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Mechanisms of propofol attenuation of ketamine-induced neonatal brain injury

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Abstract. – OBJECTIVE: We studied the mechanisms of protective effects of propofol on ketamine-induced damage to neonatal cognitive function.

MATERIALS AND METHODS: We utilized a rat model of ketamine anaesthesia. Eighty neonatal rats (7 days after birth) were divided into four groups: normal saline group, ketamine group, and low- and high-dose propofol combined with ketamine groups. Six hours after anaesthesia, we obtained hippocampal tissue, and quantified apoptotic index and total protein concentration, and assessed global proteomics changes induced by two tested drugs. The latter changes were documented by two-dimensional electrophoresis and matrix-assisted laser desorption/ ionization time of flight mass spectrometry. To evaluate cognitive functions, water maze test was applied after animals grew for 21 days. We further repeated proteomics studies at 21 days post-anaesthesia.

RESULTS: Ketamine markedly up-regulated apoptotic index and decreased total protein concentration. Propofol dose-dependently reverted these adverse changes. Six hours post-anaesthesia, combined propofol and ketamine administration up-regulated the following proteins in the hippocampus: PD1A3, NDUFB10, HSPA8, ATP5JD, and PSMA1. Furthermore, the following proteins were down-regulated: PPIA, PKM2, GFAP, NSE, PPIA, PKM2, and GFAP. After 21 days, animals treated with ketamine showed marked disturbances in cognitive function as demonstrated by increased time of the water maze test, whereas propofol diminished these changes. In addition, expression of proteins largely normalized in propofol-treated animals, with only two up-regulated proteins (FUBP3 and PRDX5) and three down-regulated proteins (GAPDH, AKR1A1, and VCP).

CONCLUSIONS: Adverse effects of ketamine on cognitive function are reverted by propofol, also through beneficial effects on protein expression in the hippocampus.

Key Words:
Propofol, Brain injury, Rats, Proteomics.

Introduction

Ketamine is an intravenous anaesthetic with strong analgesic effects and light respiratory inhibition[1-3]. It is usually administered to paediatric patients or for basal general anaesthesia[4-6]. Propofol is usually administered as a hypnotic/amnestic agent with rapid action, no accumulation and complete revival[7-10]. Ketamine can cause brain injury in the developmental phase and cause cognitive dysfunction. Propofol, on the other hand, possesses brain-protective effects. With propofol, post-operative recovery is fast, and it can alleviate the ketamine injury to neonatal neurons and decrease the damage to cognitive function. Specific mechanisms of this protective effect are not clear[11-15]. Here, we used a proteomics approach to evaluate the changes in the hippocampal tissue after ketamine anaesthesia in neonatal rats, with or without co-administration of propofol.

Materials and Methods

Animals

Eighty 7 days old male and female SD rats were used in this study. Their body weight ranged from 13 to 19 grams. The rats were SPF grade animals.

Equipment

Inverted phase contrast microscope, solid phase pH gradient isoelectric focusing apparatus, ETTAN ImageMaster 2D Elite 4.01 gel image analysis software (GE Healthcare, Amersham, UK), and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF) fingerprint detection system were from Amersham Pharmacia Biotech (Tokyo, Japan). The apoptosis kit was purchased from Beijing CellChip Biotechnology Co., Ltd. (Beijing, China). The Morris

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water maze was made by Chengdu Taimeng Technology Co. (Chengdu, China).

Study Groups

Rats were divided into the following four groups: (1) animals treated with normal saline group, (2) animals that received ketamine, and (3 and 4) animals that received low- or high-dose propofol along with ketamine. Each group comprised 20 animals. Ketamine and propofol were used at 80 mg/kg weight. Both anaesthetics were administered by intraperitoneal injection: 0.5 ml propofol and 1 ml ketamine were given to animals in the low-dose group, whereas animals in the high-dose group received 1 ml of propofol and 1 ml of ketamine. Animals in the normal saline group received an intraperitoneal injection of 1 ml of normal saline. The drugs were administered 3 times every 2 hours. Ten rats were randomly selected from each group for the collection of brain tissue 6 hours after the anaesthesia. The remaining animals were kept for 21 days after anaesthesia to conduct the water maze test. These rats were then euthanized for collection of brain tissue. This study received the animal ethics approval from the Ethics Committee.

