethesda, MD) guidelines. This article was written according to applicable ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines. Efforts were made to minimize the number of mice used in the studies.

Antibodies and reagents

Details of the antibodies used are listed in Table 1.

Primary antibodies were diluted with western blot blocking buffer (see western blot subsection for details), and secondary antibodies were diluted with 1×TBST.

Ketamine injection (Ketalar) was purchased from Hikma Pharmaceuticals PLC (London, United Kingdom).

Animals

A total of 48 8-week-old wild-type adult male C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME) with an average weight of 23 g. No significant difference in mouse weight was found between groups at the beginning of the study (Supplemental Figure S1 A1, A2). Mice were housed with four mice per cage and maintained on a 12-hour light/dark cycle (lights out at 18:00). Mice had unlimited access to water and food in their home cages. Sample size was decided by a previous pilot experiment and power analysis ($\alpha = 0.05$, $\beta = 0.1$, δ(p1-p2) = 0.51, pilot experiment p1 = 0.97). Mice were divided into 3 post-injection duration groups, 1 h group (24 mice), 2 h group (12 mice), and 6 h group (12 mice) (Fig. 1). Each post-injection duration group was divided equally into 2 treatment subgroups, a saline control group (CON) and a ketamine group (KET). Each grouping was done using the complete randomization method. Each mouse was injected with 2 μl/g via

intraperitoneal injection. The dosage of the KET group was 40 mg/kg according to the previous studies[19,20].

Behavioral tests

The animal behavioral tests battery in the present study assessed the natural behaviors with the buried food test (BFT) and open field test (OFT) to probe for attention and awareness, and the learned behaviors with the Y-maze test (YMT) to probe for cognition, which could determine the delirium-like behaviors in mice as performed in previous studies[21–23]. These tests focused on assessing delirium-like behaviors rather than only cognitive function in mice. All mice had multiple behavioral tests starting with the YMT training trail, followed by OFT, BFT, and finally the YMT retention trail at 24 h before (baseline) injection and 1 h or 2 h after injection (Fig. 1). Mice in the 6 h group only had the behavioral tests once at 2 h after the injection of ketamine. The equipment for behavioral tests was cleaned with 70% ethanol solution between trials. A video camera linked to AnyMaze (Stoelting Co., Wood Dale, IL) animal tracking system software was applied to monitor and analyze the activity of mice in YMT and OFT.

Y maze test.—The YMT was performed as described previously[20,24,25]. Precisely, the maze consists of 3 arms, including the start arm, in which the mouse starts to explore (always open); the novel arm, which is blocked at the first trial but opened at the second trial; and another familiar arm (always open). The YMT consisted of two trials separated by an inter-trial interval. The first trial (training) was 10 min in duration, which allowed the mouse to explore start arm and familiar arm of the maze, with the novel arm blocked. After a 1 h inter-trial interval, the second trial (retention) was conducted. The mouse was placed back in the maze in the same start arm with free access to all three arms for 5 min. The time spent in and entries into the novel arms were recorded and analyzed.

Open field test.—The OFT was performed as described previously[20,26]. Specifically, a mouse was gently placed in the center of an open field chamber under dim light. The total distance moved (meters), time (seconds) spent in the center of the open field, freezing time (seconds), and latency (time in seconds for the mouse to reach the location of the first attempt) to the center of the open field were recorded and analyzed.

Buried food test.—The BFT was performed as described in previous studies[27,28]. Specifically, two days before the test, we gave each mouse two pieces of sunflower seed. The test cage was prepared with clean bedding (3 cm high). We buried one sunflower seed 0.5 cm below the surface of the bedding. The location of the food pellet was changed every time randomly. We placed the mouse in the test cage and measured the latency of the mouse to eat the food. Latency was defined as the time from when the mouse was placed in the test cage until the mouse uncovered the food pellet and grasped it in its forepaws and/or teeth. Mice were allowed to consume the pellet they found and returned to their home cage. The observation time was 5 minutes. If the mouse could not find the pellet within 5 minutes, the testing session ended, and the latency was defined as 300 seconds for that mouse.

Blood collection in mice

Mice were anesthetized with 3% isoflurane for 2 minutes when retro-orbital sinus blood sampling was performed, and then mice were euthanized by cutting the head. Blood was transferred to a 4℃ refrigerator for 15 min and centrifuged at 4℃ for 15 min at $1,200 \times g$. We then extracted the supernatant as serum.

Cell culture

SH-SY5Y cells were cultured at 37°C with 5% CO_2 in DMEM/F12 1:1 (Gibco) supplemented with 10% fetal bovine serum (Gibco), 1% L-glutamine-penicillin-streptomycin solution (Sigma-Aldrich), 1% MEM non-essential amino acids solution (100X) (Gibco), and 1% sodium pyruvate (100 mM) (Gibco). When incubating with drugs, we replaced DMEM/F12 1:1 with DMEM/F12 1:1 with HEPES without phenol red and added 10% ketamine solution or saline and 0.1% DMSO.

