European Review for Medical and Pharmacological Sciences | 2018; 22: 8013-8020

Histamine H3 receptor antagonist Clobenpropit protects propofol-induced apoptosis of hippocampal neurons through PI3K/AKT pathway

W. HE[1], Q.-H. YUAN[2], Q. ZHOU[3]

[1]Department of Anaesthesiology, Mianyang Central Hospital, Mianyang, China
[2]Department of Pathology, Mianyang Central Hospital, Mianyang, China
[3]Department of Anaesthesiology, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, Chengdu, China

Abstract. – OBJECTIVE: The aim of the study was to explore whether histamine H3 receptor antagonist Clobenpropit could protect propofol-induced neurotoxicity in hippocampal neurons.

MATERIALS AND METHODS: Hippocampal neurons were extracted from neonatal rats and induced with propofol. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was performed to detect apoptotic rate of neurons. Western blot was conducted to detect protein levels of cleaved-caspase-3 and Bax/Bcl2. After LY294002 treatment, the PI3K pathway antagonist was applied in neurons, protein levels of cleaved-caspase-3 and Bax/Bcl2 were detected by Western blot as well.

RESULTS: Propofol treatment remarkably induced neuronal apoptosis. Clobenpropit alleviated cell apoptosis induced by propofol. Protein expressions of cleaved-caspase-3 and Bax/Bcl2 were remarkably downregulated in neurons treated with Clobenpropit. LY294002 induction remarkably reverses the protective role of Clobenpropit in neuronal apoptosis, manifesting as downregulated PI3K and p-AKT after LY294002 treatment.

CONCLUSIONS: Clobenpropit protects propofol-induced neuronal apoptosis through activating PI3K/AKT pathway.

Key Words:
Clobenpropit, Propofol, PI3K/AKT, Apoptosis.

Introduction

With the development of surgical techniques, the number of infants, children, and pregnant women undergoing surgeries have also increased, leading to the great concern on the side effects of anesthetics, especially on immature brains[1]. The anesthetic effect is mainly achieved by exciting gamma-aminobutyric acid (GABA) or inhibiting N-methyl-D-aspartate (NMDA)[2,3]. However, studies have found that anesthetics have toxic effects on neurons in developmental stage, which may lead to neuronal apoptosis and brain damage[4]. General anesthesia has been applied for nearly two centuries[5]. With the increased amount of surgeries, anesthesia complications such as postoperative cognitive dysfunction have been focused on. In particular, the potential influences of anesthetics on learning and memory functions of children have been well concerned throughout the world[6,7]. Propofol has been clinically applied for induction and maintenance of anesthesia since 1989. It has become an extremely useful drug for inducing and maintaining general anesthesia due to its rapid onset and recovery after withdrawal[8]. The anesthetic effect of propofol mainly relies on activating the type A receptor of GABA and inhibiting the NMDA receptor to some extent[2,9]. The balance of GABA activation and NMDA inhibition maintains the survival and apoptosis of neurons[10]. During the developmental process of central nervous system, it is very sensitive to the external stimuli and the long-term cognitive function may be influenced. Propofol exerts relative small side effects on central nervous system in comparison with other anesthetics[11]. However, recent investigations[12,13] have pointed out that propofol administration may induce neurotoxicity in both *in vivo* and *in vitro* models. It is reported that administration of propofol alone or in combination with other drugs induces neuronal death in the experimental animals, leading to subsequent learning and memory im-

