Dexmedetomidine versus ketamine in attenuating neuropathic pain through modulating STING/TBK pathway to modulate spinal ER-phagy in neuropathic pain model

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Research article

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Abstract

Background: Our previous studies suggested that stimulator of interferon genes (STING) level was altered in medial prefrontal cortex (mPFC) of spinal nerve ligation (SNL) rats.

Methods: In this study, we investigated that whether dexmedetomidine and ketamine provide antianxiety and anti-nociceptive effects via modulating spinal STING/TBK pathway to alter ER-phagy in SNL rats. We evaluated the analgesia and antianxiety effects of ketamine and dexmedetomidine in SNL rats. 2'3'-cGAMP (STING pathway agonist) was administrated to investigate whether enhanced spinal STING pathway could reverse dexmedetomidine or ketamine treatment effects in SNL rats respectively. Analgesia effects were measured with mechanical withdrawal threshold (MWT) and antianxiety effect was measured with open field test (OFT). Proteins expression levels were evaluated by Western blotting. Distribution and cellular localization of STING pathway and ER stress were evaluated by confocal immunofluorescence.

Results: SNL induced mechanical hypersensitivity and anxiety; STING pathway was involved in the modulation of ER stress and ER-phagy in SNL rats; Dexmedetomidine and ketamine provided analgesia and antianxiety effects in SNL rats; Dexmedetomidine and ketamine alleviated ER stress via inhibiting STING pathway to enhance ER-phagy in SNL rats.

Conclusions: In all, both ketamine and dexmedetomidine provided antianxiety and anti-nociceptive effects through alleviating ER stress via inhibiting STING/TBK pathway to modulate spinal ER-phagy in SNL rats.

Background

There is no denying that neuropathic pain could be a refractory disease in clinic, which resulted from nervous system dysfunction caused by nerve system damage(1, 2). The hyper-excitability and neuroplasticity may result from diverse cellular and molecular alterations in the spinal cord following peripheral nerve injury, which play an essential role in the onset and maintenance of neuropathic pain(3). Treatment for neuropathic pain is a crucial challenge in clinic because the mechanisms underlying remain far from clear and pharmacotherapy treatments available might be ineffective(4). Therefore, the combination and adjuvant pharmacotherapy for neuropathic pain demand prompt solution.

Dexmedetomidine, a selective α_2 adrenergic receptor (AR) agonist, provided analgesic potency via systemic administration, also, low doses of dexmedetomidine relieved tactile allodynia induced by neuropathic pain model(5). Moreover, study demonstrated that dexmedetomidine might be a better adjuvant for clinical procedure and pain management for neuropathic pain in clinic(6, 7).

Studies revealed that low doses and antidepressant doses level of ketamine, a sedative agent, might be a promising treatment for neuropathic pain(8). Ketamine administration showed positive analgesic and anti-nociceptive results for neuropathic conditions in animal models and in clinical patients(8, 9).

Neuropathic pain led to endoplasmic reticulum (ER) stress and autophagy impairment(10, 11). Autophagy served as a protective effect and attenuated neuropathic pain via restoring homeostasis in spinal cord(12, 13). Autophagy following ER stress mainly induced ER-phagy which could selective degrade ER via autophagy processing(14-18). Thus, ER-phagy might be a promising treatment target for neuropathic pain.

Stimulator of interferon genes (STING), an ER-resident protein, provided a novel crosstalk between ER stress and ER-phagy(19-21). STING serves as an ER adaptor, being required in elevated ER stress condition(22). Its conformational changes and phosphorylation result in its translocation from ER to distinct perinuclear endosomes near Golgi(20, 23, 24). STING phosphorylation induces TANK binding kinase 1 (TBK1) phosphorylation and subsequently leads to the phosphorylation of interferon regulatory factor3 (IRF3) production(25, 26).

Our previous studies suggested that ER-phagy was altered in neuropathic pain model(27, 28). In this study, our aim was to investigate the effects of dexmedetomidine and ketamine administration on anxiety- and nociceptive-related behaviors; investigate whether dexmedetomidine and ketamine alter spinal ER-phagy in SNL rats via modulating STING/TBK pathway.