Cell viability assays

MTT was purchased from Invitrogen (Waltham, MA), and all MTT assays were performed following the instructions provided with the kit to obtain cell viability data. LDH assay kit (Abcam, Cambridge, MA) was used to quantitatively analyze LDH concentration in the cell culture medium. Standard curves were plotted for each assay using positive control LDH provided in the kit with gradient concentration. All operations were performed according to the kit instructions.

Whole-cell and cell fraction protein extraction

All the buffers and lysates were pre-dissolved Pierce protease and phosphatase inhibitor mini-tablets (Thermo Scientific).

Whole-cell protein extraction.—We discarded cell medium, rinsed with DPBS (Lonza), discarded as much residue as possible to obtain treated cells. We added an appropriate amount of RIPA (Thermo Scientific) lysate. After incubation, we scraped off all cells and lysate and transferred them to a clean tube. The lysate was homogenized, and the tubes were shaken with a sonic dismembrator three times at 50% amplitude for 3 seconds with an interval of 3 seconds.

Cytoplasmic protein and membrane protein extraction.—We discarded cell medium, rinsed with DPBS, discarded as much residue as possible to obtain treated cells. We added an appropriate amount of M-PER (Thermo Scientific) lysate. We sealed the culture dish with plastic wrap and transferred it to a 4℃ refrigerated room for 30 min incubation on a shaker. After incubation, we scraped off all hanging cell debris and lysate and transferred them to a clean tube. We centrifuged tubes at $16,000 \times g$ for 30 min at 4℃ and carefully extracted the upper clarified solution into a clean tube. This solution contained cytoplasmic and nuclear proteins, and the pellet contained membrane protein. The membrane protein pellet was dissolved into a solution using RIPA.

Endosome isolation.—Minute endosome isolation and cell fractionation kit (Invent Biotechnologies, Plymouth, MN) was used to isolate endosomes as described

previously[29,30]. Briefly, 4×10^7 cells were collected, and 1 ml buffer A was added. The cell suspension was incubated on ice for 15 minutes, loaded into a filter cartridge, and centrifuged with $16,000 \times g$ for 30 seconds. A filter cartridge re-pass-through was performed to enrich the field. The supernatant was transferred to a fresh tube, and 500 μl buffer B was added to the tube and incubated at 4°C overnight. After incubation, vortex briefly and centrifuge with $10,000 \times g$ for 30 minutes. We removed the supernatant and washed the pellet with a 750 μl 2:1 mixture of buffer A/buffer B. After centrifugation with $10,000 \times g$ for 30 minutes, the pellet contains endosomes. We did not weigh the amounts of endosome pellets directly because the amounts were too small to be weighed out. According to the protocol document, the yield is typically 20 ~ 100 μg/sample. All the centrifugations were performed at 4°C. Endosome lumen proteins and endosome membrane proteins were extracted by a similar method as that used for cytoplasmic protein and membrane protein extraction.

ELISA

Concentrations of human total tau in the cell culture medium or protein lysate and mouse total tau in serum were measured by ELISA kits (Invitrogen). Experiments were performed according to the manufacturer's instructions.

Protein concentration

Protein concentration of lysates was obtained using the Pierce BCA protein assay kit (Thermo Scientific). Standard curves were plotted for each assay using Pierce bovine serum albumin standard (Thermo Scientific) with gradient concentration.

Western blot

Proteins were separated by NuPAGE 4–12% Bis-Tris protein gel (Invitrogen) and transferred onto PVDF membranes (Bio-Rad). Membranes were blocked in 50% SuperBlock (TBS) blocking buffer (Invitrogen), 5% bovine serum albumin (Sigma-Aldrich) in 1× TBST (8 g/L NaCl, 2.42 g/L Tris-base, 1 mL/L Tween 20, adjusted pH to 7.6 with HCl). Immunoreactive bands were detected using a SuperSignal West Pico PLUS chemiluminescent substrate (Thermo Scientific). The signal intensity was analyzed using ChemiDoc XRS + with Image Lab 5.0 software (Bio-Rad, Hercules, CA).

Composite Z-score

When pooling the battery of behavioral test data, we transformed outcome data according to the composite Z-score introduced in previous studies[21,22], with some modifications. Specifically, we used absolute values of Z-scores in our calculations to avoid researcher bias in data transformation. We first calculated the difference between the measured value after injection and the baseline value ΔX for each mouse. The ΔX from the CON group was then used to calculate the mean $M_{\Delta X_{CON}}$ and SD $S_{\Delta X_{CON}}$. The composite Z-score of each mouse was obtained by the formula $C_{Z_X} = \sum |Z_X| = \sum \left| \dfrac{\Delta X - M_{\Delta X_{CON}}}{S_{\Delta X_{CON}}} \right|$. Finally, C_{Z_X} was included in the statistical analysis.