Corresponding Author: Qin Zhou, BM; e-mail: 278523276@qq.com

pairment[14]. There are many developmental events during neurogenesis, and propofol administration may affect neuronal functions at specific stages. Propofol may be involved in various cellular processes. Although many studies have found that propofol induces neurotoxicity in the brains of growing animals, the specific mechanism, however, has not been well understood. Current studies [15-17] believed that the neurotoxic effects of propofol may involve in neural stem cell survival and neurogenesis, mitochondrial damage, calcium signal abnormalities, microRNAs-mediated mechanisms, BDNF dysregulation and inflammatory factors. In-depth studies are still required for fully investigations. Histamine is an important neurotransmitter or neuromodulator in the brain. There are four histamine receptors that have been already discovered, namely H1, H2, H3, and H4. Among them, H1 mainly presents in the postsynaptic membrane, whereas H3 receptor mainly presents in the presynaptic membrane[18-20]. H3 receptors can negatively regulate the synthesis and release of histamine, while H4 receptors are currently reported to be involved in the immune response[21, 22]. Histamine exerts various neuromodulation effects on sleep, wakefulness, learning, memory and feeding through its different receptors[23]. Histamine H3 receptor antagonist can promote the synthesis and release of histamine. Many studies[24] have shown that H3 receptor antagonist is a very promising therapeutic drug in the treatment of neurological diseases, such as epilepsy, sleep disorders and memory disorders. For example, Pitolisant was the first clinically used H3 receptor antagonist in the treatment of sleep disorders, showing a good therapeutic effect. Histamine H3 receptor antagonists can reduce neuronal apoptosis and cerebral infarction volume after ischemia[25]. The histamine receptor antagonist Clobenpropit protects NMDA-induced neuronal excitatory damage by elevating GABA concentration[26]. So far, the effects and mechanisms of histamine H3 receptor and its antagonist on propofol-induced neuronal apoptosis are rarely reported. Phosphatidylinositol 3-kinase (P13K) is an important cellular signal transduction molecule[27]. P13K can be activated by stimulation with extracellular signals, such as growth factors, cytokines, and hormones. Activation of P13K produces 3,4-diphosphophosphatidylinositol (PIP2) and 3,4,5-trisphosphonylphosphatidylinositol (PIP3). PIP3 subsequently activates multiple target proteins in cells as a second messenger. Finally, the processes of cell proliferation, differentiation, survival, etc. are regulated by PI3K[28]. AKT, also known as protein kinase B (PKB), is an essential target kinase downstream of PI3K. Phosphorylation at the Ser473 site activates AKT, which in turn activates a series of apoptosis-regulated proteins (e.g., Bad, Caspase-9, etc.) to inhibit apoptosis and promote cell survival[29]. This study aims to investigate the protective role of histamine H3 receptor antagonist in propofol-induced neuronal apoptosis, and the potential mechanism of PI3K/AKT pathway. Our results provide a reference for anesthesia medication in infants.

Materials and Methods

Extraction of Hippocampal Neurons in Rats

This study was approved by the Animal Ethics Committee of Mianyang Central Hospital Animal Center. The neonatal rats with 18-day-old were sacrificed by decapitation. After immersing in 75% alcohol for at least 2 min, the head skin was cut to expose the bilateral cerebral hemispheres. The cerebral hemispheres were placed in D-Hank's buffer on ice for harvesting hippocampal tissues. The remaining meninges, blood vessels and other tissues on the hippocampus were removed and washed with cold D-Hank's. The hippocampus tissues were gently cut and the D-Hank's containing hippocampus tissues were transferred into a centrifuge tube. The mixture was centrifuged at 900 rpm/min for 5 min. The supernatant was discarded and the centrifugation was repeated once with the same procedure. After the supernatant was discarded, 3-time volume of membrane protease was added into the hippocampus tissues, followed by digestion for 15 to 20 min. The mixture was shaken every 5 min. DMED containing 10% FBS was added to terminate the digestion. The filtered cells were collected into a centrifuge tube and centrifuged at 900 rpm/min for 5 min. The supernatant was discarded and the cell suspension was prepared by blowing. The cell suspension was transferred to a culture flask and placed in an incubator for different adherent culture conditions. Non-adherent neurons were harvested subsequently and the glial cells were isolated due to a faster adherence speed.

Extraction of Total Protein From Neurons

The medium containing neurons was discarded and dried with absorbent paper. Pre-

cooled phosphate-buffered saline (PBS) was added in each well for cell wash for a total of three times, with 1 min each time. Neurons were lysed on ice for 30 min and shaken every 3-5 min. After complete lysis, lysate and cell debris were collected in a centrifuge tube, followed by centrifugation at 4°C, 12000 rpm/min for 5 min. The supernatant was collected and preserved in -80°C.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide) Assay

Neurons were seeded in 96-well plate at density of 1000-10000 cells per well. Marginal wells were added with PBS. Each group had 5 replicates. After specific treatment, 20 μl of MTT solution (5 mg/mL) was added in each well for 4-h incubation. Subsequently, 150 μL of dimethyl sulfoxide (DMSO) were added in each well and low-speed oscillation was performed for 10 min. Finally, the optical density was detected at the wavelength of 490 nm.

