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Novel energy optimizer, meldonium, rapidly restores acute hypobaric hypoxia-induced brain injury by targeting phosphoglycerate kinase 1

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Abstract

Background Acute hypobaric hypoxia-induced brain injury has been a challenge in the health management of mountaineers; therefore, new neuroprotective agents are urgently required. Meldonium, a well-known cardioprotective drug, has been reported to have neuroprotective effects. However, the relevant mechanisms have not been elucidated. We hypothesized that meldonium may play a potentially novel role in hypobaric hypoxia cerebral injury.

Methods We initially evaluated the neuroprotection efficacy of meldonium against acute hypoxia in mice and primary hippocampal neurons. The potential molecular targets of meldonium were screened using drug-target binding Huprot™ microarray chip and mass spectrometry analyses after which they were validated with surface plasmon resonance (SPR), molecular docking, and pull-down assay. The functional effects of such binding were explored through gene knockdown and overexpression.

Results The study clearly shows that pretreatment with meldonium rapidly attenuates neuronal pathological damage, cerebral blood flow changes, and mitochondrial damage and its cascade response to oxidative stress injury, thereby improving survival rates in mice brain and primary hippocampal neurons, revealing the remarkable pharmacological efficacy of meldonium in acute high-altitude brain injury. On the one hand, we confirmed that meldonium directly interacts with phosphoglycerate kinase 1 (PGK1) to promote its activity, which improved glycolysis and pyruvate metabolism to promote ATP production. On the other hand, meldonium also ameliorates mitochondrial damage by PGK1 translocating to mitochondria under acute hypoxia to regulate the activity of TNF receptor-associated protein 1 (TRAP1) molecular chaperones.

Conclusion These results further explain the mechanism of meldonium as an energy optimizer and provide a strategy for preventing acute hypobaric hypoxia brain injury at high altitudes.

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Keywords Meldonium, Hypobaric hypoxia, Brain injury, Energy metabolism, Mitochondrial damage, Phosphoglycerate kinase 1

Background

Extreme high-altitude conditions drive biological adaptation changes while also presenting physiological challenges for those who travel to high altitudes for tourism, work, or military assignments. High-altitude environments trigger a range of hypobaric hypoxia-induced physiological changes due to its characteristic decline in atmospheric pressure, accompanied by descending temperature and humidity and increasing strength of ultraviolet rays [1–4]. Being the organ most intolerant to hypoxia, the brain often leads to individuals who ascend rapidly struggling to adjust to elevated terrains, suffering from a series of temporary or long-term symptoms that include physical, cognitive, and behavioral problems [5–8]. Multiple mechanisms regarding central nervous system (CNS) injury are complex and proposed, including cerebral hemodynamic changes, accumulation of toxic metabolites, release of cytokines and excitatory amino acids, acid-alkaline imbalance, inflammatory responses and mitochondrial damage [9–16]. In addition, there is a paucity of drugs regarding the prevention and treatment of brain injury in plateaus, mainly including acetazolamide and glucocorticoids, but both of them have more side effects. Therefore, it is crucial for medical research to explore the mechanism of hypoxia brain injury at high altitudes and develop potential therapeutic drugs.

Acute hypobaric hypoxia causes impaired bioenergetics, including mitochondrial damage and an imbalance of energy metabolism that can be fatal. Phosphoglycerate kinase 1 (PGK1), a rate-limiting enzyme in redox balance, catalyzes the conversion of 1,3-bisphosphoglycerate (1,3-BPG) to 3-phosphoglycerate (3-PG) and adenosine triphosphate (ATP) and is critical for coordinating energy metabolism and biosynthesis. Aberrant expression of PGK1 has mostly been associated with cancer-related diseases [17, 18]. PGK1 acts as a protein kinase that regulates mitochondrial metabolism, autophagy, and DNA damage in tumorigenesis [19–22]. In addition, PGK1 deficiency is linked with Parkinson's disease, hereditary aspheric hemolytic anemia, and neurological dysfunction [23–25]. Therefore, a detailed understanding of the underlying mechanisms and regulation of energy metabolism by PGK1 may have clinical implications for prevention of high-altitude acute hypoxia brain injury.

Meldonium (also called mildronate, THP, or MET-88) is a clinically cardioprotective drug that optimizes energy metabolism by inhibiting carnitine-dependent fatty acid oxidation to achieve myocardial protection. Although it is marketed for use in some countries, there are only a few clinical studies for reference. Current research shows that meldonium may act on cardiovascular, neurological, and metabolic diseases [26, 27]. In preclinical studies, meldonium has been reported to reduce the progression of myocardial infarction, COVID-19, diabetes, and brain diseases such as Huntington's disease and Alzheimer's disease and to protect the liver and kidneys from ischemia/reperfusion injury [28–32]. While the neuroprotective effects of meldonium in the CNS are noted in the literature [33], the role and mechanism of meldonium in acute high-altitude hypoxia-induced brain injury are poorly understood.

In this study, we simulated high-altitude environments in mice and primary hippocampal neuronal cells to investigate the pharmacodynamics of meldonium in acute hypobaric hypoxia-induced brain injury. Furthermore, human protein microarray combined with mass spectrometry analyses were implemented to comprehensively profile meldonium interactions with proteins, identifying PGK1 as a critical target of meldonium. Here, the effects of meldonium on signaling pathways related to PGK1 activation and transfer to mitochondria were reported. More importantly, we highlight that meldonium can be used as an energy optimizer in the field of plateau brain injury and the associated regulatory mechanisms.

Methods

Chemicals, reagents, and antibodies

Meldonium (purity 98%) was purchased from Yuanye Bio-Technology Company (Shanghai, China) and dissolved in 0.9% saline or neurobasal medium in vivo and in vitro, respectively. Acetazolamide (98% purity) provided by Selleck Chemicals (Houston, USA) was dissolved in sterilized water with 40% cyclodextrin and 1% dimethyl sulfoxide. The reagents for cell culturing, including Hank's Balanced Salt Solution (HBSS), Dulbecco's Modified Eagle Medium (DMEM), B-27 supplement, GlutaMax, and Neurobasal medium were obtained from Gibco-BRL Co. (Grand Island, NY). The use of relevant antibodies was shown in Supplementary Table S1.

Animals

Six to eight-week-old male Kunming mice (20±2 g) and pregnant Wistar rats (17–18 days of gestation) were purchased from SPF Biotechnology Co., Ltd. (Beijing, China). All animals were kept under standard housing conditions (22±2 °C, 50% humidity, 12 h/12 h dark-light cycles, provided with food and water ad libitum). All our animal experiments followed the Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Committee on the Ethics of Animal Experiments

of the Beijing Institute of Pharmacology and Toxicology (Permit Number: NBCDSER-IACUC-2018-096). This study does not contain human participants.

Primary hippocampal neurons cultures

For the primary neuron culture, hippocampi from Wistar fetus rats were dissected in cold HBSS solution and then digested with 0.25% trypsin for 10–15 min. The prepared cell suspension was centrifuged at 1000 rpm and counted. Neurons were inoculated at 8×10^5 neurons/well in DMEM high-glucose medium containing 10% FBS. After 4 h of incubation, the cell medium was replaced with Neurobasal medium including 2% B-27 supplement and 1% GlutaMax and kept in a constant temperature and CO_2 incubator at 37 °C. Adherent group cells were treated according to the experimental requirements after 7 days of growth.

Induction of acute hypobaric hypoxia brain injury and treatment

The male Kunming mice were randomized into six groups (Thirty-six, $n=6$): (1) the normoxia group (mice were kept under normal atmospheric pressure and treated with 0.9% saline, *i.g.*); (2) the hypoxia-treated 0.9% saline group; 3–5) the hypoxia-treated meldonium groups (50, 100, 200 mg/kg) and 6) the hypoxia-treated acetazolamide group (50 mg/kg, positive control). All treatments were administered intragastrically to the animals daily for 3 days prior to the induction of hypoxia. The acute hypobaric hypoxia-induced brain injury mice model was established following the methods modified from published literature [34]. Mice in the hypoxia group were placed in an animal decompression chamber to simulate the effects of an altitude of 8,000 m for 24 h, with the oxygen partial pressure from 6.8 to 7.2 kPa, humidity at 30–48%, oxygen concentration of around 5.66%, and temperature at 21–24 °C. The speed of ascent to the simulated high altitude and descent to the local horizon were both 30 m/s. For primary hippocampal neurons, the normoxia group cells were cultured in a normal environment, while the experimental groups were placed in a cellular hypoxia workstation with 5% CO_2 and 0.5% O_2 for 24 h.

Histopathological evaluations and Nissl staining

Twenty-four hours post hypobaric hypoxia exposure, mice were sacrificed by euthanasia. After perfusion with 0.9% normal saline and 4% paraformaldehyde solution, brain tissue was removed, dehydrated with graded ethanol, embedded in paraffin, and sliced into 5 μm thick sections. Sections were stained with Hematoxylin-eosin (H&E) and Nissl staining, and examined using an optical microscope (IX51, Olympus, Japan).

Doppler monitoring of cerebral blood flow

After 24 h hypoxia exposure, regional cerebral blood flow (rCBF) was measured in mouse brains using a laser doppler scan. The mouse scalp was gently removed from the cranial surface and cranial viewports were created as elaborated by Kamat et al. [35]. In addition, the cranium was cleaned with 150 μL saline to highlight blood vessels and accurately align the probe over the desired coordinates. rCBF of the parietal cortex was continuously monitored with a high-precision probe of a laser doppler blood flowmeter. Color-coded images were acquired three times continuously during the entire process, and the rCBF of each group was recorded and computed by moor FLPI-2 V50 software (Moor Instruments, Devon, UK).