Statistical analysis

Statistical analyses were performed using Prism 8 (GraphPad Software) to generate curves or bar graphs. All error bars represent standard deviation (SD). Data were first tested for conformity to a normal distribution using the Shapiro-Wilk test and then tested for equality of variances using the F test. Two-tailed unpaired Student's t-test was used for statistical analysis of two groups of samples. One-way ANOVA with Turkey's honestly significant difference test was used to evaluate the statistical significance of multiple groups of samples. Normally distributed data were described as mean ± SD. Data that did not conform to the normal distribution were combined with the experimental records to exclude outliers using the Grubbs' test and then re-examined for normality and F tests. Data that did not conform to the normal distribution were tested using the non-parametric independent samples Mann-Whitney exact probability test. Data were then described using median (interquartile spacing). All tests were set at $\alpha = 0.05$ and $\beta = 0.1$.

Results

Ketamine induces delirium-like behavior and increases serum tau amount in mice.

We used our established animal model, consisting of the YMT, BFT, and OFT [21,22], to study the effects of ketamine on delirium-like behavior in mice (Supplemental Figure S1). A single injection of ketamine in mice significantly increased the latency to eat food in the BFT and freezing time in the OFT compared to control mice at 1, but not 2, h after ketamine administration (Supplemental Figure S2). Composite Z-scores were obtained by pooling data from six measurements. The composite Z-score increased in the ketamine-treated mice at 1, but not 2, h after the ketamine treatment compared to control mice [KET: 7.899 (10.03–6.927) versus CON: 4.657 (6.371–3.365), P = 0.0005, Mann-Whitney test; Fig. 2A, B]. These data suggest that ketamine may induce delirium-like behaviors in mice. Blood was collected from the mice 1, 2, and 6 h after administration of a single injection of ketamine, and serum was extracted. We found increased amounts of tau in the serum of mice at 1, but not 2 nor 6, h after ketamine administration compared to control mice (KET: 90.36 pg/mL ± 47.41 pg/mL versus CON: 53.81 pg/mL ± 11.64 pg/mL, P = 0.0232, Student's t-test; Fig. 2C–E).

Ketamine increases tau amounts in the endosome lumen and culture medium of SH-SY5Y cells.

Ketamine decreased viability of cultured SH-SY5Y cells in a dose-dependent manner (F = 155.7, P < 0.0001, one-way ANOVA; Fig 3A). Hence, we chose 156.3 μg/mL ketamine (KET1), 312.5 μg/mL ketamine (KET2), 625 μg/mL ketamine (KET3), and a control condition (CON) to treat SH-SY5Y cells. We specifically assessed the effects of ketamine on the amount of tau inside the endosome lumen and outside of the SH-SY5Y cells. Ketamine increased the amounts of tau in whole-cells (Fig. 3B, C). ELISA showed that ketamine also increased the amounts of tau in the endosomal lumen (Fig. 3D) and the cell culture medium (Fig. 3E). However, a lactate dehydrogenase (LDH) assay showed that treatment with ketamine did not increase LDH amounts in the cell culture medium (F = 1.015, P = 0.4068, one-way ANOVA; Fig. 3F), indicating that the ketamine-induced increase in tau in

the cell culture medium was not due to rupture of cell membranes. These data indicate that ketamine increases endosomal uptake and extracellular release of tau.

Ketamine increases early and late endosome accumulation in SH-SY5Y cells.

Given that ketamine increased tau concentration in both the endosome and extracellular space, next, we assessed the effects of ketamine on endosome amounts and activation in SH-SY5Y cells. Quantitative western blot analysis demonstrated that after 24 h of incubation, whole-cell protein amounts of EE markers EEA1 ($F = 36.48$, $P < 0.0001$, one-way ANOVA; Fig. 4A, B) and Rab5 ($F = 31.23$, $P < 0.0001$, one-way ANOVA; Fig. 4A, C) were significantly increased, suggesting that ketamine increased amounts of EEs in the cells. Moreover, ketamine increased whole-cell protein amounts of LE marker Rab7 ($F = 32.05$, $P < 0.0001$, one-way ANOVA; Fig. 4A, E) but not CD63 ($F = 2.358$, $P = 0.1102$, one-way ANOVA; Fig. 4A, D). In addition, ketamine decreased whole-cell protein amounts of lysosome marker lysosome-associated membrane protein 2 (LAMP2) ($F = 4.892$, $P = 0.0323$, one-way ANOVA; Fig. 4A, F). These data demonstrated that ketamine increased the accumulation of EEs and LEs and decreased lysosome amounts in SH-SY5Y cells.

Ketamine decreases endosome membrane-bound Rab5 and Rab7 but increases Rab5 and Rab7 in the cytoplasm of SH-SY5Y cells.