Western Blot

Neurons were added with lysis buffer and shaken on ice for 30 min. The total protein was separated after the centrifugation at 14,000 g/min for 15 min at 4°C. Protein concentration was calculated by the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA). The extracted proteins were separated on a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Western blot analysis was performed according to standard procedures.

LY294002 Treatment

To explore whether PI3K/AKT pathway was involved in the neuroprotection, neurons were treated with 20 μM LY294002 (PI3K/AKT pathway antagonist) for 30 min prior to Clobenpropit treatment.

Statistical Analysis

Data were analyzed by Statistical Product and Service Solutions (SPSS) 22.0 statistical software (IBM, Armonk, NY, USA). The quantitative data were represented as mean ± standard deviation ($\bar{x} \pm s$). The continuous variables were analyzed by the *t*-test. $p < 0.05$ was considered statistically significant.

Results

Effects of Propofol on Neuronal Apoptosis

MTT assay was conducted after neurons were exposed to different concentrations of propofol for detecting apoptotic rate. The data showed that the apoptotic rate of neurons remarkably increased in a concentration-dependent manner (Figure 1). Specifically, neurons were induced with 0.1 μM, 1 μM, 10 μM, 100 μM and 1000 μM propofol for 24 hours. Neuronal apoptosis was significantly observed except for those treated with 0.1 μM propofol. In the subsequent experiments, we selected 100 μM propofol as the induction concentration.

Clobenpropit Protected Propofol-Induced Neuronal Apoptosis

To elucidate the potential role of Clobenpropit in propofol-induced neuronal apoptosis, neurons were treated with both 100 μM propofol and different concentrations of Clobenpropit (1 nM, 10 nM and 100 nM). MTT assay elucidated that Clobenpropit treatment significantly protects propofol-induced neuronal apoptosis in a concentration-dependent manner (Figure 2A). In particular, induction of 10 nM Clobenpropit has already exerted significant anti-apoptotic effect. Subsequently, neurons were induced with 100 μM propofol and 100 nM Clobenpropit for 24 h, followed by detection of apoptosis-related genes with Western blot. Protein expressions of cleaved-caspase-3

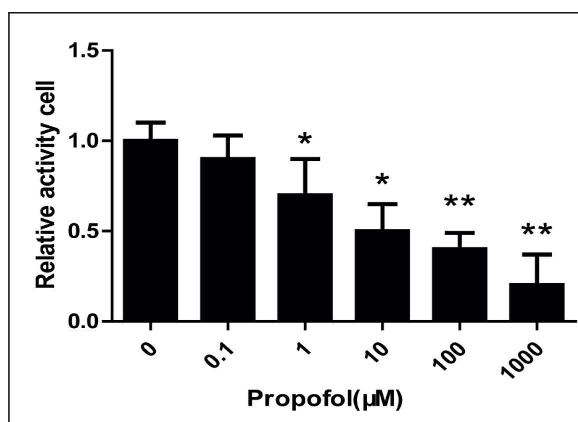

Figure 1. Effects of propofol on neuronal apoptosis. Neurons were induced with 0.1 μM, 1 μM, 10 μM, 100 μM and 1000 μM propofol for 24 hours, followed by MTT assay for detecting apoptotic rate.