Multiple microelectrode array (MEA) analysis

Spontaneous activity in primary hippocampal neurons under acute hypoxia and meldonium treatment was recorded using the Maestro system (Axion Biosystems, Atlanta, GA, USA). Primary hippocampal neuron cells were seeded in 24-well MEA plates. The mean spike rate per active electrode was calculated using the spike count file generated by the Axion Integrated Studio program (Axion Biosystems, Atlanta, GA, USA). The 6-minute raster plots were used to qualitatively observe neuronal pro-discharge under acute hypoxia. The black lines indicate single spikes defined in a burst, and the time surrounded by rose red indicates the moment when a network burst occurs.

Biochemical analysis

After 24 h hypoxia exposure, mice brain tissues were homogenized in pre-cold 0.9% saline and centrifuged at 3,500 rpm for 10 min at 4 °C. The supernatant was collected and analyzed for hydroxyl radical, malondialdehyde (MDA), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and adenosine triphosphate (ATP) using the respective kits (Jiancheng Bioengineering Institute Co., Ltd., Nanjing, China). Protein concentrations were quantified using the bicinchoninic acid (BCA) method (Keygen Biotech, Nanjing, China).

Transmission electron microscope (TEM)

Kunming mice hippocampi were fixed for 20 h in 2.5% (v/v) glutaraldehyde followed by 1% (w/v) osmium tetroxide (OsO_4) for 1 h. Fixed slices were dehydrated in an ascending series of ethanol solutions, finishing with absolute alcohol, and then embedded in EPON resin. Ultra-thin Sect. (70 nm thick) were cut from the tissue and stained with uranyl acetate and lead citrate, and then examined using TEM (H-7650, Tokyo, Japan). Ultrastructural changes were observed in mitochondria and neuron synapses in the hippocampus.

Mitochondrial permeability transition pore (MPTP)

Mouse brain tissue mitochondrial suspension was prepared using a mitochondrial extraction kit (Keygen Biotech, Nanjing, China). Isolated mitochondria were immersed in 10 mM HEPES buffer containing 395 mM sucrose, 120 mM KCl, and 5 mM KH_2PO_4. Afterward, calcium chloride solution was added to the solution to reach a final concentration of 200 μM. The change in absorbance value at 520 nm wavelength in mitochondrial solution for 10 consecutive minutes was measured.

Fluorescent probe labeling and measurement of neuronal morphology, mitochondrial membrane potential (MMP)

Changes in neuronal morphology and mitochondrial membrane potential (MMP) under acute hypoxia and pretreatment with meldonium were assessed by labeling fluorescent probe neuron-specific microtubule-associated protein 2 (MAP2) and JC-1, respectively. Briefly, primary hippocampal neurons were fluorescently labeled with probe working solution of MAP2. After 1 h incubation at 37 °C, 2 μg/mL Hoechst 33,342 (Sigma-Aldrich, USA) was added for 10 min at 37 °C to label cell nuclei. For MMP measurement, the purified mitochondrial suspension was stained with a JC-1 fluorescent probe solution at 37 °C for 30 min and then washed twice with JC-1 dyeing buffer. Cell images were captured and quantified using High Content Analysis (Molecular Devices, Sunnyvale, USA). The Neurite Outgrowth module in High Content Analysis can automatically and rapidly identify neuronal bodies and their protrusions (length and branching), thus obtaining the morphological characteristics of neuronal cells and the cell mean outgrowth results.

Proteome microarrays analysis

Screening and identification of meldonium binding proteins were done with the human proteome microarray (HuProt™ 20 K, Wayen Biotechnology, Shanghai, China). Briefly, the proteome microarrays were immersed in 5% BSA buffer, and added 10 μM Biotin and 10 μM Biotin-meldonium to incubate at 25 °C for 1 h. After washing, the proteome microarray was put into 0.1% Cy5-Streptavidn solution to react for 20 min at 25 °C under light-proof conditions. After centrifugation (1000 g for 2 min) and drying, the microarrays chips were scanned at 635 nm with a GenePix 4000B scanner (Axon Instruments, USA), and the results were analyzed with the GenePix™ Pro v6.0 software (Axon Instruments, USA).

Pull-down assay and mass spectrometry analysis

A pull-down assay was performed using bio-meldonium as baits to detect interacting proteins of meldonium in primary hippocampal neuron cell lysates. Briefly, the purified biotin or biotin-conjugated meldonium is coupled to positively charged resins and rotated at 4 °C for 2 h. Afterward, the formed small molecule-resin mixture was incubated with primary hippocampal neuron cell lysate for 2 h. After incubation, the complex resin was washed three times with PIPA buffer and then boiled for 5 min in 2.5 × SDS loading buffer. This was followed with SDS-PAGE and Coomassie staining.

Gel bands were sheared and de-stained, followed by a reduction in 20 mM DTT/50 mM NH_4HCO_3, alkylation in 40 mM IAA/50 mM NH_4HCO_3, and trypsin digestion overnight at 37 °C. The recovered peptides were assayed in a liquid mass spectrometer (Thermo Fisher) at a flow rate of 0.6 μL/min for 90 min. Mass spectrometry analysis was performed using Thermo™ Proteome Discoverer 2.1 software. The obtained protein was searched using the Protein Resource (UniProt) database. Unipeptides ≥ 4 and Coverage rate ≥ 50% were used as the criteria for identifying proteins during screening.

Molecular docking

The molecular docking program (Molecular Operating Environment, MOE, Chemical Computing Group ULC., Canada) was used to predict whether there were any meldonium-protein or protein-protein interactions. The 3D crystal structure of PGK1 and TRAP1 were downloaded from the Protein Data Bank (PDB ID: PGK1, 4O33; TRAP1, 5HPH). Then, 3D protonation and energy minimization of meldonium and PGK1 or PGK1 and TRAP1 was done using the docking program to assess the possibility of their binding.

Surface plasmon resonance (SPR) analysis

The binding affinity of meldonium to recombinant human PGK1 (rhPGK1) was assessed using the Protein Interaction Assay system (OpenSPR™, Nicoya, Canada) with a nanogold sensor chip. rhPGK1 protein was dissolved in 10 mM acetate acid buffer (pH 5) and immobilized on the chip. Different concentrations of meldonium (8, 4, 2, 1, 0 mM, and 500 μM) were prepared with PBS buffer (pH 7.5). The interactions were measured at a flow rate of 20 $\mu L \cdot min^{-1}$ for 240 s during the binding phase, followed by 480 s for the dissociation phase at 25 °C. The results were analyzed with the Trace Drawer software (Ridgeview Instruments, Uppsala, Sweden). Meldonium binding kinetic parameters were calculated and fitted using a 1:1 Langmuir binding model.

Seahorse energy metabolism analysis

Mitochondrial respiratory function and glycolytic stress were measured using Seahorse XF Bioscience energy metabolism analyzer (Agilent, USA). Mitochondrial respiration, which consumes oxygen and oxidizes fatty acids or other substrates to generate ATP, can be roughly estimated using the oxygen consumption rate (OCR).

During glycolysis, glucose is broken down into lactate and the resulting protons are exported to the extracellular medium, which can be measured as the extracellular acidification rate (ECAR). Primary hippocampal neuron cells were plated at a density of 5×10^5/well in an XF96 plate and immersed in a culture medium supplemented with 2 mM glutamine, 1 mM sodium pyruvate, and 10 mM glucose. Additionally, different inhibitor complexes were added into different ports of the cartridge. For the OCR assay, the inhibitors added were 1.5 μM oligomycin, 4 μM FCCP, and 0.5 μM rotenone/antimycin A. The EACR assay required the addition of 30 mM saturating glucose dose, 4 μM oligomycin, and 50 mM 2-deoxyglucose. Data were analyzed using the Mito Stress and Glycolysis Stress Test Report Generator (Agilent Technologies).

Western blotting analysis

Mouse brain tissue and primary hippocampal neuron cells were lysed in radio Immuno-precipitation Assay (RIPA) lysis buffer. Total protein, nucleoprotein and mitochondrial purified proteins were extracted using standard protocols and quantified with the BCA protein assay kit. The extracted protein was then mixed with 2.5 × SDS loading buffer and boiled at 100 °C for 5 min. Protein samples (20 μL/lane) were electrophoresed and transferred to a polyvinylidene fluoride (PVDF) membranes. After blocking with 5% skimmed milk powder for 2 h, the membranes were incubated with primary antibodies (anti-HIF-1α, anti-PGK1, anti-TRAP1, anti-CyP-D, anti-Caspase 3, anti-Cyt C, anti-COX-IV, anti-PDK1, anti-GLUT1, anti-MCT4, and anti-β-actin) at 4 °C. After washing three times with a TBST buffer shaker for 5 min each, PVDF membranes were incubated with horseradish peroxidase-labeled secondary antibody for 1 h at 25 °C and then washed three more times. Blots were detected with hypersensitive electrochemiluminescence (ECL) reagent and quantified using the ChemiDoc XRS$^+$ chemiluminescence imaging system.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 (GraphPad, CA, USA). Each experiment was repeated at least six times, and the results are expressed as mean ± SD. Comparison between the two groups used unpaired Student's t-tests, while multiple comparisons were performed using a one-way ANOVA followed by a post-hoc Turkey analysis. $P<0.05$ was considered statistically significant.