Given that ketamine increased the amounts of proteins associated with EEs in whole-cells, we further assessed the effects of ketamine on the amounts of these proteins bound with the endosome membrane or in the cytoplasm. First, we demonstrated that ketamine did not significantly change the amounts of sodium-potassium ATPase (Na-K ATPase) ($F = 1.483$, $P = 0.2569$, one-way ANOVA; Fig. 5A, B), indicating that Na-K ATPase could be used as an internal loading control for the amounts of the protein associated with endosome membranes. Quantitative western blot analysis demonstrated that ketamine significantly increased the amounts of cytoplasmic Rab5 ($F = 11.15$, $P = 0.0003$, KET3: 237.2% ± 84.99% versus CON: 100% ± 16.32%, one-way ANOVA; Fig. 5C, D) and Rab7 ($F = 9.200$, $P = 0.0009$, KET3: 277.6% ± 113.5% versus CON: 100% ± 26.04%, one-way ANOVA; Fig. 5C, E) 24 h after ketamine administration. Conversely, ketamine treatment significantly decreased the amounts of membrane-bound Rab5 ($F = 7.410$, $P = 0.0025$, KET1: 69.00% ± 26.86% and KET3: 55.86% ± 6.633% versus CON: 100% ± 7.792%, one-way ANOVA; Fig. 5C, F) and Rab7 ($F = 8.174$, $P = 0.0016$, KET2: 55.23% ± 16.50% and KET3: 28.56% ± 11.05% versus CON: 100% ± 41.50%, one-way ANOVA; Fig. 5C, G). These data demonstrated that ketamine could decrease endosome membrane-bound Rab5 and Rab7 but increased cytoplasmic amounts of Rab5 and Rab7, suggesting that ketamine may impair EE and LE maturation.

Discussion

Our results show that ketamine induced delirium-like behavior and increased serum tau amounts in mice. In the mechanistic studies, ketamine impaired intracellular tau trafficking, evidenced by increasing amounts of tau inside the endosome lumen and extracellular space of SH-SY5Y cells. Moreover, ketamine might impair intracellular tau trafficking by increasing the number of endosomes but inhibiting endosome maturation. These studies,

using ketamine as a clinically relevant tool, demonstrate that tau trafficking could contribute to the pathogenesis of delirium, promoting future research to study delirium pathogenesis. These data show that anesthetic ketamine could induce tauopathy and delirium-like behavior, pending confirmative studies.

We firstly found time-dependent effects of ketamine in inducing delirium-like behavior, evidenced by the findings that ketamine increased composite Z-score compared to controls. Consistently, previous studies show anesthesia/surgery induces time-dependent[21] and age-dependent[22] delirium-like behavior in mice. However, previous studies did not assess anesthetic effects without surgery on the delirium-like behavior in mice.

The present study showed that anesthetic ketamine without surgery could still induce delirium-like behavior in mice. Ketamine induced delirium-like behavior and increased serum tau amount 1 h after administration, suggesting an association between delirium-like behavior and increased serum tau. Consistently, a clinical observational study in 2021 concluded that postoperative plasma tau amounts could serve as a biomarker for postoperative delirium in patients[7].

Endosomes are responsible for the intracellular transport of cargo, including proteins, lipids, and nucleic acids, essential for cell function[31]. Cell function is severely impaired when endosomes accumulate[13]. We found that ketamine increased endosome accumulation, which could explain the findings that ketamine increased endosomal tau amounts because more endosomes could uptake more tau. However, ketamine may also enhance the ability of single endosomes to uptake tau. We will test this hypothesis in future studies.

Previous studies showed that increased Rab5 or Rab7 in the cytoplasm and decreased amounts of membrane-bound Rab5, or Rab7 indicate inhibition of endosome maturation[17,18]. Endosomes become exosomes when they bind to the cell membrane[32]. Moreover, it has been suggested that inhibition of the endosome maturation process will increase protein release via exosomes[33]. In the present study, ketamine inhibited endosome maturation, evidenced by increased cytoplasm amounts of Rab5 and Rab7 and decreased membrane-bound Rab5 and Rab7. Meanwhile, ketamine also increased amounts of tau in the extracellular space. These findings indicate that ketamine inhibits endosome maturation while increasing tau uptake by endosomes, causing migration of endosomes to the cell membrane to form exosomes and releasing more tau into the extracellular space.

The mechanism of tau release into the extracellular space is unclear. However, increasing evidence shows that tau relies mainly on the non-classical protein secretion pathway and extracellular vesicles for extracellular transport[34,35]. Our previous study also demonstrated that the trafficking of tau to the extracellular space depended on tau phosphorylation and the generation of extracellular vesicles[11]. In the present study, we illustrated that ketamine increased endosome accumulation but inhibited endosome maturation, leading to more tau release into the extracellular space. Moreover, we postulate that the effect of ketamine on EEs includes blocking the conversion of EEs to LEs but does not affect the conversion of other organelles, such as TGN, to EEs.