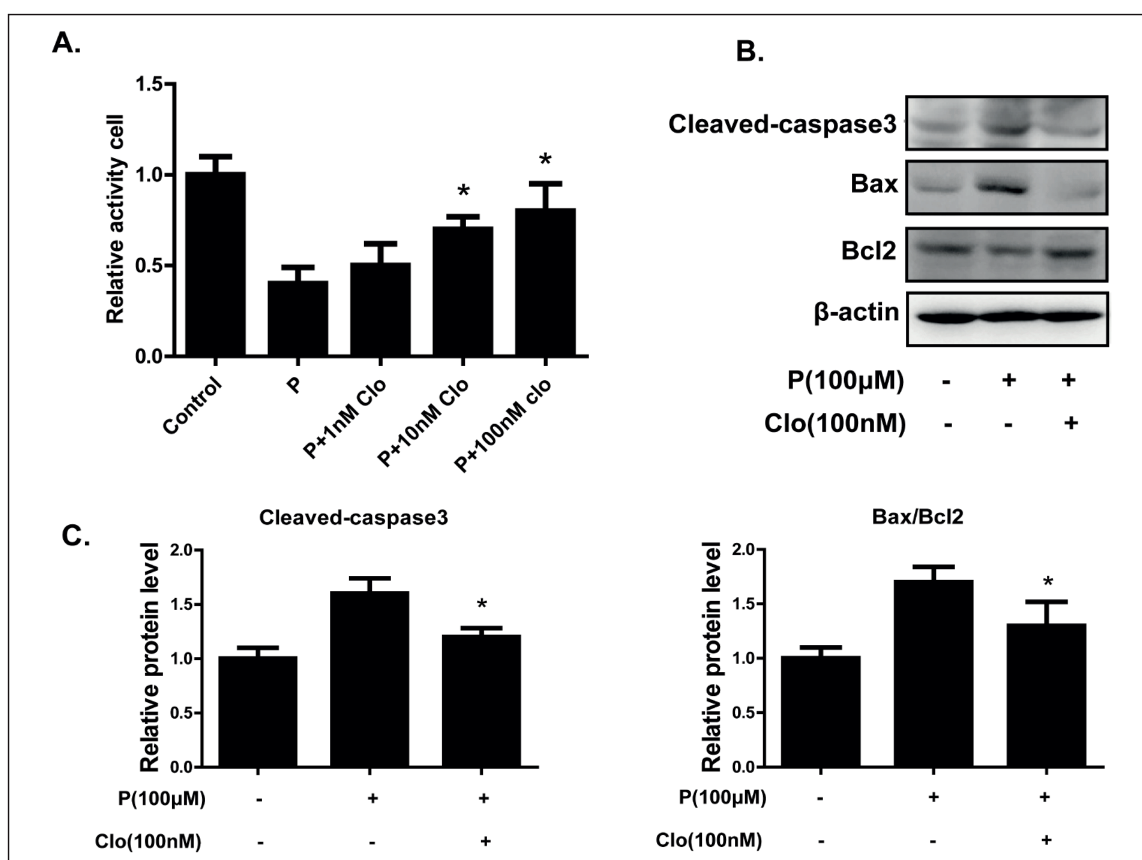

Figure 2. Clobenpropit protected propofol-induced neuronal apoptosis. Neurons were treated with 0 nM, 1 nM, 10 nM and 100 nM, respectively. ***A,*** Apoptotic rate detected by MTT assay. ***B, C,*** Protein levels of cleaved-caspase-3 and Bax/Bcl2 (*$p < 0.05$).

and Bax/Bcl2 were remarkably downregulated in neurons treated with Clobenpropit (Figure 2B and 2C).

Clobenpropit Treatment Activated PI3K/AKT Pathway In Neurons

To further verify whether PI3K/AKT pathway was involved in the neuroprotective effect of Clobenpropit on propofol-induced neuronal apoptosis, we detected protein levels of PI3K and p-AKT. Compared with those of controls, protein expressions of PI3K and p-AKT decreased in propofol-induced neurons (Figure 3A). By comparison, neurons induced with 100 μM propofol and 100 nM Clobenpropit presented similar levels of PI3K and p-AKT as the baseline (Figure 3B). Subsequently, LY294002, the PI3K pathway antagonist was applied in neurons. As shown in Figure 4, neurons were divided into four group, namely group 1 (negative controls), group 2 (treated with 100 μM propofol), group 3 (treated with 100 μM propofol and 100 nM Clobenpropit) and group 4 (treated with 100 μM propofol, 100 nM Clobenpropit and 20 μM LY294002). Western blot was conducted for accessing the protein levels of PI3K and p-AKT. It is shown that LY294002 induction remarkably reverses the protective role of Clobenpropit in neuronal apoptosis, manifesting as downregulated PI3K and p-AKT in group 4 compared with those in group 3. It is concluded that Clobenpropit protects propofol-induced neuronal apoptosis through activating PI3K/AKT pathway.

