



RESEARCH ARTICLE

Ketamine Produces Anesthesia and Analgesia in Miniature Pigs Via NO-cGMP Signaling Pathway

Wenhan Liu, Yu Chen, Yu Zhang, Xinran Li, Yanan Li, Jinghua Zhao and Li Gao*

Heilongjiang Key Laboratory for Laboratory Animals and Comparative Medicine, College of Veterinary Medicine, Northeast Agricultural University, Harbin, China

*Corresponding author: gaoli43450@163.com

ARTICLE HISTORY (18-166)

Received: May 13, 2018
Revised: October 07, 2018
Accepted: October 08, 2018
Published online: November 13, 2018

Key words:

Ca²⁺-Mg²⁺-ATPase
Ketamine
Na⁺-K⁺-ATPase
NO-cGMP signaling pathway
Swine

ABSTRACT

Ketamine is a commonly used anesthetic for injection in pigs. However, how ketamine produces anesthesia and analgesia is unclear. Twenty *Bama miniature* pigs were randomly divided into four groups: saline control, induction (T1=15 min), deep anesthesia (T2=45 min) and recovery (T3=75 min). The cerebrum cortex, cerebellum, brainstem, hippocampus, and thalamus were collected. Results indicated that the activities or content of the indicators in different regions was inhibited by ketamine at different periods. The Na⁺-K⁺-ATPase activity in the cerebral cortex, hippocampus, thalamus and brainstem decreased by 48.69, 20.27, 51.18 and 36.44%, respectively, while the Ca²⁺-Mg²⁺-ATPase activity in each region decreased by 28.75, 28.59, 46.58, 64.11 and 34.68%, respectively. The NOS activity in regions except thalamus decreased by 49.76, 13.12, 13.77 and 15.96%, respectively. The content of No in the cerebral cortex, cerebellum, hippocampus and thalamus decreased by 33.25, 46.93, 44.44 and 50.00%, respectively, and the cGMP content in the cerebral cortex, cerebellum and hippocampus decreased by 28.25, 29.87 and 40.60%, respectively. The changes of each index were consistent with the physiological responses of pigs when they are anesthetized. In summary, ketamine is suitable for pig anesthesia. It produces anesthetic and analgesic effects via the NO-cGMP signaling pathway, however, more drugs need to be studied for an effective balanced anesthetic protocol that meets demands.

©2018 PVJ. All rights reserved

To Cite This Article: Liu W, Chen Y, Zhang Y, Li X, Li Y, Zhao J and Gao L, 2018. Ketamine produces anesthesia and analgesia in miniature pigs via NO-cGMP signaling pathway. Pak Vet J. <http://dx.doi.org/10.29261/pakvetj/2018.107>

INTRODUCTION

Pig anesthesia is an indispensable part of breeding, husbandry, transportation and clinical treatment. In addition, anesthesia management for pigs as pets sometimes takes place in animal hospitals. As commonly used experimental animals, pigs often need to be anesthetized for surgery or other examinations during biomedical research (Pehbock *et al.*, 2015). Moreover, animal welfare organizations and protectors have called for porcine anesthesia during surgery, such as castrations. Surgical piglet castration without pain relief has been banned in organic farming in the EU (Heid and Hamm, 2013). However, a few of them are still performed without anesthesia.

Inhalation anesthesia has many advantages, such as highly controllable, easy to monitor, short induction

period and high safety. In practical use, equipment and consumables, such as respiratory anesthetic machines, are needed and workers also need to be trained. Inhaled anesthesia may be less suitable for short-term anesthesia than injected anesthesia. Moreover, some swine farmers reported headaches or dizziness during or after castration work with inhalation anesthesia (Enz *et al.*, 2013). Last but not least, the porcine oropharynx is special in its anatomical structure, as the maxilla is wide and thick, and the mandible is small and short. When performing tracheal intubation, it is possible to be blocked as a result of irritation. Therefore, it is very necessary to develop a reasonable swine injection anesthesia program.

Currently used injectable anesthetic drugs for pigs include: ketamine, azaperone, xylazine, midazolam, remifentanyl and diazepam (Santos *et al.*, 2013; Daş *et al.*, 2016; Zacharioudaki *et al.*, 2017). Due to the clinical

features of pigs, such as their physiological characteristics and tolerance to sedatives, balanced anesthesia is usually needed and a combination of two or more drugs meets the actual requirements better. However, if the effect of a single anesthetic on pigs is not yet clear, how can a complex balanced anesthetic protocol be formulated? Thus, this study investigated the anesthetic effect of ketamine, one of the commonly used drugs for pig anesthesia.