Results

Pretreatment with meldonium rescued acute hypobaric hypoxia-induced pathological injury of brain

To verify the pharmacological effect of pretreatment with meldonium on acute high-altitude brain injury, acute hypoxia models in mice and primary hippocampal neurons were established (Fig. 1A and Additional file 1: Fig. S1A). In the mouse model, we used a mortality rate of about 30% as an evaluation criterion for modeling success, both to elaborate acute plateau-induced injury and to examine the effect of meldonium on survival rate. As shown in Fig. 1B and C-F, pretreatment with meldonium reduced the mortality of mice in a dose-dependent manner and alleviated pathological changes in the hippocampus cornu ammonis 3 (CA3) area of the brain, such as nuclear pyknosis, Nissl body dissolution, and reduced blood flow in brain tissue. Then, we investigated the effects of meldonium pretreatment on primary hippocampal neuron cell outgrowth through the immunofluorescent tracking of neuron dendritic marker MAP2. The results showed that neurons appeared swollen or deformed, the outline of the cell body was blurred, or even completely disintegrated, and the density of dendritic spines decreased in hypoxia group, however, pretreated with meldonium groups significantly improved the above pathological changes (Fig. 1G and H). Similar results were also found in neurons firing rate that meldonium markedly promoted the mean firing rate and communication of primary hippocampal neurons under acute hypoxia (Additional file1: Fig. S1D). Additionally, the results show that groups pretreated with meldonium had significantly increased cell viability in a dose-dependent manner (Additional file1: Fig. S1B) and inhibited cell cytotoxicity (Additional file1: Fig. S1C) under hypoxia, similar to that observed in the acetazolamide treatment group. The results indicate that the application of meldonium improved hypoxia outcomes and recovered pathological damage at the histological and cellular levels.

Pretreatment with meldonium mitigated oxidative stress and mitochondrial damage under acute high-altitude exposure

Oxidative stress (OS) is a powerful initiator of apoptosis, leading to neuronal cell death under hypoxia. In the pathological condition of hypoxia, the imbalance between the generation of ROS and the antioxidant system can induce excessive lipid peroxide production and decreased antioxidant enzyme activity. As shown in Fig. 2A and B, pretreatment with meldonium significantly reduced the excessive production of MDA, hydroxyl radicals, and ROS, and increased the activity of the antioxidant enzymes, GSH-Px and SOD. Meanwhile, we observed the changes in $^1O_2$, as a strong oxidative reactive oxygen species, in primary hippocampal neuron cells. The

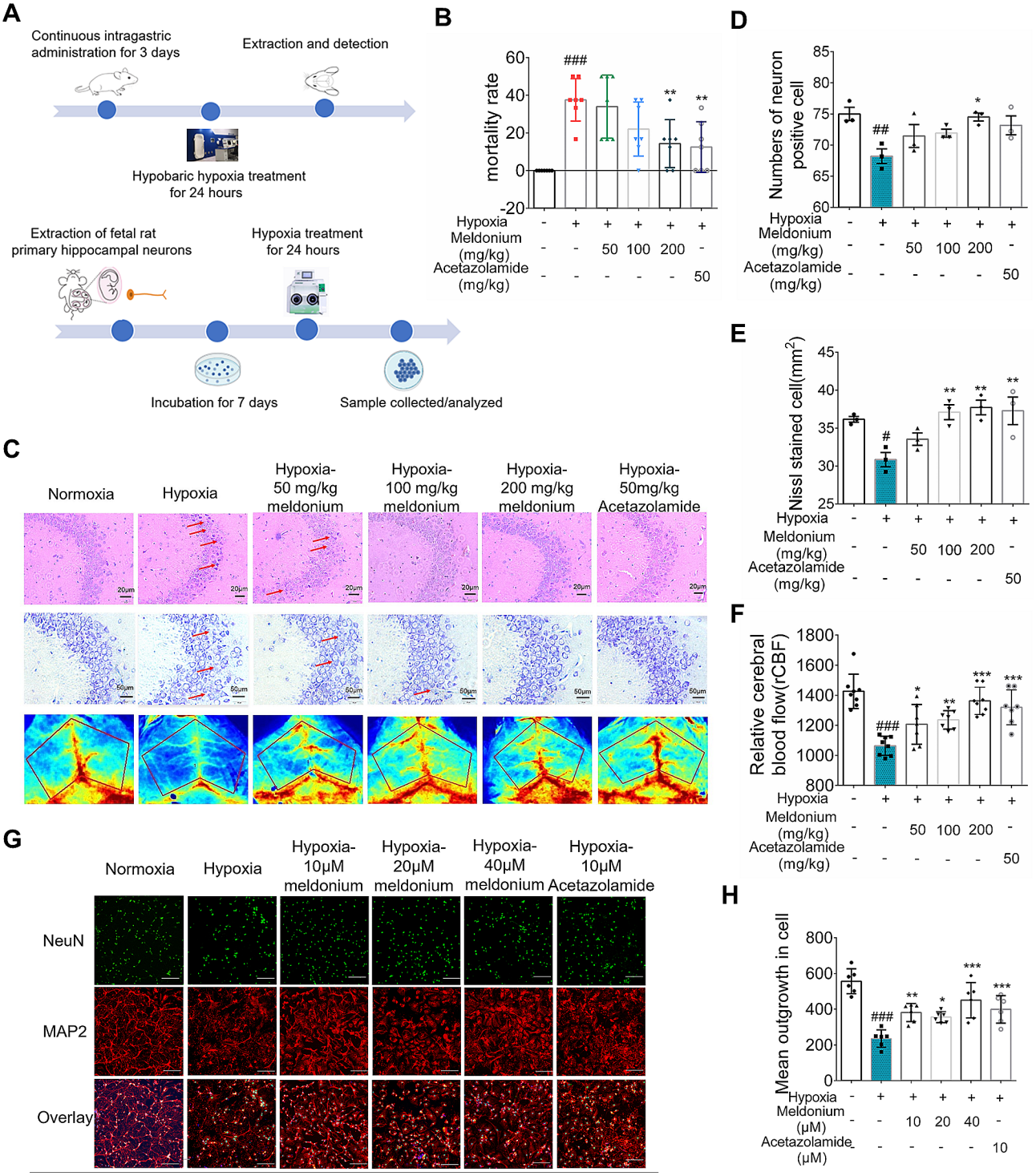

Fig. 1 Pretreatment with meldonium alleviates acute high-altitude brain pathological damage. **A** Models establishment timeline of acute hypoxia experiment in mouse and primary hippocampal neuron. **B** Mortality rate under hypoxia exposure. $n=8$. **C-F** Representative images and quantification of pathological changes in mice brain tissue after hypoxia and pretreatment with meldonium detected by H&E, Nissl staining (CA3 area, Scale bars: 20 μm and 50 μm, respectively, $n=3$), and cranial Doppler flow analyses and quantitative analysis of relative blood flow changes, $n=8$. HE staining: red arrows represent pyknosis of neuron, Nissl staining: red arrows represent disappearance of Nissl bodies. **G-H** Representative immunofluorescence images and quantitative analysis in neuron dendritic markers MAP2 and neuronal markers NeuN (green). $n=6$. Data are presented as the mean ± SD. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ versus the normoxia group, * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus the hypoxia group using the one-way ANOVA followed by Tukey's multiple comparison tests