In LEs, we found that ketamine did not significantly change CD63 protein amounts but increased Rab7 amounts. Further, ketamine decreased membrane Rab7 yet increased cytoplasmic amounts of Rab7. These data suggest that ketamine inhibits LE maturation, as demonstrated in previous studies[36,37].

As an NMDA receptor antagonist, ketamine affects intracellular calcium homeostasis[1]. Calcium homeostasis is associated with lysosome function[38], and the impaired lysosome function and calcium dysregulations contribute to the pathogenesis of Alzheimer's disease and cognitive dysfunction[39,40]. Therefore, it is conceivable that ketamine may regulate endosome trafficking via impairing calcium homeostasis and lysosome function, leading to delirium-like behavior. Future studies will test this hypothesis.

There are several limitations of this study. First, we did not study whether inhibition of the ketamine-induced increase in serum tau could mitigate ketamine-induced delirium-like behavior. We could not find a specific inhibitor of tau trafficking from the intracellular to extracellular space. We will use tau knockout mice to determine the role of tau in delirium-like behavior using the established system in future studies. Second, we did not determine the effects of ketamine on the intracellular trafficking of phosphorylated tau in the present study because we wanted to focus on tau. We will use the established system to study the intracellular trafficking of both tau and phosphorylated tau in future studies. Finally, we only assessed the effects of ketamine on membrane Rab5 and Rab7 but not the specific endosome membrane Rab5 and Rab7. However, Rab5 and Rab7 are only known to bind to the endosome membrane.

In conclusion, the present study showed that ketamine induced delirium-like behavior and increased serum tau amounts in mice. In the *in vitro* studies, we found ketamine increased tau uptake in endosomes by increasing endosome accumulation. Ketamine then increased the release of tau to the extracellular space by inhibiting endosomal maturation. These findings will promote future studies of tau trafficking and delirium and anesthetic effects on tauopathy and delirium-like behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Glossary of Terms

BFT	buried food test
CD63	cluster of differentiation 63
CON	control group
EE	early endosome

EEA1	early endosome antigen 1
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
KET	ketamine group
LAMP2	lysosome-associated membrane protein 2
LDH	lactate dehydrogenase
LE	late endosome
Na-K ATPase	sodium potassium ATPase
NMDA	N-methyl-D-aspartic acid
OFT	open field test
TGN	trans-Golgi network
Tau	microtubule-associated protein τ
YMT	Y-maze test

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Key Points Summary

Question:

What are the effects and mechanism of ketamine on delirium-like behavior in mice and on tau trafficking in cultured cells?

Finding:

Ketamine induced delirium-like behavior, increased tau concentration in mouse serum, and induced accumulation of endosomes as evidenced by increased amounts of cytoplasmic Rab5 and Rab7.

Meaning:

Ketamine may induce delirium-like behavior by interfering with intracellular tau trafficking. These findings will promote future research regarding the neurotoxicity of anesthetics.
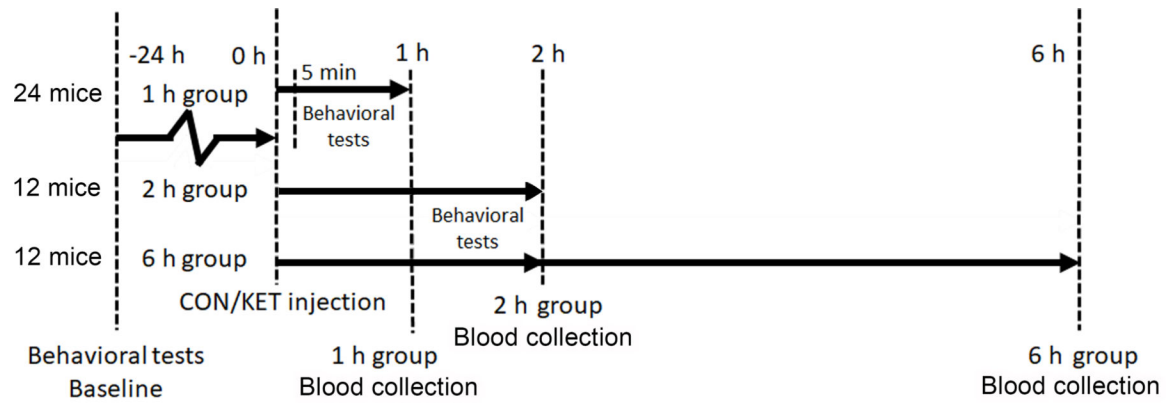
Figure 1. The diagram of experimental design.