Specimens and Outcomes

The cortex was separated, and the hippocampus was collected for paraffin embedding. Paraffin sections were prepared. Neuron apoptosis was detected according to the protocol of the assay kit. Cell apoptosis detection kit was used to detect the apoptosis of hippocampus neurons.

In other 10 rats, hippocampus proteins were prepared as follows. Fresh brain tissue was collected, and the cortex and hippocampus were rapidly separated. The tissue was washed with distilled water, and excess fluid was absorbed using a filter paper. Tissue specimens were preserved in liquid nitrogen and subsequently homogenized at low temperature. Total proteins were extracted with lysis buffer and kept at -80° C pending analyses.

The 2D Quant protein quantification kit was used to assess protein concentration. Proteins were separated using 2D gel electrophoresis, and MALDI-TOF fingerprint detection system, database searches, and Western blot analyses were used to identify differentially expressed proteins.

Statistical Analysis

Data were analyzed using SPSS16.0 (SPSS Inc., Chicago, IL, USA). Numerical data were presented as mean ± SD and analyzed by the *t*-test. Animal groups were compared with the one-way ANOVA analysis and LSD test. The $p < 0.05$ was considered as statistically significant.

Results

Apoptosis of Hippocampal Neurons

Apoptotosis index of hippocampal neurons in the group treated with normal saline was 3.71 ± 2.12%. Treatment with ketamine significantly increased the number of apoptotic cells (14.98 ± 5.65%, $p < 0.01$ vs. normal saline group; Table I). Propofol dose-dependently decreased cell apoptosis caused by ketamine (Table I).

Protein Concentration in Hippocampal Tissue

Treatment with ketamine significantly decreased protein concentration in hippocampal tissue compared with treatment with normal saline (Table II). However, treatment with propofol dose-dependently abrogated damaging effects of ketamine (Table II).

Modulation of Protein Expression by Treatment with Propofol in Combination with Ketamine

To detect global protein changes, we used a proteomics approach and evaluated expression of hippocampal proteins at the time point of 6 hours post-anaesthesia (Table III). There were up-regu-

Table I. Apoptosis of hippocampal neuronal cells.

	Number of animals	Apoptosis index
Normal saline group	10	3.71 ± 2.12
Ketamine group	10	14.98 ± 5.65*
Low-dose propofol + ketamine group	10	10.23 ± 4.82$^{*\#}$
High-dose propofol + ketamine group	10	6.79 ± 6.48$^{*\#}$

Data are mean ± SD. $p < 0.05$ vs. normal saline group $^{*}p < 0.01$ vs. normal saline group; $^{\#}p < 0.05$ vs. ketamine group.

Table II. Protein concentration in hippocampus tissue.

	Number of animals	Protein concentration in the hippocampus (μg/μl)
Normal saline group	10	2.11 ± 0.32
Ketamine group	10	1.78 ± 0.28*
Low-dose propofol + ketamine group	10	1.88 ± 0.27
High-dose propofol + ketamine group	10	1.93 ± 0.21

Data are mean ± SD. *$p < 0.05$ vs. normal saline group.

lated proteins including PD1A3, NDUFB10, HSPA8, ATP5JD, and PSMA1, as well as down-regulated proteins (PPIA, PKM2, GFAP, NSE, PPIA, PKM2 and GFAP). Proteomics changes on day 21 post-anaesthesia were more modest and included up-regulation of FUBP3 and PRDX5, and down-regulation of GAPDH, AKR1A1 and VCP (Table III).

Water Maze Test

The next test assessed changes in cognitive function in study animals. We observed that animals that were exposed to ketamine anaesthesia showed a significantly increased time of crossing the annulus compared with animals exposed to normal saline (Table IV). These negative effects of ketamine anaesthesia were reverted by propofol (Table IV), such that the parameters in both propofol-treated groups became comparable to those in the normal saline group.