Discussion

Side effects of anesthesia have been revealed in different animal models. Based on previous studies, brain regions involving learning, mem-

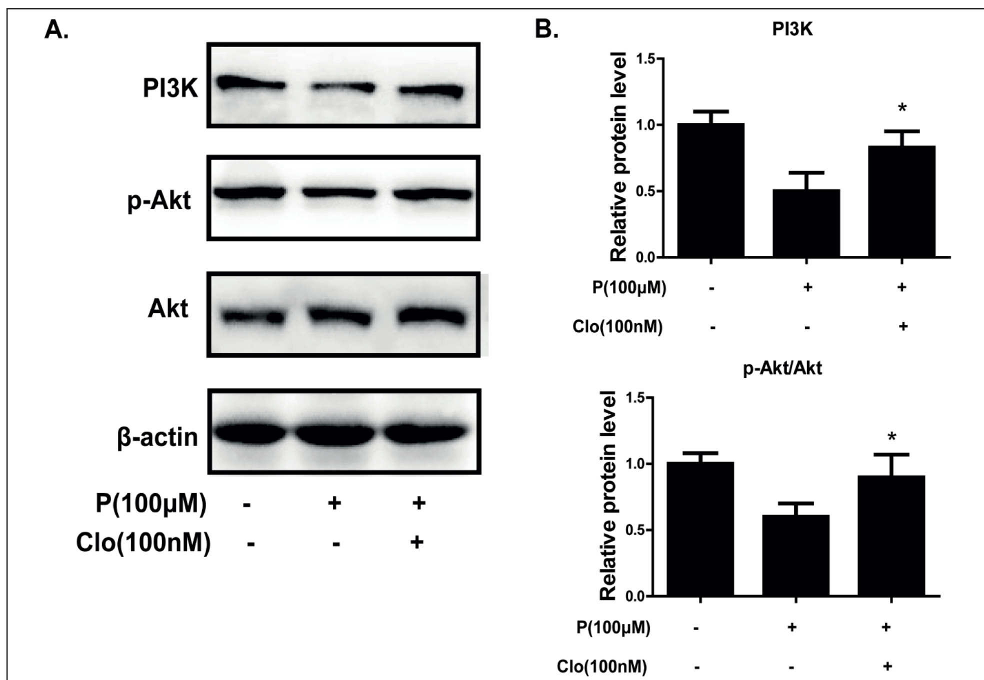

Figure 3. Clobenpropit treatment activated PI3K/AKT pathway in neurons. ***A, B,*** Protein levels of PI3K and p-AKT in neurons with different treatments (*$p < 0.05$).

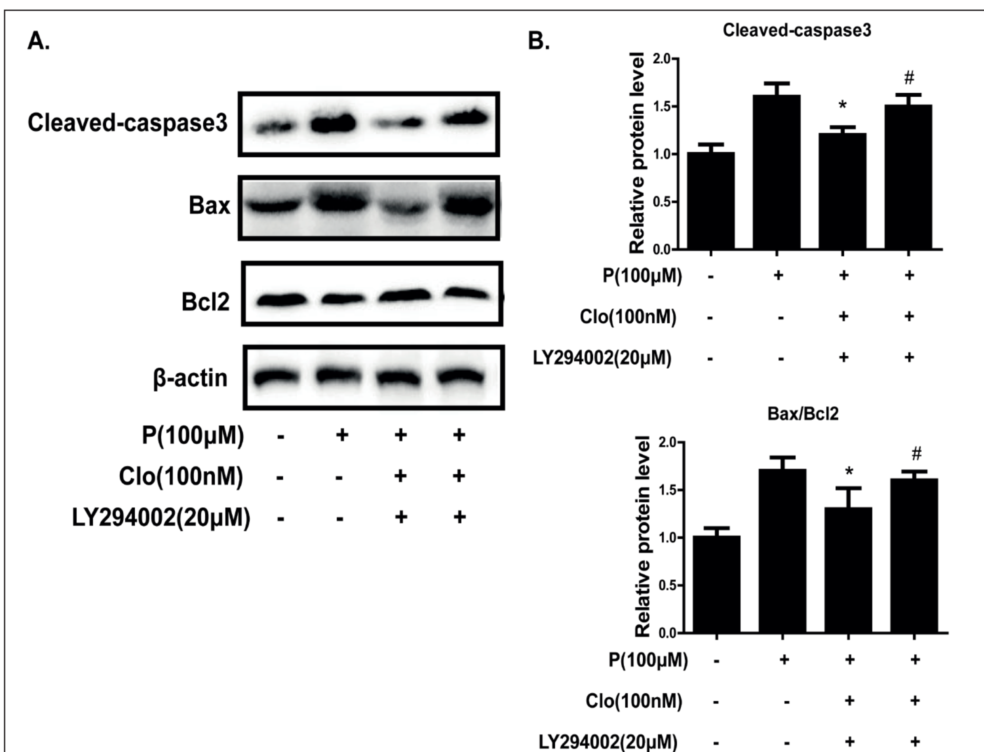

Figure 4. PI3K/AKT pathway in propofol-induced apoptotic neurons. Neurons were divided into group 1 (negative controls), group 2 (treated with 100 μM propofol), group 3 (treated with 100 μM propofol and 100 nM Clobenpropit) and group 4 (treated with 100 μM propofol, 10 n0M Clobenpropit and 20 μM LY294002). Protein levels of PI3K and p-AKT in neurons were detected by Western blot (*$p < 0.05$ compared with group 2, $^{\#}p < 0.05$ compared with group 3).