Mice had behavioral tests 24 hours before and then 1 and 2 hours after the ketamine treatment or control condition. We collected the blood of mice at 1, 2, and 6 hours after the ketamine treatment or control condition in the mice.
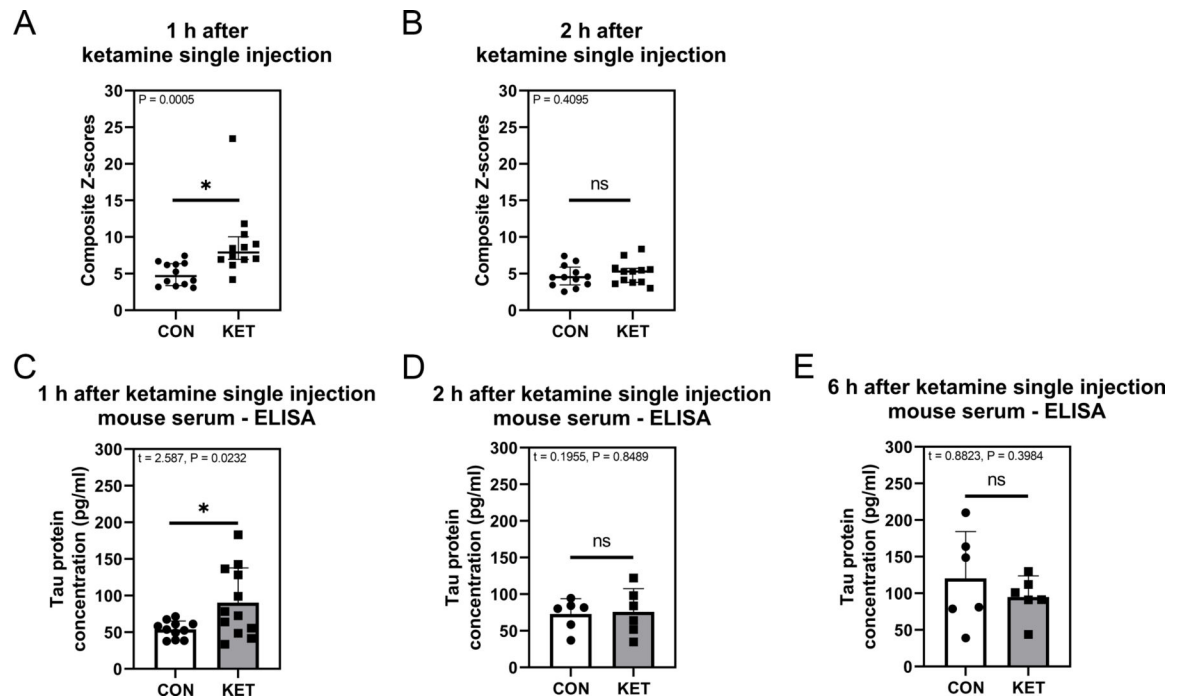
Figure 2. Ketamine induces delirium-like behavior and increases serum total tau amounts in mice.