Methods

Animals

Male Sprague Dawley (SD) rats (180–230g) were obtained from the Changsheng biological company and housed in Shengjing Hospital Benxi experiment base. This study was in accordance with the ethical guidelines of China Medical University for the use of laboratory animals and approved by the Animal Ethics Care and Use Committee of China Medical University Shengjing Hospital (approved number 2016PS013K). All surgery was performed under 1% pentobarbital sodium anesthesia (35mg/kg i.p.). Rats were randomly separated into different groups. n=6 rats per group. Timeline of experimental procedure was demonstrated as Figure 1.

Spinal nerve ligation (SNL)

Spinal nerve ligation procedure was carried out as described previously(29). Rats were placed in the prone position under anesthesia. A 2 cm incision was made on the left lumbar 5 level, 0.5 cm approximately to midline. The left L5 and L6 spinal nerve were separated and ligated tightly with 4-0 silk thread, distal end of ligation was transected.

Behavior tests

Mechanical withdrawal threshold (MWT)

To examine the mechanical hypersensitivity of rats, the mechanical withdrawal threshold (MWT) test was measured with Von Frey filaments (Stoelting Company, Wood Dale, IL, USA) as demonstrated

previously(10, 30). Rats were habituated 30 min before MWT test in plexiglass chamber. A positive withdrawal threshold of the hind paw was measured with up and down procedure. For each MWT trial, stimulation duration was 5s approximately; interval time was 5 min; cut-off value was 15g.

Open field test (OFT)

The anxiety and locomotor level was evaluated with open field test as reported previously(31). The open field arena consisted of an aluminum plate base (100 cm×100 cm) surrounded by walls of 45 cm. The interior was painted black. Open field arena was equipped with infrared detectors and analyzed by Noldus software. Rats were put in open field for 10 min. The distance, the proportion of the path in the center, travel trace and heat map were demonstrated. The field was cleaned with 75% ethanol after each trial.

Drug delivery

5μg 2'3'-cGAMP (STING agonist, Cat: #tlrl-nacga23, InvivoGen, USA) was intrathecal injected with 5 μl Hamilton microsyringe at 4h before operation and postoperative day 2, 4, 6. Single intrathecal injections were administrated via percutaneous lumbar puncture through 5th or 6th intervertebral space. A rapid tail flick manifested that the microsyringe penetrated the dura mater. The injection speed was 0.5s/μl approximately and microsyringe was hold still for 30 s after injection.

Ketamine (20 mg/kg, Cat. #1709291, Fujian Gutian Pharmaceutical Co., Ltd. China) or vehicle was intraperitoneally (i.p.) injected at 4 h before operation and postoperative day 2, 4, 6.

Dexmedetomidine (20 μg/kg, Cat. #181017BP, Jiangsu Hengrui Pharmaceutical Co., Ltd. China) or vehicle was intraperitoneally (i.p.) injected at 4h before operation and postoperative day 2, 4, 6.

Western blot

At postoperative day 7, SD rats were deeply anaesthetized and sacrificed. The L4~L6 spinal cord were rapidly dissected and frozen at -80°C. The tissues were homogenized in RIPA buffer (p0013B, Beyotime, China) and phosphatase inhibitors (1:100, Solarbio, China) for 30 min on ice, then centrifugation at 14,000 rpm for 40 min at 4 °C. The supernatant fraction after centrifugation was collected. The lysate with loading buffer (Beyotime, China) were separated with 12% SDS/PAGE gel and transferred to PVDF membranes (GE, USA). Primary antibodies were incubated at 4 °C overnight (12h at least) respectively after 1h room temperature blocking in 5% BSA with TBST (0.1%Tween 20 in Tris-buffered saline). Bands were incubated with HRP-conjugated second antibodies for 1.5 h at room temperature. After 3 times TBST washing, signal was detected with ECL plus kit (Tanon, China bands), captured with the chemiluminescence imaging systems (GE, USA; c300, Azure biosystems, USA) and quantified with Image J software (NIH, USA).

The following antibodies were used in this study: rabbit Grp78 (1:2000, Abcam, USA), rabbit LC3 (1:1000, CST, USA;), rabbit p62 (1:2000, CST, USA), rabbit FAM134B (1:1000, Abcam, USA), rabbit p-STING (1:1000, CST, USA), rabbit STING (1:1000, CST, USA), rabbit p-TBK (1:1000, CST, USA), TBK (1:1000, CST, USA),

mouse GAPDH (1:8000, Solarbio, China), goat anti-rabbit/goat anti-mouse IgG horseradish peroxidase (1:5000, Beyotime, China).