Ketamine, (2-(*o*-chlorophenyl)-2-(methylamino)cyclohexanone)), is primarily known as an effective N-methyl-D-aspartic acid receptor (NMDAR) blocker. It is widely used for anesthesia and sedation, because of its rapid induction, quick recovery, low impact on respiratory and circulatory system and high-level of safety (Morgan *et al.*, 2012). The anesthetic effects of ketamine are mainly attributed to its ability to decrease inter-cortical communication, thereby generating dissociative anesthesia while simultaneously activating the limbic system (Craven, 2007). In addition, ketamine can produce anesthetic and analgesic effects through hyperpolarization-activated cyclic-nucleotide gated channels (HCN), especially subtype 1 (HCN1). Additionally, the NO/cGMP pathway in postsynaptic can increase NMDAR currents via HCN (Chen *et al.*, 2009; Zhou *et al.*, 2015).

The NO-cGMP signaling pathway can help alter the physiological functions of various organs and systems, such as smooth muscle relaxation, inhibition of platelet aggregation, and neuronal transmission (Russwurm *et al.*, 2013). It is also widely found in the central nervous system (CNS), especially in neurons. NO is an important information factor that is, closely related to neurotoxicity, memory, consciousness, learning and pain mediated by excitatory amino acids (Hao *et al.*, 2016). It is produced by NOS catalyzed by L-arginine and acts as an endogenous activator of soluble guanylyl cyclase (sGC), which can further activate sGC to catalyze the generation of cGMP by GTP. As a central part of the NO-cGMP pathway, cyclic GMP (cGMP) can act on cGMP ligand-gated ion channels and exert biological effects by regulating cGMP dependent protein kinase (PKG) production and phosphodiesterase (PDE) activity which depends on a Ca/CaM signal, and directly controls channel opening via cyclic nucleotide-gated ion channel (CNG) (Neitz *et al.*, 2014; Karaçay and Bonthius, 2015; Shen *et al.*, 2016). Interestingly, the NO-cGMP pathway plays an important role in a variety of anesthetics (Ding *et al.*, 2017; Wang *et al.*, 2017). Therefore, we studied the effect of porcine anesthesia with ketamine via NO-cGMP signaling pathway in an effort to provide ideas for clinical practice.

MATERIALS AND METHODS

Animals: Twenty male and female *Bama miniature* pigs, eight months of age, weighing 32.2 ± 4.3 kg, were purchased from Sanyuan Swine Breeding Center (Harbin, China). Prior to the experiment, the pigs were quarantined for 6 weeks at the Northeast Agricultural University (Harbin, China). To prevent vomiting or other possible adverse effects, food was withheld for 24 h and water was withheld for 4 h before the administration of drugs. All experiments were performed in accordance with the guidelines outlined by the Ethical Committee for Animal Experiments (Northeast Agricultural University, Harbin, China).

Grouping and drug administration: Five pigs were randomly selected as the normal saline control group (C group), and the rest were used in the experimental groups. All pigs in the experimental groups were anesthetized with 10 mg/kg ketamine. The experimental animals were euthanized at the induction stage (T1=15 min), the deep anesthesia stage (T2=45 min) or the recovery stage (T3=75 min)

All brain tissues were collected and the residual blood was washed with sterile 4°C saline. The brain tissues were then immediately separated into cerebral cortex, cerebellum, thalamus, hippocampus, and brainstem on saline ice plates. All samples were separately stored in liquid nitrogen.

Na⁺-K⁺-ATPase and Ca²⁺-Mg²⁺-ATPase activity: The ATPase activity was assessed by measuring the release of inorganic phosphate (Pi) from ATP according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).