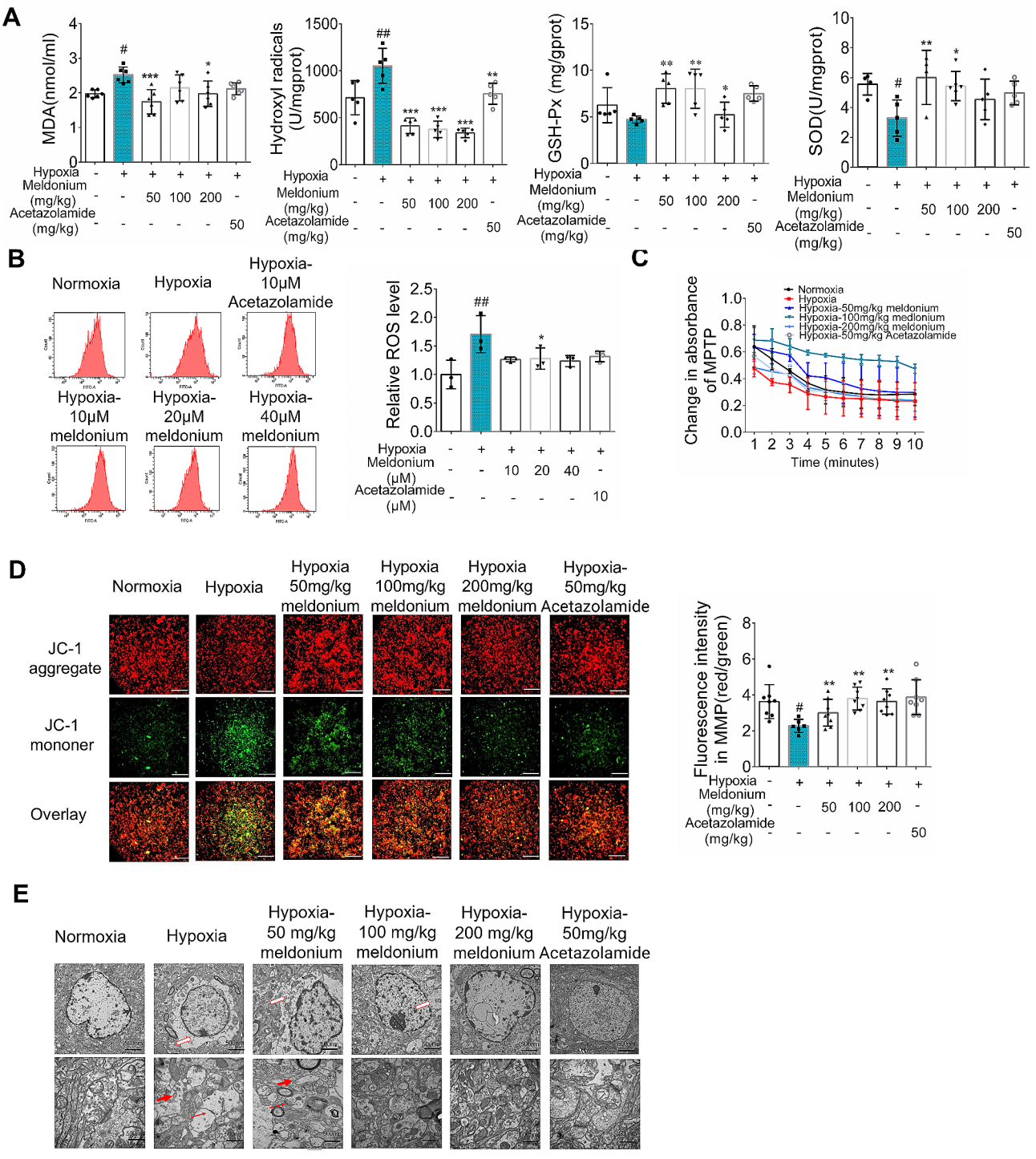

Fig. 2 Pretreatment with meldonium ameliorates oxidative stress and mitochondrial damage under acute hypoxia exposure. **A** Quantitative analysis of lipid metabolites (MDA, hydroxyl radicals) and antioxidant enzymes (GSH-Px, SOD). $n = 6$. **B** Representative figures and quantification of ROS by Flow cytometry analysis. $n = 3$. **C** Effects of meldonium on isolated mPTP opening under hypoxia exposure. Sloping curves indicate mPTP opening induced by $CaCl_2$. **D** Representative fluorescence images and quantitative analysis of MMP under hypoxia exposure. JC-1 aggregates in healthy mitochondria are represented by red fluorescent surrogates, while JC-1 monomers are indicated by green fluorescence for MMP dissipation. $n = 8$. **E** Ultrastructural changes visible under a transmission electron microscope in the hippocampus of mice. Open arrows indicate cytoplasmic vacuolization. Solid short arrows indicate swelling of mitochondria burst and solid long arrows indicate narrowing of synaptic intercellular space. Data are presented as the mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus the normoxia group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus the hypoxia group by one-way ANOVA followed by Tukey's multiple comparison tests

fluorescence intensity of the Si-DMA probe labeled in the hypoxia group was higher than that of the normoxia group, indicating that the presence of singlet oxygen and the degree of cell damage increased under hypoxia. However, pretreatment with meldonium groups prevented the damage mentioned above, and the antioxidant effect was significantly superior to that of acetazolamide (Additional file1: Fig. S2A).

Mitochondrial respiratory chain complexes I and III are the gatekeepers of the mitochondrial respiratory chain and the main sources of ROS. The results showed that meldonium prevented oxidative stress damage from the source in the primary hippocampal neurons under acute hypoxia exposure (Additional file1: Fig. S2B). Excessive accumulation of oxygen radicals disrupts mitochondrial structural and functional integrity. As indicated in Figu. 2 C and D, acute hypoxia leads to the opening of mitochondrial membrane permeability transition pore, which in turn leads to disturbance of the mitochondrial membrane potential, however, pretreatment with meldonium contributed to a significantly inhibited excessive MPTP opening and MMP depolarization. Similar results were obtained in the acetazolamide-treated group. Furthermore, TEM imaging confirmed that pretreatment with meldonium significantly reduced cytoplasmic vacuolations, mitochondrial swelling with loss of internal cristae, and constricted intercellular space of synaptic under hypobaric hypoxia exposure, similar to the effect of acetazolamide (Fig. 2E). These data show that meldonium attenuated mitochondrial damage and the resultant oxidative stress during hypoxia challenge.

Pretreatment with meldonium improved energy metabolism under acute high-altitude exposure

Mitochondrial damage further affects energy metabolism. As expected, pretreatment with meldonium reversed acute hypoxia-induced reduction in ATP production (Fig. 3A). Mitochondrial respiration and glycolysis are the two main pathways by which cells produce energy. Next, to assess the response of pretreatment with meldonium to changes in glycolysis under acute hypoxia, we measured glycolytic capacity by the extracellular acidification rate (ECAR). Basal glycolysis, maximal glycolytic capacity, and reserve glycolysis were all up-regulated under 24 h hypoxia exposure, but pretreatment with meldonium significantly improved glycolytic function after hypoxia (Fig. 3B). Measuring the effects of hypoxia on the mitochondrial respiration function in primary hippocampal neurons revealed that mitochondrial oxygen consumption rate in the hypoxia group was significantly inhibited in basal OCR, maximal OCR, spare respiratory capacity, and ATP-linked, which indicated hypoxia-induced impaired mitochondrial function and reduced availability of mitochondrial oxidative phosphorylation substrates. However, pretreatment with meldonium substantially protected against these hypoxia-induced defects in mitochondrial respiration and improved non-mitochondrial respiration (Fig. 3C). This promotion of energy metabolism was significantly better than that of the positive control drug acetazolamide. The metabolic regulator hypoxia-inducible factor-1 (HIF-1α) and the metabolic enzymes, including glucose transporter-1 (GLUT1), monocarboxylate transporter 4 (MCT4) and recombinant pyruvate dehydrogenase α1 (PDHA1) in brain tissue by western blotting assays further noted that pretreatment with meldonium significantly increased the expression of HIF-1a, GLUT1 and PDHA1 while inhibiting the production of MCT4 (Fig. 3D). The above results show that meldonium exerts neuroprotection advantage through energy metabolism under acute hypoxia brain injury.

Identification of PGK1 as a direct meldonium-binding protein

To elucidate the underlying mechanism of neuroprotection conferred by meldonium under acute hypoxia, we screened for meldonium binding proteins using the HuProt™. The synthesized biotin-labeled meldonium reacted with recombinant proteins constructed on the HuProt™ (Fig. 4A). Proteins bound to biotin-meldonium or biotin were captured using Cy5-streptavidin (Fig. 4B). We then performed microarray analysis to gain a comprehensive understanding of the biological function of meldonium binding proteins. To further clarify the meldonium binding proteins, mass spectrometry analysis was performed following the pull-down assay with affinity streptomycin in primary hippocampal neuronal cells treated with biotin or biotin-meldonium (Fig. 4C). HuProt™ microarray and mass spectrometry screening results were enriched for analysis. The top 15 enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were mostly involved in energy metabolism, including carbon metabolism, glycolysis/gluconeogenesis, and pyruvate metabolism, indicating that meldonium binding proteins were primarily involved in metabolic processes (Fig. 4D). Meldonium binding proteins screened based on both methods showed that meldonium may bind to PGK1 (Coverage rate = 53%, Unipeptide = 20, Imean Ratio = 2.27, Table S3 and Additional file1: Fig. S3A).

PGK1, as an energy metabolism enzyme, plays a crucial role in the progression of acute hypoxia. We, therefore, considered the mechanism by which meldonium binds to PGK1. The SPR assay revealed that meldonium bound to PGK1 with an equilibrium dissociation constant of $9.72e^{-3}$ M at steady-state fit (Fig. 4E). Immunofluorescence showed co-localization of meldonium and PGK1 in the cytoplasm and nucleus (Fig. 4F). In addition,