ory, sensory information processing, and cognitive function are affected by the side effects of anesthetics[30]. Neuronal damage caused by neuronal apoptosis in these functional areas may impair the development of neuronal networks, leading to long-term decline in neurocognitive function[31]. Anesthesia exposure is capable of inducing apoptosis in different regions of immature brain in different levels. However, only a certain threshold level of neuronal apoptosis in important functional areas may induce behavioral effects, such as hippocampus damage. In the existing studies of impaired learning and memory function, cognitive deficits are mainly related to hippocampus dysfunction. Hippocampal neuronal apoptosis is more severe than those occurring in other sites[32]. This may be the reason why neuronal apoptosis occurs immediately after anesthesia, and long-term neurocognitive impairment has only been validated in some studies. It is significant to recognize that cognitive impairment is caused by neuronal loss resulted from cell death, not by cell death itself. Propofol is a very useful drug for inducing and maintaining general anesthesia due to its rapid onset and recovery after withdrawal, which has been applied since 1989[33]. In the past researches, no serious complications were found during the maintenance or recovery after anesthesia. In addition, propofol has fewer side effects than other anesthetics. However, studies have shown that propofol can induce neurotoxicity in a variety of models in recent years, which has caused concern about the safety of propofol in pediatric anesthesia. With the advancement of medicine, clinical application of pediatric anesthesia has also increased[34]. Since it is difficult to directly obtain histological evidences of anesthesia neurotoxicity from pediatric patients, more and more studies analyzed the neurotoxicity of anesthetics using stem cell models[35]. Although the responses of anesthetics in different *in vitro* and *in vivo* models vary a lot, researchers confirmed that propofol administration would lead to axonal retraction and reduced dendritic growth. Clobenpropit, the histamine H3 receptor antagonist, is capable of alleviating NMDA-induced neuronal damage[36]. Histamine H3 receptor antagonists can negatively regulate the synthesis and release of histamine. They can also exert neuroprotection in a histamine-independent manner[37]. Due to experimental conditions and time constraints, this study was conducted using *in vitro* neuron model. Further *in vivo* studies are still required for elucidating the specific molecular mechanism involving in the neuroprotective role of Clobenpropit.

Conclusions

We found that clobenpropit protects propofol-induced neuronal apoptosis through activating PI3K/AKT pathway and it may decrease anesthetics toxic effects.

Conflict of Interest
The Authors declare that they have no conflict of interests.

References

1) Madl JE, Royer SM. Glutamate dependence of GABA levels in neurons of hypoxic and hypoglycemic rat hippocampal slices. Neuroscience 2000; 96: 657-664.
2) Forman SA, Miller KW. Mapping general anesthetic sites in heteromeric gamma-aminobutyric acid type A receptors reveals a potential for targeting receptor subtypes. Anesth Analg 2016; 123: 1263-1273.
3) Myhrer T, Nguyen NH, Enger S, Aas P. Anticonvulsant effects of GABA(A) modulators microinfused into area tempestas or substantia nigra in rats exposed to soman. Arch Toxicol 2006; 80: 502-507.
4) Li Q, Wang S, Xiao W, Huang C, Li H, Sun M. BBB: permeable conjugate of exogenic GABA. ACS Omega 2017; 2: 4108-4111.
5) Vaught JL, Pelley K, Costa LG, Setler P, Enna SJ. A comparison of the antinociceptive responses to the GABA-receptor agonists THIP and baclofen. Neuropharmacology 1985; 24: 211-216.
6) Sun W, Zhou Q, Ba X, Feng X, Hu X, Cheng X, Liu T, Guo J, Xiao L, Jiang J, Xiong D, Hao Y, Chen Z, Jiang C. Oxytocin relieves neuropathic pain through GABA release and presynaptic TRPV1 inhibition in spinal cord. Front Mol Neurosci 2018; 11: 248.
7) Xu F, Armstrong R, Urrego D, Qazzaz M, Pehar M, Armstrong JN, Shutt T, Syed N. The mitochondrial division inhibitor Mdivi-1 rescues mammalian neurons from anesthetic-induced cytotoxicity. Mol Brain 2016; 9: 35.
8) Zaouter C, Hemmerling TM, Mion S, Leroux L, Remy A, Ouattara A. Feasibility of automated propofol sedation for transcatheter aortic valve implantation: a pilot study. Anesth Analg 2017; 125: 1505-1512.
9) Shishido T, Watanabe Y, Suzuki H, Kato K, Niwa S, Hanoune J, Matsuoka I. Effects of repeated meth-