A: Composite Z-scores 1 h after ketamine administration, n = 12 in each group. **B**: Composite Z-scores 2 h after ketamine administration, n = 12 in each group. **C**: Serum total tau protein concentration 1 h after ketamine administration, n = 11 in CON group, n = 12 in KET group. **D**: Serum total tau protein concentration 2 h after ketamine administration, n = 6 in each group. **E**: Serum total tau protein concentration 6 h after ketamine administration, n = 6 in each group. Mann-Whitney test was applied to analyze data in panels A and B. Grubbs' test was applied to identify an outlier owing to hemolysis in data in panel C. Student's t-test was applied to analyze data in panels C, D, and E. Error bars represent 25%–75% interquartile range in panels A and B, and SD in panels C, D, and E; * indicates $P < 0.05$ by statistical analysis; ns indicates no significant difference between groups ($P > 0.05$) by statistical analysis.
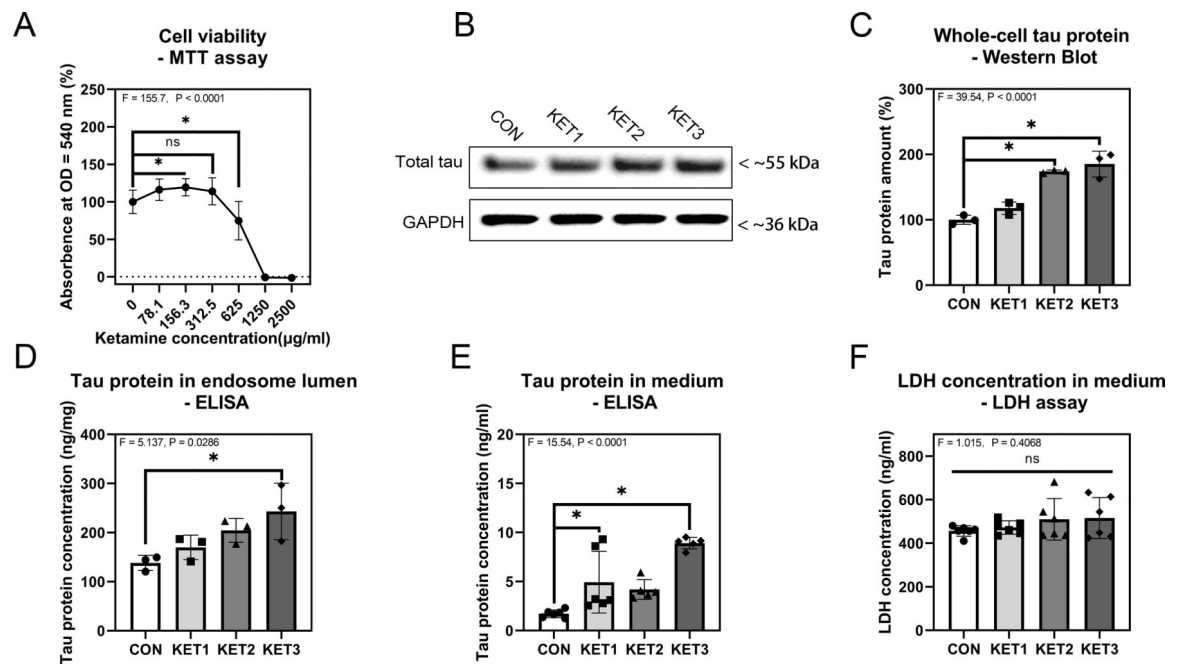
Figure 3. Ketamine induces changes in total tau amounts in endosomes and the medium.
A: Cell viability via MTT assay, n = 12 biological variables in each group. **B**: Western blot analysis of tau in whole-cells and the internal loading control glyceraldehyde-3-phosphate dehydrogenase (GAPDH). One band is shown for each experimental condition. **C**: Quantitation of the western blot analysis of tau in whole-cells, n = 3 biological variables in each experimental group. **D**: ELISA of tau concentrations in the endosomal lumen, n = 3 biological variables in each group. **E**: ELISA of total tau concentrations in the cell culture medium, n = 6 biological variables in the CON and KET1 groups, n = 5 biological variables in KET2 and KET3 groups. **F**: LDH concentration in the cell culture medium, n = 6 biological variables in each group. One-way ANOVA was applied to analyze data in panels A and C–F. Dunnett's test was applied for ANOVA post-hoc. SH-SY5Y cells were treated with gradient concentrations of ketamine medium. Grubbs' test was applied to identify outliers in data in KET2 and KET3 groups in panel D. Error bars represent SD; * indicates P < 0.05 by statistical analysis.
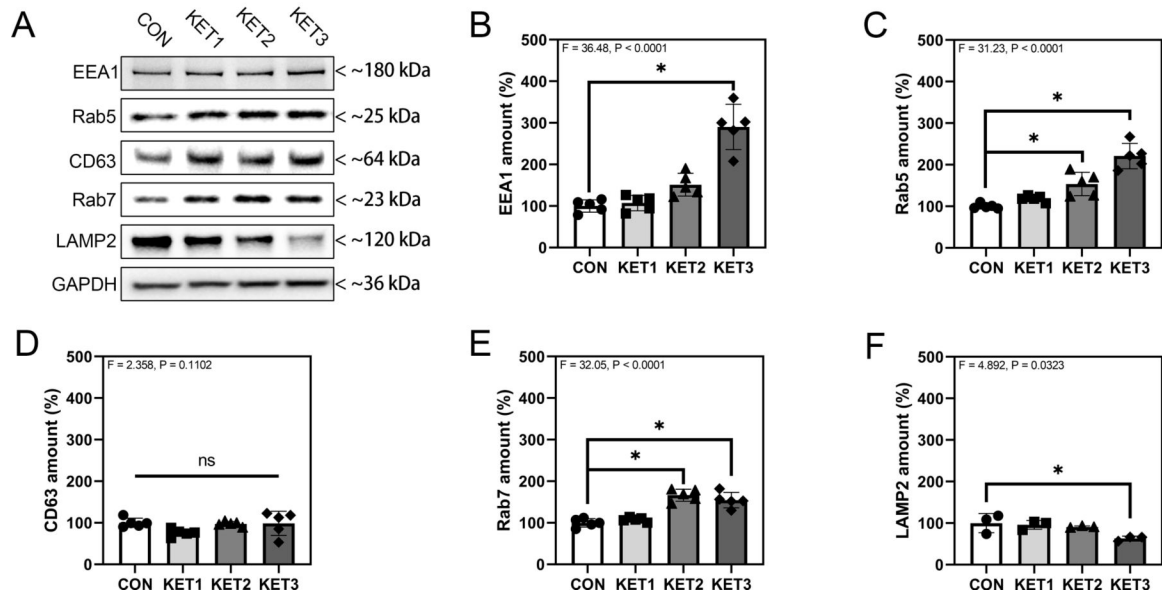
Whole-cell protein - Western Blot