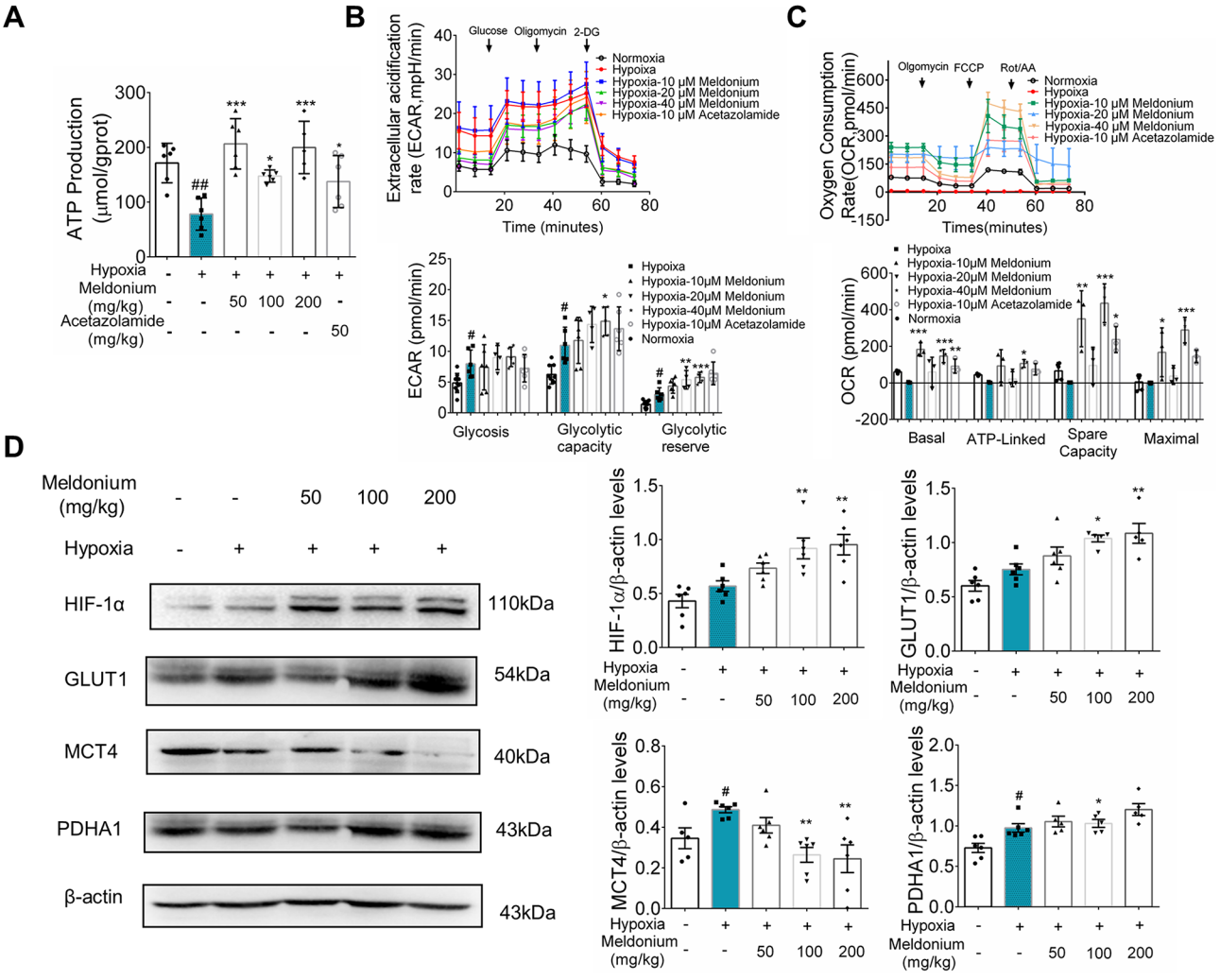

Fig. 3 Pretreatment with meldonium improves energy metabolism under acute hypoxia exposure. **A** Relative content of ATP Change under normoxia and hypoxia treatments in primary hippocampal neurons. $n=6$. **B** Analysis of ECAR in primary hippocampal neurons in normoxia and hypoxia to assess glycolysis by Seahorse XF-96 metabolic flux analyses. $n=6$. **C** Analysis of OCR in primary hippocampal neurons in normoxia and hypoxia to assess mitochondrial function by Seahorse XF-96 metabolic flux analyses. **D** Representative western blotting images and quantitative analysis in metabolic regulatory factor and enzymes. Data are presented as the mean ± SD. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ versus the normoxia group, * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus the hypoxia group by one-way ANOVA followed by Tukey's multiple comparison tests

molecular docking analysis (Molecular Operating Environment software, MOE) showed that meldonium may bind to Lys 132 in the pocket region of PGK1 with hydrogen and ionic bonds (Fig. 4G). We verified the interaction of meldonium with PGK1 and HIF-1α (an upstream protein of PGK1) using a pull-down assay under normoxia and hypoxia exposure (Fig. 4H); HIF-1α did not bind to meldonium, suggesting that meldonium binds directly to PGK1 instead of to upstream factors.

The binding mechanism of meldonium to PGK1 prompted us to examine how meldonium regulates PGK1 under acute hypoxia. We found that the 100 mg/kg and 200 mg/kg meldonium and the 20 μM meldonium group increased PGK1 expression in brain tissue and primary hippocampal neurons under acute hypoxia exposure, respectively (Additional file1: Fig. S3B-C). Furthermore, when primary hippocampal neurons were pretreated with meldonium (20 μM) for 24 h and then stimulated with hypoxia exposure for 6, 12, 24, 36, and 48 h, neurons pretreated with meldonium had significantly up-regulated PGK1 expression after ≥24 h of hypoxia (Fig. 4I). Elisa assay showed that PGK1 activity increased in a meldonium dose-dependent manner under acute hypoxia conditions (Fig. 4J). Taken together, these data show that meldonium may exert neuroprotective effects by directly targeting and activating PGK1.

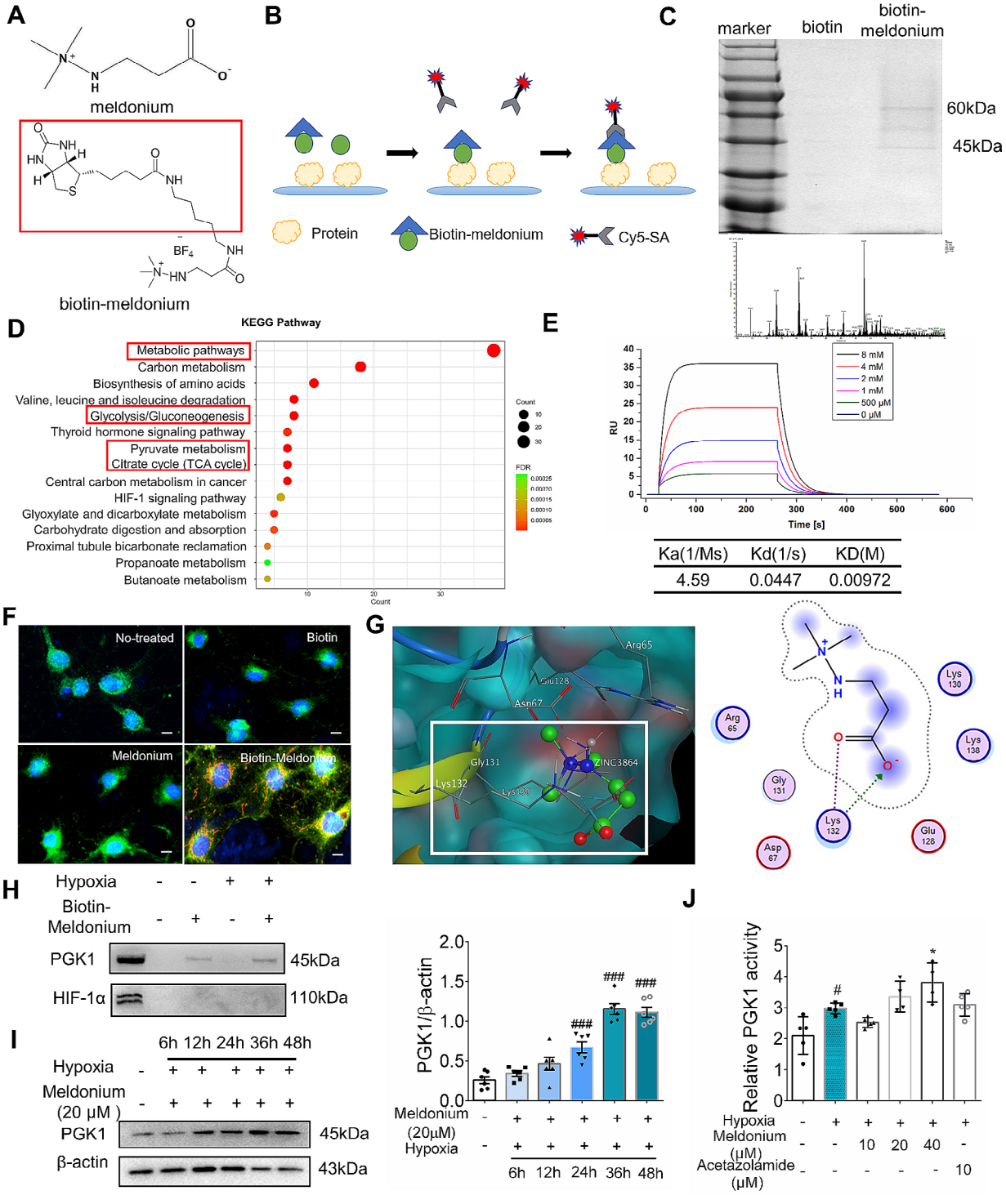

Fig. 4 Meldonium binds and activates PGK1 protein. **A** Molecular structure of meldonium and biotin-labelled meldonium (Biotin-meldonium). **B** Schematic representation of the detection of meldonium-binding proteins using the Human Proteome Microarray. **C** The processing of meldonium binding protein by pull-down and mass spectrometry analysis. **D** KEGG pathway of meldonium-binding proteins. **E** Concentration-response SPR experiment showing the binding of meldonium to PGK1. **F** Confocal microscope image showing colocalization of meldonium with PGK1 in cytoplasm and nucleus. **G** MOE virtual molecular docking indicates meldonium binds to lysine 132 of PGK1 pocket by hydrogen bond and ionic bond. **H** Western blotting validation of pull-down assay of PGK1 and HIF-1a binding in meldonium treatments. **I** Representative immunoblotting images and quantitative analysis of PGK1 exposed to hypoxia for 6, 12, 24, 36 and 48 h in primary hippocampal neurons $n = 6$. **J** The PGK1 enzyme activity in primary hippocampal neurons under hypoxia exposure was detected by ELISA ($n = 5$). Data are expressed as mean ± SD. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ versus the normoxia group; * $P < 0.05$ versus the hypoxia group

Meldonium promoted energy metabolism by activating PGK1

Although up-regulation of the glycolytic pathway has been reported to benefit cell survival, whether meldonium affects energy metabolism by activating PGK1 has yet to be further elucidated. We first assessed the effect of meldonium on NADH, the immediate downstream product of PGK1, and changes in glucose uptake and lactate production in the glycolysis pathway where PGK1 is located after knockdown of PGK1 (Additional file1: Fig. S4). The upregulated expression of NAD^+/NADH in the hypoxia group was reversed after PGK1 knockdown, while the group pretreated with meldonium had up-regulated the expression of NAD^+/NADH after PGK1 knockdown (Fig. 5A). Moreover, pretreatment with meldonium promoted glucose uptake while reducing lactate overproduction after PGK1 knockdown under hypoxia (Fig. 5B). Considering that activation of PGK1 also promotes ATP production, the alteration in ATP content of primary hippocampal neurons under acute hypoxia exposure was evaluated. Total cellular ATP level was significantly reduced under hypoxia relative to the normoxia group. Notably, the group pretreated with meldonium simultaneously improved ATP production in glycolysis and mitochondrial oxidative phosphorylation after PGK1 knockdown and hypoxia (Fig. 5C).