Immunofluorescence staining

Rats were deeply anesthetized and perfused with 0.9% NaCl solution transcardially, followed by cold 4% paraformaldehyde in 0.1M PBS. L5 spinal cords were removed, post-fixed in fixative solution for 24h, and dehydrated with 30% sucrose in ddH₂O at 4°C for 24h. The brains were embedded with optimal cutting temperature (OCT, SAKURA, USA) compound. Embedded L5 tissue was coronal sectioned in cryostat at 10µm thickness. For confocal immunostaining, sections were incubated with anti-NeuN (neuronal marker, 1:200, MAB377, Millipore, USA)/GFAP (glial cell marker, 1:200, Abcam, China) and anti-Grp78 (ER stress marker, 1:200, Abcam, USA)/p-TBK (1:200, CST, China). Cell nuclei were counterstained with DAPI (Beyotime, China) for 5 minutes.

Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). Analysis was measured with IBM SPSS Statistics 22 software (SPSS Inc., Armonk, New York, USA). Western blot and open field test results were analyzed by one-way analysis of variance (ANOVA) following post hoc multiple comparison; MWT data were analyzed by two-way analysis of variance (ANOVA) following post hoc multiple comparison. P values < 0.05 were considered significant.

Results

Spinal nerve ligation led to severe mechanical hypersensitivity, anxiety and STING/TBK activation

Our data confirmed that spinal nerve ligation resulted in mechanical hypersensitivity. A significant decrease of MWT was observed at postoperative day 2, 4, 6. Since anxiety is a frequent co-morbidity of neuropathic pain, anxiety level was evaluated with OFT. Our data demonstrated that the distance, the proportion of time spent in the center zone were significant decreased in compared with sham group. Representative heat maps and travel traces were shown in Figure 2. Expressions of related proteins were evaluated with Western blotting tests. The up-regulation of LC3, FAM134b, Grp78 and down-regulation of p62 confirmed that SNL induced ER-phagy impairment and ER stress. Furthermore, p-STING/STING and p-TBK/TBK was increased in SNL group compared with sham group, indicating STING/TBK pathway was activated in SNL rats.

Distribution and cellular localization of p-TBK and Grp78 in SNL rats

In this study, we evaluated the distribution of STING/TBK and ER stress in SNL rats. Our data demonstrated that p-TBK mainly expressed in astrocytes of lamina III-IV in SNL rats (Figure 3A, B). Furthermore, ER stress marker, Grp78, mainly expressed in neurons of lamina I-IV (Figure 3C, D). Our data suggested that STING/TBK pathway in astrocytes was activated following SNL induced ER stress in neurons.

Dexmedetomidine neutralized SNL induced nociception, anxiety and STING/TBK pathway activation

Dexmedetomidine administration resulted in significant analgesic and antianxiety effects. In SNL+D group, MWT was increased at postoperative day 4, 6 compared with SNL group (Figure 4A). Dexmedetomidine injection significantly increased the proportion of time spent in the center zone compared with SNL group (Figure 4B). p-STING, p-TBK was significantly decreased indicating STING/TBK pathway was down-regulated following dexmedetomidine administration. Up-regulation of FAM134b and down-regulation of LC3, p62, Grp78 suggested ER-phagy level was enhanced and ER stress was attenuated. Moreover, 2'3'-cGAMP administration resulted in reversed the analgesic and antianxiety effects induced by dexmedetomidine administration (Figure 4A, B). In SNL+D+G group, p-STING, p-TBK, FAM134b, LC3, p62, Grp78 were increased compared with SNL+D group, indicating 2'3'-cGAMP administration induced ER-phagy impairment and ER stress via activating STING/TBK pathway (Figure 4D). Representative heat maps and travel traces of con group, SNL group, SNL+D, SNL+D+G group were shown in Figure 4C.

Ketamine neutralized SNL induced nociception, anxiety and STING/TBK pathway activation

Ketamine administration led to significant analgesic and antianxiety effects. In SNL+K group, MWT was increased at postoperative day 2, 4, 6 compared with SNL+V group. Ketamine injection significantly increased that the distance and the proportion of time spent in the center zone compared with SNL group. Representative heat maps and travel traces were shown in Figure 5. Ketamine administration decreased the expression level of p-STING, p-TBK, p62, Grp78 and increased the expression level of FAM134b, LC3 compared with SNL group. Data suggested that ketamine ameliorated ER stress via increasing ER-phagy through inhibiting STING/TBK pathway. Furthermore, 2'3'-cGAMP neutralized the analgesic and antianxiety effects induced following ketamine administration assayed using MWT and OFT. Moreover, in SNL+K+G group, the protein expression level of p-STING, p-TBK, FAM134b, p62, Grp78 was increased and the expression level of LC3 was decreased compared with SNL+K group. Data revealed that 2'3'-cGAMP activated STING/TBK pathway and resulted in blocking the degradation of ER-phagy to enhance ER stress.