amphetamine administration on dopamine D1 receptor, D2 receptor and adenylate cyclase type V mRNA levels in the rat striatum. Neurosci Lett 1997; 222: 175.

10) Romuk EB, Szczurek W, Nowak PG, Hudziec E, Chwalinska E, Birkner E. Effects of propofol on the liver oxidative-antioxidant balance in a rat model of Parkinson's disease. Adv Clin Exp Med 2016; 25: 815-820.

11) Rayadurg V, Uttarwar A, Surve R. Is propofol safe in patients with phenylketonuria? J Neurosurg Anesthesiol 2018; 30: 85-86.

12) Zhu A, Wei X, Zhang Y, You T, Yao S, Yuan S, Xu H, Li F, Mao W. Propofol provides cardiac protection by suppressing the proteasome degradation of caveolin-3 in ischemic/reperfused rat hearts. J Cardiovasc Pharmacol 2017; 69: 170-177.

13) Monni L, Ghezzi F, Corsini S, Nistri A. Neurotoxicity of propofol on rat hypoglossal motoneurons in vitro. Neurosci Lett 2017; 655: 95-100.

14) Montero-Tinnirello J, Magaldi M, Fontanals J, Masgoret P, Bravo JC. Sinusal reversion of supraventricular tachyarrhythmias after propofol administration. A case series. Med Intensiva 2017; 41: 499-501.

15) Xing SG, Zhang KJ, Qu JH, Ren YD, Luan Q. Propofol induces apoptosis of non-small cell lung cancer cells via ERK1/2-dependent upregulation of PUMA. Eur Rev Med Pharmacol Sci 2018; 22: 4341-4349.

16) Meng C, Song L, Wang J, Li D, Liu Y, Cui X. Propofol induces proliferation partially via downregulation of p53 protein and promotes migration via activation of the Nrf2 pathway in human breast cancer cell line MDA-MB-231. Oncol Rep 2017; 37: 841-848.

17) Tahamtan A, Teymoori-Rad M, Nakstad B, Salimi V. Anti-inflammatory MicroRNAs and their potential for inflammatory diseases treatment. Front Immunol 2018; 9: 1377.

18) Lu CW, Lin TY, Chang CY, Huang SK, Wang SJ. Ciproxifan, a histamine H3 receptor antagonist and inverse agonist, presynaptically inhibits glutamate release in rat hippocampus. Toxicol Appl Pharmacol 2017; 319: 12-21.

19) Mena-Avila E, Marquez-Gomez R, Aquino-Miranda G, Nieto-Alamilla G, Arias-Montano JA. Clobenpropit, a histamine H3 receptor antagonist/inverse agonist, inhibits [(3)H]-dopamine uptake by human neuroblastoma SH-SY5Y cells and rat brain synaptosomes. Pharmacol Rep 2018; 70: 146-155.

20) Tanaka S, Sakaguchi M, Yoneyama H, Usami Y, Harusawa S. Histamine H3 receptor antagonist OUP-186 attenuates the proliferation of cultured human breast cancer cell lines. Biochem Biophys Res Commun 2016; 480: 479-485.

21) Delay-Goyet P, Blanchard V, Schussler N, Lopez-Grancha M, Menager J, Mary V, Sultan E, Buzy A, Guillemot JC, Stemmelin J, Bertrand P, Rooney T, Pradier L, Barneoud P. SAR110894, a potent histamine H3-receptor antagonist, displays disease-modifying activity in a transgenic mouse model of tauopathy. Alzheimers Dement (N Y) 2016; 2: 267-280.