To further ascertain the effect of meldonium-mediated PGK1 on energy metabolism under hypoxia, mitochondrial respiration function and glycolytic stress following PGK1 knockdown using the Seahorse XF96 Bioanalyzer were analyzed. As shown in Fig. 5D, acute hypoxia significantly decreased glycolysis, glycolytic capacity, and glycolytic reserve after PGK1 knockdown, while pretreatment with meldonium significantly limited glycolytic

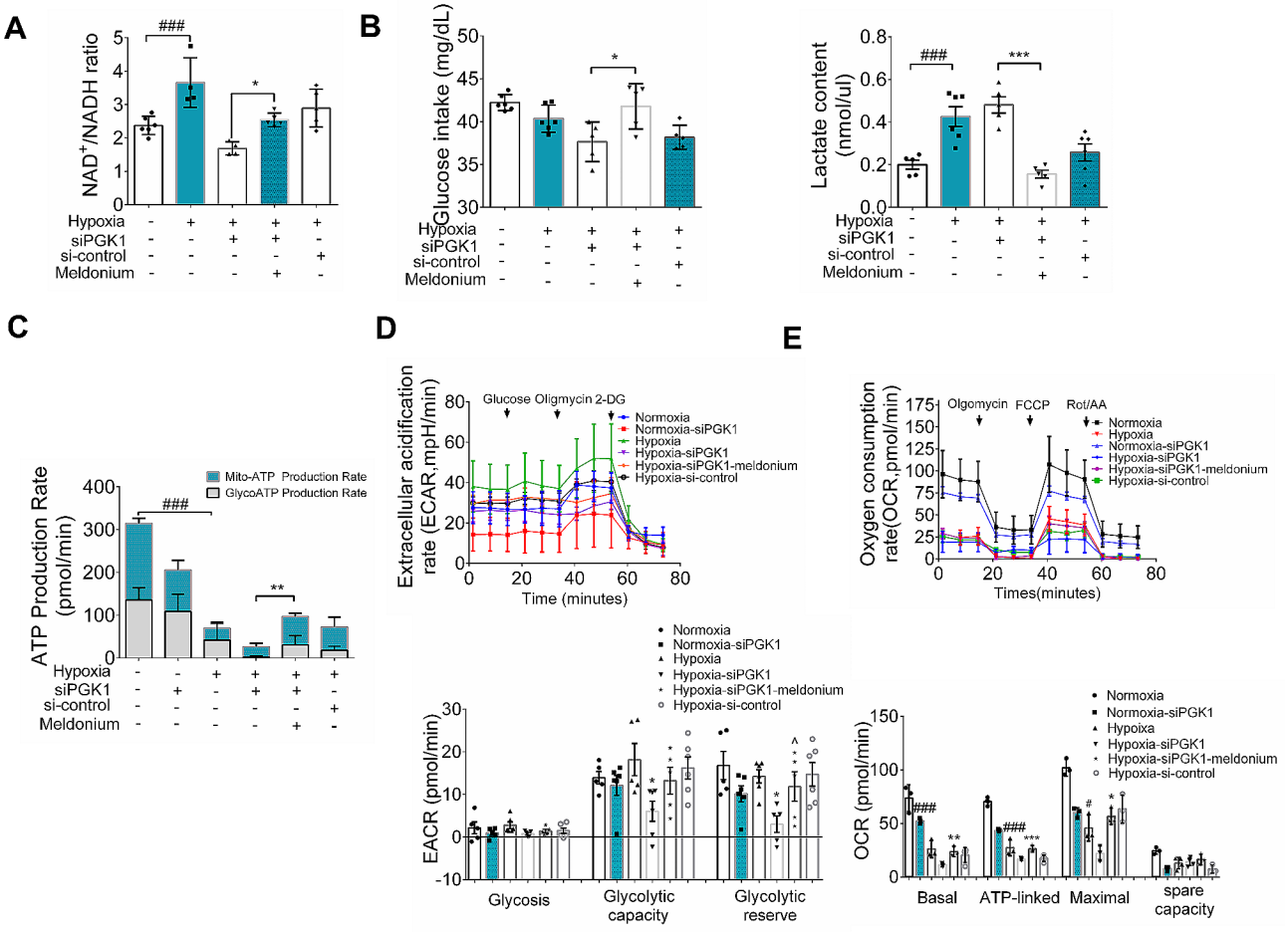

Fig. 5 Pretreatment with meldonium reverses PGK1 knockdown and hypoxia-elicited energy metabolism disorder in vitro. Primary hippocampal neuron cells were treated under hypoxia exposure and/or transfected with siPGK1, followed by measures of **A** the NAD^+/NADH ratio; **B** Glucose uptake and lactate content; **C** ATP level; $n = 4–6$. **D** Analysis of ECAR in primary hippocampal neurons transfected with control and siPGK1 to assess glycolysis, glycolytic capacity, and glycolytic reserve. $n = 5–6$. **E** Analysis of OCR in primary hippocampal neurons transfected with control and siPGK1 to assess rates of basal respiration, maximal respiration, respiration linked to ATP production and spare respiratory capacity. $n = 3–4$. Data are expressed as mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus the normoxia group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus the siPGK1 group, $^{\wedge}P < 0.05$ versus the hypoxia group by one-way ANOVA followed by Tukey's multiple comparison tests

reserve reduction and tended to up-regulate expression in glycolysis and glycolytic capacity. In agreement with an increase in glycolytic activity, overall mitochondrial respiration, as measured by OCR, increased in primary hippocampal neurons. As quantified in Fig. 5E, after PGK1 knockdown, basal respiration, ATP-linked, and maximal respiration are all significantly increased under acute hypoxia in the group pretreated with meldonium. These results suggest that meldonium targeted PGK1 promoted glycolysis while improving mitochondrial respiratory function under acute hypoxia.

Meldonium ameliorated mitochondrial injury by PGK1 translocating to mitochondrial and interacting with TRAP1

To explore the possible neuroprotective mechanism of PGK1 activated by meldonium, the protein-protein interaction (PPI) network of PGK1 was constructed by STRING and Cytoscape data sorting of the proteins screened by meldonium mass spectrometry. Bioinformatics analysis showed that PGK1 interacts with some proteins from the heat shock protein family (Fig. 6A and Table S4). TRAP1 is a mitochondrial homolog of the heat shock chaperone family, which protects cells from oxidative stress and maintains mitochondrial integrity. We further assessed the relationship between PGK1 and TRAP1. Primary hippocampal neuron cell lysates were immunoprecipitated with isotype IgG or anti-PGK1 antibody, followed by western blotting analysis with TRAP1 antibody, confirming the endogenous interaction between PGK1 and TRAP1 (Fig. 6B). The direct interaction between His-PGK1 and GST-TRAP1 was further confirmed with a pull-down assay and immunoblotting (Additional file1: Fig. S5A). Moreover, we verified the binding of PGK1 lysine 322 sites to TRAP1 glutamate 407 sites with hydrogen bonds and ionic bonds by MOE computer virtual docking technology (Fig. 6C and Table S5).

Studies have shown that PGK1 undergoes mitochondrial translocation in certain cancer progressions, which in turn affects glycolytic activity and downstream reactions [36]. The interaction between PGK1 and TRAP1 prompted us to verify the possible role of PGK1 in mitochondria. Mitochondrial fluorescent probe tracking in neuronal cells revealed that PGK1 was translocated to mitochondria under acute hypoxia exposure (Fig. 6D) and co-localized with TRAP1 (Additional file1: Fig. S5B). The results of the protein levels showed that PGK1 was significantly up-regulated in mitochondria protein of brain tissue and primary hippocampal neurons (Fig. 6E). Given this activated PGK1 in mitochondria and the co-localization of PGK1 and TRAP1, we sought to determine the response of meldonium to mitochondrial membrane potential after the overexpression of PGK1. The results showed that hypoxia-induced mitochondrial membrane depolarization in PGK1 overexpressing cells is still better ameliorated by meldonium pre-treatment as compared to non-treatment group (Fig. 6F). To better quantify the effects of meldonium modulation of PGK1 on the TRAP1 signaling pathway, we performed an immunoblot analysis which suggested that pretreatment with meldonium could promote the expression of PGK1 and TRAP1, and reduce the overexpression of the downstream factors, cyclophilin-D (CyP-D), cytochrome C (Cyt-C). and caspase 3 in hypoxia (Fig. 6G). Collectively, these data confirmed that meldonium pretreatment significantly inhibited hypoxia-induced mitochondrial damage in primary hippocampal neurons by modulating PGK1 interaction with TRAP1 after PGK1 hypoxia translocation.