Discussion

In this study, our results showed that SNL induced significant mechanical allodynia and anxiety in rats, furthermore, ER stress and ER-phagy impairment via STING pathway activation. The mechanical allodynia and anxiety in SNL rats can be attenuated by injection of dexmedetomidine and ketamine respectively. Of note, both dexmedetomidine and ketamine relieved allodynia and anxiety induced by SNL via down-regulating STING/TBK pathway to ameliorate spinal ER stress. However, ER-phagy levels were altered differently following dexmedetomidine and ketamine treatment respectively.

Dexmedetomidine and ketamine produced analgesic and anxiolytic effects as premedication before general anesthesia(32, 33). Furthermore, administration of dexmedetomidine and ketamine both could reverse central sensitization induced by neuropathic pain(8, 34). In this study, our data demonstrated that dexmedetomidine and ketamine served antiallodynic and anxiolytic effects in animal neuropathic pain condition(9, 35-37), suggesting dexmedetomidine and ketamine might be promising pharmacotherapies.

Dexmedetomidine is a highly selective α -2 agonist, its affinity for α -2 AR is greater compared with clonidine(35, 38), which could enhancing norepinephrine (NE) concentration in the spinal cord of neuropathic pain patients together with amitriptyline and duloxetine. In addition, treatments such as amitriptyline and duloxetine are regard as first-line pharmacotherapies for neuropathic pain in clinical guideline nowadays(39, 40). In this case, dexmedetomidine might be widespread used as neuropathic pain treatment in the future. However, little is known about the underlying molecular mechanisms in which dexmedetomidine attenuates neuropathic pain induced allodynia and anxiety.

Studies focus on the neurotoxicity of high dosing ketamine administration and long-term ketamine abuse(41, 42). However, ketamine served as proper rescue, pharmacotherapy and, also, adjuvant for neuropathic pain with promising application prospect(8). Sub-anesthetic dosing of Ketamine provided clinical and experimental benefits for chronic pain and depression(43, 44). Since it is so, it is essential to investigate the mechanisms of ketamine administration in neuropathic pain model.

Our data demonstrated that dexmedetomidine and ketamine reversed SNL induced allodynia and anxiety significantly. As shown in Figure 4, 5, mechanical allodynia was attenuated in SNL+D group and SNL+K group compared with SNL group respectively. Dexmedetomidine and ketamine administration induced anxiolytic effect measured with open field tests. To be specific, dexmedetomidine improved the behavior tests in the proportion of time spent in the center. On the other hand, ketamine improved SNL-rats behaviors in the distance and the proportion of time spent in the center. Our data suggested that ketamine could both improve the performance of locomotion and antianxiety. In addition, dexmedetomidine and ketamine both provided a satisfactory analgesic effect in SNL induced neuropathic pain rats.

To determine whether ER-phagy was modulated following dexmedetomidine and ketamine administration in SNL rats, the expression of FAM134b, LC3 and p62 were measured with immunoblotting. Interestingly, our data revealed ER-phagy processing were modulated differently following dexmedetomidine and ketamine administration, respectively.

Dexmedetomidine treatment resulted in FAM134b significant increased, LC3 and p62 decreased significantly, indicating the level of ER bind to autophagosomes and ER degradation by ER-phagy was increased, however, the autophagosome level was decreased. The autophagosome decreased might result from the unbalance of formation and effective degradation of autophagosome.

Ketamine treatment induced FAM134b, LC3 significant increased and p62 significant decreased, suggesting spinal ER-phagy significant increased. Data suggested that more ER was selectively removed

by autophagosomes and degradation by ER-phagy processing. Dexmedetomidine rendered the degradation of ER-phagy increase, and ketamine induced both the formation and degradation of autophagosomes swallowed ER increased. Even so, both dexmedetomidine and ketamine administration led to enhance ER-phagy process. Moreover, 2'3'-cGAMP administration induced LC3 increased in SNL+D+G group compared with SNL+D group, indicating autophagosome level being increased following 2'3'-cGAMP injection. On the other hand, 2'3'-cGAMP decreased the expression level of LC3 in SNL+K+G group compared with SNL+K group.