22) Eissa N, Khan N, Ojha SK, Lazewska D, Kiec-Kononowicz K, Sadek B. The histamine H3 receptor antagonist DL77 ameliorates MK801-induced memory deficits in rats. Front Neurosci 2018; 12: 42.

23) Hagenow S, Stasiak A, Ramsay RR, Stark H. Ciproxifan, a histamine H3 receptor antagonist, reversibly inhibits monoamine oxidase A and B. Sci Rep 2017; 7: 40541.

24) Luo T, Wang Y, Qin J, Liu ZG, Liu M. Histamine H3 receptor antagonist prevents memory deficits and synaptic plasticity disruption following isoflurane exposure. CNS Neurosci Ther 2017; 23: 301-309.

25) Alachkar A, Lazewska D, Kiec-Kononowicz K, Sadek B. The histamine H3 receptor antagonist E159 reverses memory deficits induced by dizocilpine in passive avoidance and novel object recognition paradigm in rats. Front Pharmacol 2017; 8: 709.

26) Femenia T, Magara S, DuPont CM, Lindskog M. Hippocampal-dependent antidepressant action of the H3 receptor antagonist clobenpropit in a rat model of depression. Int J Neuropsychopharmacol 2015; 18: 1-16.

27) Zhou RP, Lin SJ, Wan WB, Zuo HL, Yao FF, Ruan HB, Xu J, Song W, Zhou YC, Wen SY, Dai JH, Zhu ML, Luo J. Chlorogenic acid prevents osteoporosis by Shp2/PI3K/Akt pathway in ovariectomized rats. PLoS One 2016; 11: e166751.

28) Tian JH, Xue B, Hu JH, Li JX, Cheng XY, Hu JS, Li FC, Chen YH, Li B. Exogenous substances regulate silkworm fat body protein synthesis through MAPK and PI3K/Akt signaling pathways. Chemosphere 2017; 171: 202-207.

29) Nakano N, Matsuda S, Ichimura M, Minami A, Ogino M, Murai T, Kitagishi Y. PI3K/AKT signaling mediated by G proteincoupled receptors is involved in neurodegenerative Parkinson's disease (Review). Int J Mol Med 2017; 39: 253-260.

30) Fechner J, Ihmsen H, Hatterscheid D, Schiessl C, Vornov JJ, Burak E, Schwilden H, Schuttler J. Pharmacokinetics and clinical pharmacodynamics of the new propofol prodrug GPI 15715 in volunteers. Anesthesiology 2003; 99: 303-313.

31) Li H, Lu Y, Pang Y, Li M, Cheng X, Chen J. Propofol enhances the cisplatin-induced apoptosis on cervical cancer cells via EGFR/JAK2/STAT3 pathway. Biomed Pharmacother 2017; 86: 324-333.

32) Purtell K, Gingrich KJ, Ouyang W, Herold KF, Hemmings HJ. Activity-dependent depression of neuronal sodium channels by the general anaesthetic isoflurane. Br J Anaesth 2015; 115: 112-121.

33) Ratnakumari L, Hemmings HJ. Inhibition by propofol of [3H]-batrachotoxinin-A 20-alpha-benzoate

binding to voltage-dependent sodium channels in rat cortical synaptosomes. Br J Pharmacol 1996; 119: 1498-1504.

34) Oncul S, Karabiyik L, Coskun E, Kadioglu E, Gulbahar O. Comparisons of the effects of the sevoflurane and propofol on acute ischemia reperfusion and DNA damages in rabbits. Braz J Anesthesiol 2017; 67: 35-41.

35) Nishizawa T, Suzuki H. Propofol for gastrointestinal endoscopy. United European Gastroenterol J 2018; 6: 801-805.

36) Tanaka T, Kochi T, Shirakami Y, Mori T, Kurata A, Watanabe N, Moriwaki H, Shimizu M. Cimetidine and clobenpropit attenuate inflammation-associated colorectal carcinogenesis in male ICR mice. Cancers (Basel) 2016; 8: 25.

37) Tanaka T, Kochi T, Shirakami Y, Mori T, Niwa A, Watanabe N, Moriwaki H, Shimizu M. Erratum: Tanaka, T. et al. Cimetidine and clobenpropit attenuate inflammation-associated colorectal carcinogenesis in male ICR mice. Cancers, 2016, 8, 25. Cancers (Basel) 2017; 9(7). pii: E80.