Figure 4. Ketamine increases endosome accumulation in SH-SY5Y cells.
A: Effects of ketamine on whole-cell proteins (EEA1, Rab5, CD63, Rab7, LAMP2) and the internal loading control GAPDH. One band is shown for each condition. **B–F**: Quantitation of western blot analysis of whole-cell EEA1 (**B**), Rab5 (**C**), CD63 (**D**), Rab7 (**E**), and LAMP2 (**F**) proteins. n = 5 biological variables for panels B – E, n = 3 biological variables for panel F in each group. One-way ANOVA and post-hoc analysis with Dunnett's test were applied for statistical analysis. Error bars represent SD; * indicates P < 0.05 by statistical analysis; ns indicates no significant difference between groups (P > 0.05) by statistical analysis.
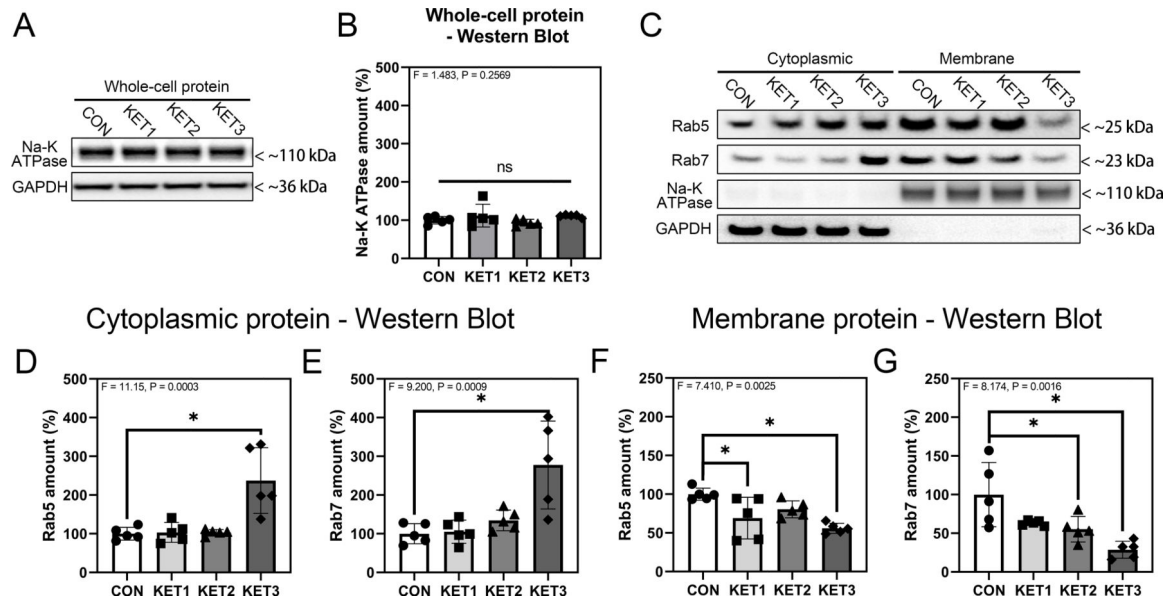
Figure 5. Ketamine decreases endosome membrane-bound Rab5 and Rab7 but increases cytoplasmic amounts of Rab5 and Rab7.
A: Effects of ketamine on whole-cell Na-K ATPase and the internal loading control GAPDH. One band is shown for each experimental condition. **B**: Quantitation of western blot analysis of whole-cell Na-K ATPase, n = 5 biological variables. **C**: Western blot analysis of cytoplasmic and membrane proteins Rab5 and Rab7 and internal loading control proteins Na-K ATPase and GAPDH. One band is shown for each experimental condition. **D–G**: Quantitation of western blot analysis of cytoplasmic Rab5 (**D**), cytoplasmic Rab7 (**E**), membrane Rab5 (**F**), and membrane Rab7 (**G**), n = 5 biological variables in each group. One-way ANOVA and post-hoc analysis with Dunnett's test were applied to analyze data in panels D–G. Error bars represent SD; * indicates P < 0.05 by statistical analysis; ns indicates no significant difference among groups (P > 0.05) by statistical analysis.

Table 1.

Antibody information

Antigen	Host species	Dilution	Manufacturer	Catalog No.
CD63	Rabbit	1:1000	Abcam	ab216230
EEA1	Mouse	1:1000	Cell Signaling Technology	48453S
GAPDH	Rabbit	1:2000	Cell Signaling Technology	5174S
LAMP2	Rabbit	1:1000	Abcam	ab199947
Na-K ATPase	Rabbit	1:20000	Abcam	ab76020
Rab5	Mouse	1:1000	Cell Signaling Technology	46449S
Rab7	Rabbit	1:1000	Abcam	ab126712
Total tau	Mouse	1:1000	Abcam	ab80579
Mouse (secondary antibody)	Goat	1:2500	Sigma-Aldrich	SAB3701095
Rabbit (secondary antibody)	Goat	1:10000	Invitrogen	G-21234