Recently studies demonstrated that STING pathway had tremendous potential for immunotherapy from bench to bedside(24, 45-47). However, whether STING pathway involved in neuropathic pain process and whether STING pathway regulate ER-phagy process still remain unknown. In this study, our data indicated that STING/TBK pathway was activated in the spinal cord of neuropathic pain model.

Activated STING signaling triggers ER stress(48). STING-containing endoplasmic reticulum-Golgi intermediate compartment (ERGIC) served as a membrane source for the lipidation of LC3(20, 49, 50). STING directly interacted with LC3 and regulated autophagy process(19). Thus, STING modulates autophagy, an essential mechanism to keep homeostasis following ER stress, via localizing to autophagosomes(15, 17, 51, 52). Specifically, Studies suggested that altered STING pathway driving ER-phagy to resolves ER stress(21, 53, 54). Our data revealed that dexmedetomidine and ketamine up-regulated the level of ER-phagy via inhibiting STING/TBK pathway. Moreover, activating STING pathway via 2'3'-cGAMP resulted in neutralizing the treatment effects of dexmedetomidine and ketamine induced through impairing ER-phagy. We investigated the distribution and cellular localization of spinal STING/TBK and ER stress using immunofluorescence. p-TBK mainly expressed in astrocytes, Grp78 mainly expressed in neurons, indicating that SNL induced spinal ER stress in neurons through activating spinal STING pathway in astrocytes. These results expanded our understanding of the interaction of neurons and astrocytes in the spinal cord of SNL rats.

However, this study failed to demonstrate whether dexmedetomidine and ketamine provide does-dependent analgesic and antianxiety effects in SNL rats. Furthermore, STING pathway might be involved in immune response, the crosstalk between ER-phagy and immune response in neurons or glial cells still needs further researches to illustrate.

Conclusions

STING/TBK signaling pathway in astrocytes was activated in the spinal cord of SNL rats. Our data suggested that both dexmedetomidine and ketamine could elevate the level of ER-phagy via inhibiting STING pathway to attenuate ER stress in SNL rats. Nevertheless, dexmedetomidine and ketamine raised the level of spinal ER-phagy in SNL rats differently. 2'3'-cGAMP elevated STING pathway to attenuate dexmedetomidine and ketamine treatment effects via modulating ER-phagy.

Abbreviations

STING: stimulator of interferon genes

mPFC: medial prefrontal cortex

SNL: Spinal nerve ligation

MWT: mechanical withdrawal threshold

OFT: open field test

AR: selective $\alpha 2$ adrenergic receptor

ER: endoplasmic reticulum

TBK: TANK binding kinase

IRF3: interferon regulatory factor3

SD: Sprague Dawley

ANOVA: analysis of variance

NE: norepinephrine

ERGIC: endoplasmic reticulum-Golgi intermediate compartment

Declarations

Ethics approval consent to participate

All procedures were approved by the Animal Ethics Care and Use Committee of China Medical University Shengjing Hospital (approved number 2016PS013K).

Consent of publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article

Competing interests

None.

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Authors' contributors

Yongda Liu designed the study and wrote the manuscript. Jiao Guo, Xingyue Li, Guang Han analyzed data and revised the manuscript. Yongda Liu, Zhibin Wang, Mengmeng Ding performed the research. Ping Zhao supervised the study.

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Figures

Figure 1

Timeline of experimental procedure Abbreviation: D: demedetomidine administration; K: ketamine administration; G: 2'3'-cGAMP administration; O: spinal nerve ligation operation; TC: tissue collection; M: mechanical withdrawal threshold; OF: open field test.

Figure 2

Spinal nerve ligation activated STING/TBK pathway (A) Mechanical withdrawal threshold in sham group and SNL group (two-way ANOVA). (B) The trace map and heat map of open field test in sham group and SNL group. (C) Quantification of open field test (one-way ANOVA, distance: $F=16.781$, $p=0.002$; the proportion of time spent in the center: $F=12.512$, $p=0.005$). (D) Western blotting in sham group and SNL group. (E) Quantification of immunoblotting in sham group and SNL group (one-way ANOVA, p-STING/STING: $F=16.75$, $p=0.002$; p-TBK/TBK: $F=10.786$, $p=0.008$; FAM134b/GAPDH: $F=19.265$, $p=0.001$; LC3/GAPDH: $F=10.756$, $p=0.008$; p62/GAPDH: $F=8.957$, $p=0.014$; Grp78/GAPDH: $F=10.897$, $p=0.008$). $n=6$ rats per group, $*p<0.05$ compared with sham group. Abbreviation: SNL: spinal nerve ligation; sham: sham operation of spinal nerve ligation; BL: baseline