The brain tissues were added to a pre-cooled 0.32 M sucrose buffer (4°C, PH=7.4) containing 0.32 M sucrose, 1 mM Na₂HPO₄, 1 mM EDTA, 10 mM Tris-HCl, at a ratio of 1:10 (w/v). The mixture was placed in a glass homogenizer in an ice-water bath, and then ground to obtain brain homogenate. Density gradient centrifugation was used to prepare synaptosomes (Bermejo *et al.*, 2014). Briefly, the homogenate was centrifuged at $112 \times g$ for 10 min at 4°C and then the procedure was repeated. The supernatants were collected and 1.2 M pre-chilled sucrose solution was slowly added along the wall of the tube. Next, the mixture was centrifuged at $7155 \times g$ for 20 min. The middle gradient zone containing the synaptosomes was carefully removed and diluted 1:3 (v/v) with 0.32 M pre-cooled sucrose buffer. Finally, the dilution was slowly spread on the surface of 0.8 M pre-cooled sucrose buffer and, centrifuged at $11180 \times g$ for 20 min. The resulting precipitate was the synaptosomes.

NO content and NOS activity: Brain homogenate was made according to above method, and then centrifuged for 10 min at $699 \times g$ at 4°C to collect the supernatant. The experiment was carried following the instructions of the NO and NOS kits (Nanjing Jiancheng Bioengineering Institute, China). The principle of these methods is as follows:

Determining NO content was achieved using the principle that nitrate reductase can specifically reduce NO₃⁻ to NO₂⁻, allowing for the NO content to be determined by measuring the color depth.

NOS can catalyze the reaction of L-Arginine with O₂ to produce NO, which forms a colored compound with nucleophilic species. Therefore, following the reaction, the absorbance was measured spectrophotometrically at a wavelength 530 nm. The change in NOS activity was calculated based on the measured absorbance.

cGMP content: Brain homogenate was also produced and then centrifuged at $699 \times g$ for 10 min at 4°C. The cGMP concentration was determined by ELISA (Calvin Biotechnology Co., Ltd., China).

Statistical analysis: All data were analyzed with SPSS 19.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 7.0 (GraphPad Software Inc., USA) via one-way ANOVA, followed by Turkey's post hoc test or unpaired two-tailed Student's t-test. Data are presented as the mean \pm standard deviation, unless otherwise noted. Values were considered to be statistically significant for $P < 0.05$ and extremely statistically significant for $P < 0.01$.

RESULTS

The $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in the cerebral cortex at T1 decreased by 26.15% ($P < 0.01$) (Table 1, Fig. 1), however, there was no significant difference in other brain regions at T1. At T2, it was significantly inhibited in the cerebral cortex, hippocampus, thalamus and brain stem and decreased by 48.69% ($P < 0.01$), 20.27% ($P < 0.05$), 51.18% ($P < 0.01$), and 36.44% ($P < 0.01$), respectively, compared with C group. During T3, a tendency of recovery appeared. The $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity was significantly restored in the brainstem ($P < 0.05$), but in the cerebral cortex, hippocampus and thalamus, it was still significantly inhibited ($P < 0.01$ or $P < 0.05$). $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in the cerebellum was not significantly affected throughout the anesthesia.

The $\text{Ca}^{2+}\text{-Mg}^{2+}\text{-ATPase}$ activity in cerebellum, thalamus, and brainstem was significantly decreased by 13.86% ($P < 0.05$), 34.33% ($P < 0.05$) and 14.06% ($P < 0.05$) at T1 (Table 1, Fig. 2). The activity decreased further at T2 in different regions by 28.75% ($P < 0.01$), 28.59% ($P < 0.01$), 46.58% ($P < 0.01$), 64.11% ($P < 0.01$), and 34.68% ($P < 0.01$). At T3, $\text{Ca}^{2+}\text{-Mg}^{2+}\text{-ATPase}$ activity in all regions tended to recover, with no significant difference to the normal cerebral cortex and cerebellum activity, whereas, it was still significantly inhibited in other regions ($P < 0.01$ or $P < 0.05$). The $\text{Ca}^{2+}\text{-Mg}^{2+}\text{-ATPase}$ activity in the cerebral cortex, thalamus, and brainstem were significantly different compared to that in T2 ($P < 0.01$).

The NOS activity in the cerebral cortex, cerebellum, hippocampus and brainstem during T1 was reduced by 37.56% ($P < 0.01$), 13.12% ($P < 0.05$), 13.77% ($P < 0.05$) and 15.96% ($P < 0.05$), respectively (Table 1, Fig. 3). In the hippocampus, it significantly changed compared with T2 ($P < 0.01$). At T2, only the NOS activity of the cerebral cortex had a decrease of 49.76% ($P < 0.01$), while there were no significant changes ($P > 0.05$) in the other brain regions. At T3, NOS activity in the hippocampus and brainstem decreased by 11.80% ($P < 0.05$) and 23.40% ($P < 0.05$) compared with the C group. NOS activity in thalamus did not change significantly. Interestingly, there was an upward trend during T2 ($P > 0.05$).