Discussion

In this study, we identified a previously unexplored role of meldonium in regulating acute high-altitude brain injury. Although changes in energy metabolism have been noted upon meldonium treatment, the mechanisms and underlying targets of meldonium remains elusive. Here, we make several key observations, summarized in Fig. 7. We demonstrated in a constructed simulated acute high-altitude hypobaric hypoxia brain injury model in vivo and in vitro that pretreatment with meldonium significantly ameliorated oxidative stress response and mitochondrial dysfunction, and prompted energy metabolism. Using human protein chips, mass spectrometry, SPR, MOE docking, and pull-down techniques, we show that meldonium may activate PGK1 to enhance glycolysis and pyruvate metabolism. Moreover, PGK1 transfers to mitochondria under acute hypoxia where it interacts with the molecular chaperone TRAP1, and pretreatment with meldonium promotes PGK1/TRAP1-regulated mitochondrial dysfunction.

Currently, available preventive and therapeutic options for acute high-altitude sickness preclude long-term application due to non-specificity and side effects [4, 37]. For example, the carbonic anhydrase inhibitor acetazolamide not only has gastrointestinal reactions but also risks of liver and kidney damage; the steroidal drug, dexamethasone, the main drug used to treat HACE, can have teratogenic effects. Thus, it is critical to develop prevention drugs with fewer side effects against hypobaric hypoxia-induced brain injury. Human pharmacokinetic data show that meldonium has good efficacy and safety in cardiovascular and cerebrovascular diseases [38]. As a well-known anti-myocardial ischemia drug, meldonium can improve renal and liver ischemia-reperfusion (I/R) injury in male Wistar rats and subarachnoid hemorrhage of the brain in New Zealand rabbits [31, 39]. Consistent with earlier research [40], our current experiment also observed that meldonium restored the reduction in CBF (cerebral blood flow is disrupted due to impaired autoregulation

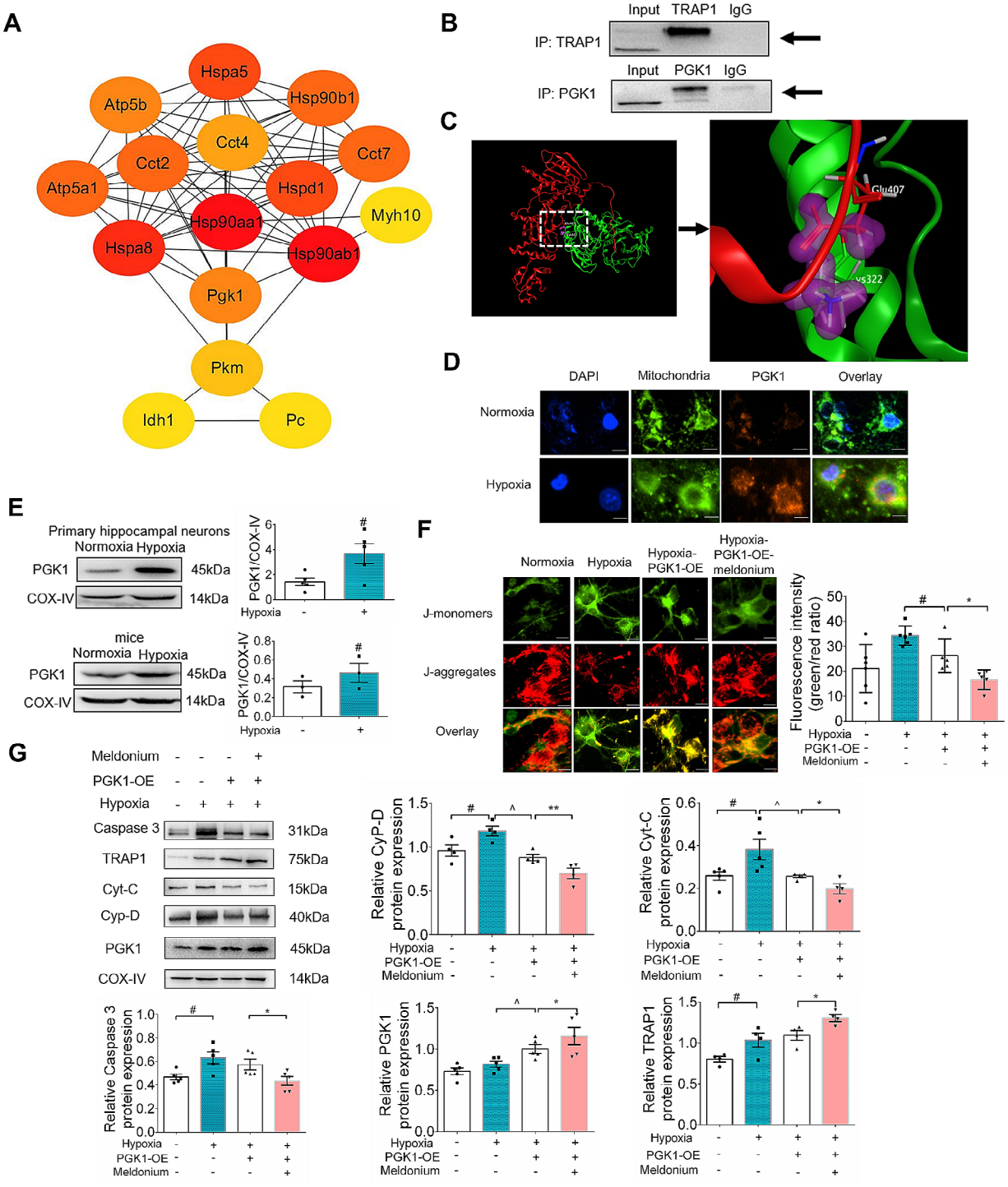

Fig. 6 Meldonium regulates mitochondrial function via the PGK1/TRAP1 signaling pathway. **A** Biological interaction networks of PGK1 for the top 15 connections. **B** Co-IP between PGK1 and TRAP1 in primary hippocampal neurons was detected by western blotting. **C** Molecular docking of PGK1 active site (PDB-ID: 4O33) and the ligand binding site of TRAP1 (PDB-ID: 5HPH) was performed in MOE. **D** Primary hippocampal neurons were stimulated with or without hypoxia for 24 h and stained with an anti-PGK1 antibody, Mito-Tracker, and DAPI. **E** Mitochondria fractions were prepared from primary hippocampal neurons and mice brain tissue after hypoxia for 24 h and then PGK1 protein expression was analyzed using western blotting. $n = 3–5$. **F** Representative fluorescence images and quantitative analysis of MMP after primary hippocampal neurons were treated with siPGK1. Red fluorescence represents JC-1 aggregates in normal physiological state, while green fluorescence represents JC-1 monomers indicating mitochondrial depolarization. $n = 6$. **G** Representative images and quantitative analysis of the immunoblots of PGK1, TRAP1, CyP-D, Cyt-C, Caspase 3, and COX-IV were detected using western blotting analysis in primary hippocampal neurons cells treated with PGK1 overexpression (PGK1-OE). $n = 4–6$. Data are expressed as mean ± SD. $^{\#}P < 0.05$ versus the normoxia group; $^{\wedge}P < 0.05$ versus the hypoxia group. * $P < 0.05$, ** $P < 0.01$ versus the PGK1-OE group

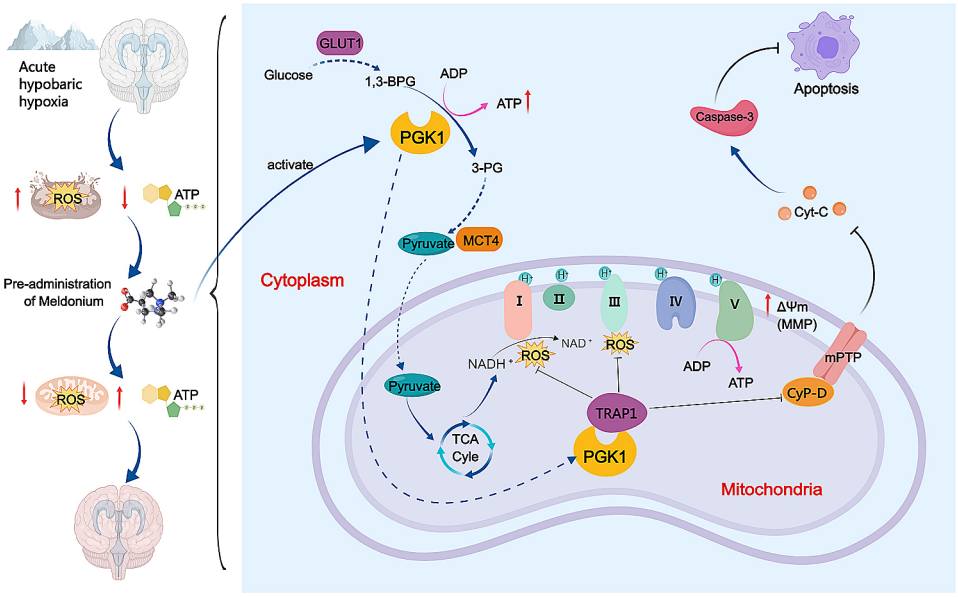

Fig. 7 A graphic illustration of the mechanism by which meldonium ameliorates acute high-altitude brain injury

during hyperventilation in hypoxia), thereby confirming the protective effect of meldonium on cerebrovascular function. Our previous studies have shown that meldonium alleviates acute high-altitude lung injury [41]. Thus, meldonium could be a potential prevention drug for acute hypobaric hypoxia-induced brain injury. Studies have reported that the hippocampal CA3 area neurons are highly susceptible to insults such as ischemia, inflammation, hypoglycemia, or excitotoxicity [42–44]. In this study, we observed that meldonium significantly inhibited hippocampal CA3 area cell swelling and rupture, disintegrating Nissl bodies, similar to that observed in the positive control drug acetazolamide treatment group. In addition, results of in vitro studies also showed that meldonium significantly alleviated neuronal morphology and restored neuronal firing in hypoxia, which was superior to acetazolamide. This could be due to meldonium preserving neuronal activity in low-oxygen conditions by obstructing the synthesis of carnitine. And the sustaining neuronal activity aids in the cerebrovascular control of cerebral blood flow. Consequently, these findings reveal for the first time that meldonium acts as a strong neuroprotective compound, ameliorating brain histopathological harm due to acute plateau hypobaric hypoxia.