Figure 3

Co-localization of p-TBK/Grp78 with GFAP and NeuN in ipsilateral spinal dorsal horn of SNL rat. (A) Confocal immunofluorescence of p-TBK and GFAP in laminae I-III of SNL rats L5 level. (B) Confocal immunofluorescence of p-TBK and NeuN in laminae I-III of SNL rats L5 level. (C) Confocal immunofluorescence of Grp78 and GFAP in laminae I-III of SNL rats L5 level. (D) Confocal immunofluorescence of Grp78 and NeuN in laminae I-III of SNL rats L5 level. n=6 rats per group. Scale bar = 50 μ m.

Figure 4

Dexmedetomidine provided antianxiety effect via inhibiting STING/TBK pathway. (A) MWT tests of con group, SNL group, SNL+D and SNL+D+G group (two-way ANOVA) (B) Quantification of open field of con group, SNL group, SNL+D and SNL+D+G group (one-way ANOVA, distance: $F=13.260$, $p=0.003$; the proportion of time spent in the center: $F=16.520$, $p=0.006$). (C) Trace map and heat map of con group, SNL group, SNL+D and SNL+D+G group. (D) Western blot of con group, SNL group, SNL+D and SNL+D+G

group. (E) Western blot quantification of con group, SNL group, SNL+D and SNL+D+G group (one-way ANOVA, p-STING/GAPDH: $F=6.092$, $p=0.006$; p-TBK/GAPDH: $F=3.709$, $p=0.034$; FAM134b/GAPDH: $F=13.979$, $p=0.007$; LC3/GAPDH: $F=9.265$, $p=0.001$; p62/GAPDH: $F=4.268$, $p=0.022$; Grp78/GAPDH: $F=8.613$, $p=0.001$). $n=6$ rats per group, $*p<0.05$ compared with con group; $\#p<0.05$ compared with SNL group; $\Delta p<0.05$ compared with SNL+D group. Abbreviation: BL: baseline; Dex: dexmedetomidine; con group: rats received sham operation, vehicle of dexmedetomidine and 2'3'-cGAMP administration; SNL group: rats received SNL procedure and vehicle of dexmedetomidine and 2'3'-cGAMP administration; SNL+D group: rats received SNL and dexmedetomidine and vehicle of 2'3'-cGAMP. SNL+D+G group: rats received SNL and dexmedetomidine and 2'3'-cGAMP.

Figure 5

Ketamine provided antianxiety effect via inhibiting STING/TBK pathway. (A) MWT tests of con group, SNL group, SNL+K and SNL+K+G group (two-way ANOVA) (B) Quantification of open field of con group, SNL group, SNL+K and SNL+K+G group (one-way ANOVA, distance: $F=16.034$, $p=0.002$; the proportion of time spent in the center: $F=11.637$, $p=0.005$). (C) Trace map and heat map of con group, SNL group, SNL+K and SNL+K+G group. (D) Western blot of con group, SNL group, SNL+K and SNL+K+G group. (E) Western blot quantification of con group, SNL group, SNL+K and SNL+K+G group (one-way ANOVA, p-

STING/GAPDH: $F=10.419$, $p=0.001$; p-TBK/GAPDH: $F=12.993$, $p=0.003$; FAM134b/GAPDH: $F=16.606$, $p=0.005$; LC3/GAPDH: $F=11.014$, $p=0.005$; p62/GAPDH: $F=4.047$, $p=0.026$; Grp78/GAPDH: $F=7.520$, $p=0.002$). $n=6$ rats per group, $*p<0.05$ compared with con group; $\#p<0.05$ compared with SNL group; $\Delta p<0.05$ compared with SNL+K group. Abbreviation: BL: baseline; Ket: ketamine; con group: rats received sham operation, vehicle of ketamine and 2'3'-cGAMP administration; SNL group: rats received SNL procedure and vehicle of ketamine and 2'3'-cGAMP administration; SNL+K group: rats received SNL and ketamine and vehicle of 2'3'-cGAMP. SNL+K+G group: rats received SNL and ketamine and 2'3'-cGAMP.