Discussion

The hippocampus is the brain region participating in cognitive function [16-19]. Ketamine is the non-competitive antagonist of N-methyl-D-aspartate (NMDA) receptor which is mostly expressed in the brain cortex and hippocampus. Ketamine affects the NMDA receptor-mediated CaMKs-ERK-EIKI/CREB-LTP signaling pathway, thereby negatively modulating learning and memory functions[20-23]. Ketamine can also cause brain damage by inducing apoptosis in neural cells, and this contributes to the damage of short-term and long-term cognitive functions[24]. Ketamine can be applied independently or combined with other anesthetics. Because it has respiratory inhibition, respiration stimulant is not recommended. Ketamine is not recommended during the operation on pharynx, larynx or bronchus, and muscle relaxant must be administered. Ketamine has some adverse effects (hallucinogen, schrute dependence, etc.) which act through the inhibition of NMDA receptor, and propofol can attenuate these effects. Thus, to reduce the adverse effects, the stimulus must be avoided, and short-term effect barbital can be administered. Propofol can inhibit up-regulation of caspase-3 induced by ketamine and, thereby, block the ketamine-induced neuron apoptosis[25]. Propofol further decreases the cerebral metabolic rate of oxygen and intracranial pressure, diminishes lipid peroxidation, blocks the transduction pathway of glutamic acid, inhibits calcium overload, prevents the cell injury[26], and reduces free radical formation[27].

In our study, we observed that young animals exposed to ketamine anaesthesia exhibited increased apoptosis indexes in hippocampal tissue. This significantly affected the cognitive function of these animals, as demonstrated by the water maze test. However, these adverse effects of ketamine were reverted by co-administration of

Table III. Changes in protein expression induced by propofol in combination with ketamine.

Time post-anaesthesia	Up-regulated proteins	Down-regulated proteins
6 hours	PD1A3, NDUFB10, HSPA8, ATP5JD, PSMA1, isoform-CRA -c	PPIA, PKM2, GFAP, NSE, SYN1
21 days	FUBP3, PRDX5	GAPDH, AKR1A1, VCP, TUBULIN A1 B

Table IV. Water maze test.

Time	1 day	2 days	3 days	Time to cross the annulus
Normal saline group	102 ± 29	89 ± 13	54 ± 14	6.1 ± 2.2
Ketamine group	113 ± 21	101 ± 19	99 ± 17*	1.4 ± 1.3*
Low-dose propofol + ketamine group	107 ± 22	95 ± 18	80 ± 21*#	3.3 ± 1.9*#
High-dose propofol + ketamine group	98 ± 19	87 ± 17	63 ± 16¶	4.6 ± 1.7

Footnote: Data are mean ± SD. *$p < 0.01$ vs. normal saline group; #$p < 0.05$ vs. ketamine group; ¶$p < 0.5$ vs. low-dose propofol + ketamine group.

propofol, especially when the latter was co-administered at a high dose.

Propofol also diminished protein loss induced by ketamine. On a global scale, using 2D gel electrophoresis, we observed that propofol modulated protein expression in the hippocampus 6 hours post-administration, such as up-regulated expression of PD1A3, NDUFB10, HSPA8, ATP5JD and PSMA1, and down-regulated expression of PPIA, PKM2, GFAP, NSE, PPIA, PKM2 and GFAP. After 21 days, there were two up-regulated proteins (FUBP3 and PRDX5) and four down-regulated proteins (GAPDH, AKR1A1, VCP and TUBULIN A1 B) in animals treated with ketamine and propofol. Ketamine anaesthesia is known to cause down-regulation of synaptic vesicle transport proteins SYNI and DNMr. Down-regulation of SYNI affects the transport efficiency of the synapse, whereas down-regulation of VCP after 21 days both affects the protein degradation by the ubiquitin-proteasome pathway and inhibits the apoptotic activity of Nuclear Factor-κB [28]. Furthermore, 6 hours post-anaesthesia, down-regulation of NSE, PKMZ and AfPSB affects the aggregation of protein microtubulin, which is not beneficial for the energy metabolism. An overdose of ketamine can cause respiratory inhibition, mechanical ventilation or artificial respiration should be applied instead of respiration stimulant. Muscle relaxant must be added during the operation on pharynx, larynx or bronchus. Besides, some patients have nausea, vomiting or even illusion, sometimes complicated with delirium and agitation. To reduce these adverse effects, the outside stimulus must be avoided, and short-term effect barbital can be administered. However, after 21 days, beneficial mechanisms of propofol led to normalization of expression of most of these proteins, even though continuing down-regulation of VCP and TUBULIN A1 B could not be advantageous to protein stability and may still cause the neuron apoptosis.

Conclusions

Adverse effects of ketamine on cognitive function are reverted by propofol, also through beneficial effects on protein expression in the hippocampus.

Funding

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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