In most instances, factors such as low pressure, hypoxia, fast-forwarding, and poor physical fitness can induce high-altitude brain injury. Available evidences suggest that acute hypoxia not only induces apoptosis, but also affects mitochondrial dysfunction and its secondary damage [45–47]. Mitochondria, as biosynthetic sites and energy generators, are sensitive to intracellular oxygen interference and produce ROS in the process of biological oxidation and energy conversion [48, 49]. When there is an imbalance between the production of ROS and the body's antioxidant defense system, mitochondrial oxidative stress occurs. Consistent with previous reports, we detected a dramatic increase in ROS in acute hypoxia-exposed brain tissues and primary hippocampal neuronal cells, accompanied by the disruption of mitochondrial structure and function. Notably, meldonium can significantly reduce the hydroxyl free radicals and lipid peroxides MDA, increase the activity of antioxidant enzymes (SOD and GSH-Px) in brain tissue, and maintain free radical metabolism homeostasis in the body. Moreover, the intuitive observation of ROS and their source (mitochondrial respiratory chain complex I and III) from primary hippocampal neuronal cells supports previous work on the antioxidant effects of meldonium. Oxidative stress promotes the opening of the MPTP. Once the opening of MPTP is triggered, it allows molecules smaller than 1.5 kDa to cross mitochondrial membrane, leading to respiratory chain uncoupling and MMP dissipation, followed by progressive mitochondrial swelling and outer membrane rupture, ultimately resulting in metabolic disturbance [48, 50]. Of note, pretreatment with meldonium significantly protected against impaired mitochondrial function under hypobaric hypoxia by improving membrane potential and decreasing in the MPTP opening threshold. These results suggest that meldonium may rescue mitochondrial function and prevent excess ROS generation, a potentially critical intervention for interrupting subsequent energy metabolism disorders.

Mitochondria play a pivotal role in the maintenance of acute high-altitude brain injury as a major regulator of redox homeostasis and a center of cellular metabolism

[51]. Based on the evidence of structural and functional protection of mitochondria in acute hypoxia conditions by meldonium, we propose that meldonium may also respond to energy metabolism. In support of this hypothesis, we report changes in extracellular acidification rate and oxygen consumption in primary hippocampal neurons following hypoxia, with significant increases in various parameters of compensatory glycolysis, glycolytic capacity, and reserve capacity after meldonium prophylaxis administration. In addition, meldonium maintained basal respiratory function and maximum capacity of the mitochondrial electron transport system and maintained ATP conversion in primary hippocampal neurons under acute hypoxia. These results further demonstrate that meldonium not only enhances glycolysis as described in previous studies [26], but also promotes mitochondrial oxidative phosphorylation. Studies reported that as a transcription factor, the activation of HIF-1α may be involved in the regulation of energy metabolism [52–55]. Our investigation of HIF-1α and related metabolic enzymes (GLUT1, MCT4, and PDHA1) in glycolysis and oxidative phosphorylation pathways further illustrates the above changes. We tentatively hypothesized that the protective effect of meldonium against hypoxia brain injury might be through the activation of HIF-1α signaling pathway to regulate energy metabolism.

To decipher mitochondrial damage and metabolism mechanism of meldonium as prophylactics against acute high-altitude brain injury, HuProt™ protein chip analysis combined with mass spectrometry was performed. We found that meldonium interacts with a variety of energy metabolism-related proteins. Based on the above evidence, we identified PGK1 as a potential target of meldonium. The SPR analysis showed that meldonium binds to recombinant PGK1 protein. Furthermore, immune colocalization and MOE virtual molecules docking verified the location and site of their binding. To note, the pull-down assay confirmed that meldonium binds directly to PGK1 without binding to the upstream factor HIF-1α. This further validates the above hypothesis. Based on our results, meldonium appears to act as a PGK1 activator. This view is supported by the fact that meldonium improved the protein expression in brain tissue and primary hippocampal neurons and promoted PGK1 enzyme activity under acute hypoxia. To confirm the hypothesis that meldonium affects energy metabolism through PGK1 activation under acute hypoxia exposure, we assessed glycolysis, mitochondrial oxidative phosphorylation, and the overall ATP levels. By treating acute hypoxia-exposed primary hippocampal neuronal cells with PGK1 knockdown or in combination with meldonium, we found that meldonium restored energy homeostasis to similar levels. Pretreatment with meldonium to enhance glycolysis after PGK1 knockdown validates previous studies that meldonium may promote anaerobic oxidation of glycolysis [50]. Of note, in terms of ATP production from mitochondrial oxidative phosphorylation levels, prophylactic administration with meldonium also ameliorated the damage to mitochondrial respiratory function after PGK1 knockdown and promoted mitochondrial oxidative phosphorylation. Undeniably, while PGK1 activity plays a crucial role, it may not be the solely explanation for the neuroprotective effects of medonium. A comprehensive comparison of the effects of meldonium on hypoxia and the effects of meldonium after PGK1 knockdown may provide a more complete picture of the effects of meldonium on high-altitude sickness.

PGK1, as an essential enzyme of glycolysis, not only participates in the regulation of energy metabolism but also acts as a protein kinase to regulate apoptosis, autophagy, ion channels, etc., according to the emerging evidence [56, 57]. This prompted us to further explore how meldonium regulates mitochondrial functions through the activation of PGK1. The protein-protein interaction analysis showed that the heat shock protein family interacted with PGK1. TRAP1 is known as a mitochondrial homolog of the heat shock family, and its ATPase activity acts as a molecular chaperone activity in an active state and interacts with other proteins. TRAP1 is also involved in the regulation of oxidative stress and mitochondrial bioenergetics [58]. Consistent with our hypothesis, TRAP1 demonstrated binding to PGK1. PGK1 has been shown in previous studies to be regulated by the tumor microenvironment and possibly translocated to mitochondria to affect pyruvate metabolism. We found that mitochondrial translocation of PGK1 may also be induced under acute hypoxic stress. Through immunofluorescence, we further verified that PGK1 and TRAP1 co-localized in mitochondria and participated in the mitochondrial damage pathway regulated by TRAP1. Meanwhile, meldonium ameliorates TRAP1 pathway-regulated mitochondrial damage after PGK1 mitochondrial translocation. In this regard, this is the first of meldonium-afforded PGK1-dependent energy metabolism. PGK1 activation has also been shown to interact with TRAP1 in acute hypoxia to participate in mitochondrial injury protection.

In summary, we first established that meldonium plays a neuroprotective role against acute high-altitude brain injury. Mechanistically, meldonium activates PGK1 to promote energy metabolism, and on the other hand, it ameliorates mitochondrial damage by PGK1 translocating to mitochondria and regulating TRAP1 molecular chaperones activity in response to acute hypoxia, thereby improving pathological injury and survival rate in hypoxia stress. Therefore, this study not only provides insight into the mechanism of meldonium as an energy optimizer but also identifies a novel and potential

preventive drug to rapidly prevent acute high-altitude brain injury.

Abbreviations

SPR	Surface plasmon resonance
PGK1	Phosphoglycerate kinase 1
TRAP1	Tumor necrosis factor receptor-associated protein 1
ROS	Reactive oxygen species
GLUT1	Glucose transporter 1
MCT4	Monocarboxylate transporter 4
RCBF	Regional cerebral blood flow
MMP	Mitochondrial membrane potential
MPTP	Mitochondrial permeability transition pore
KEGG	Kyoto encyclopedia of genes and genomes
ECAR	Extracellular acidification rate
OCR	Oxygen consumption rate
MAP2	microtubule-associated protein-2
H&E	Hematoxylin-eosin

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12964-024-01757-w.

Supplementary Material 1

Supplementary Material 2

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Not applicable.

Author contributions
L.Y. and W.Y.A. conceived the presented idea. L.F.Y., H.H.H., Y.W.J., and W.D.H. performed the experiments and interpreted the data. S.X., S.Y.Y., W.S., Y.Y., X.Z.H. and Y.J. supervised this work. L.F.Y. wrote the manuscript. L.Y. and W.Y.A. contributed to funding acquisition. L.Y. and L.F.Y. revised the manuscript critically. All authors discussed the results and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All our animal experiments followed the Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Beijing Institute of Pharmacology and Toxicology (Permit Number: NBCDSER-IACUC-2018-096). This study does not contain human participants.

Consent for publication
Not applicable.

Competing of interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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