During T1, the NO content in the cerebral cortex, cerebellum, hippocampus, thalamus and brainstem showed differing degrees of reduction at 33.25% ($P < 0.01$), 46.93% ($P < 0.01$), 44.44% ($P < 0.01$), 50.00% ($P < 0.01$), and 11.00% ($P > 0.05$), respectively (Table 1, Fig. 4). During T2, the NO content in each region showed a significant downward trend, at 52.79% ($P < 0.01$), 46.67% ($P < 0.01$), 33.09% ($P < 0.01$), 52.58% ($P < 0.01$) and 26.08% ($P < 0.01$) lower than that of the C group. During T3, the NO content in the hippocampus returned to a normal level, but in the cerebral cortex, cerebellum, thalamus and brainstem, it was still at a low level, which was decreased by 19.30% ($P < 0.05$), 31.20% ($P < 0.01$), 38.36% ($P < 0.01$), and 31.20% ($P < 0.01$). Compared with T2, the NO content in regions except brainstem recovered ($P < 0.01$ or $P < 0.05$).

The cGMP content in the brain regions was reduced to varying degrees during T1 ($P > 0.05$) (Table 1, Fig. 5). At T2, the cGMP levels in the cerebral cortex, cerebellum, and hippocampus decreased by 28.25% ($P < 0.05$), 29.87% ($P < 0.01$) and 40.60% ($P < 0.01$), respectively. During T3, cGMP returned to a normal level in each brain region. At the same time, compared with T2, the cGMP content in the cerebral cortex, cerebellum and hippocampus showed a significant or extremely significant increase ($P < 0.01$ or $P < 0.05$).

Table 1: Indicators (means \pm standard deviation) in pigs controlled by saline (C group, $n=5$) or anesthetized intramuscularly with ketamine (10 mg/kg) at T1, T2 and T3 period (T1=15 min, $n=5$; T2=45 min, $n=5$; T3=75 min, $n=5$)

Parameters	Groups	The effect of ketamine on different encephalic regions of miniature pigs in the study.				
		Cerebral cortex	Cerebellum	Hippocampus	Thalamus	Brainstem
$\text{Na}^+\text{-K}^+\text{-ATPase}$ (U/mgprot)	C group	9.98 \pm 0.69	6.91 \pm 0.59	5.13 \pm 0.28	3.81 \pm 0.34	3.54 \pm 0.56
	T1 period	7.37 \pm 0.15***	6.61 \pm 0.34	4.89 \pm 0.30	3.62 \pm 0.49##	3.17 \pm 0.69#
	T2 period	5.12 \pm 0.12**	6.21 \pm 0.37	4.09 \pm 0.46*	1.86 \pm 0.69**	2.25 \pm 0.23**
	T3 period	8.44 \pm 1.13###	7.04 \pm 0.64	4.32 \pm 0.57*	2.45 \pm 0.49**	3.24 \pm 0.87#
$\text{Ca}^{2+}\text{-Mg}^{2+}\text{-ATPase}$ (U/mgprot)	C group	7.06 \pm 0.60	6.82 \pm 0.06	3.80 \pm 1.02	4.18 \pm 0.26	3.20 \pm 0.19
	T1 period	6.90 \pm 0.17###	5.87 \pm 0.52*	3.76 \pm 0.29###	2.74 \pm 0.49***	2.75 \pm 0.33###
	T2 period	5.03 \pm 0.55**	4.87 \pm 0.11**	2.03 \pm 0.19**	1.50 \pm 0.19**	2.09 \pm 0.25**
	T3 period	6.89 \pm 0.53###	6.22 \pm 0.59	2.97 \pm 0.17***	2.65 \pm 0.27***	2.76 \pm 0.13###
NOS (U/mgprot)	C group	4.10 \pm 0.28	2.82 \pm 0.42	3.05 \pm 0.21	2.62 \pm 0.22	3.76 \pm 0.31
	T1 period	2.56 \pm 0.13***	2.45 \pm 0.12*	2.63 \pm 0.13###	2.75 \pm 0.27	3.16 \pm 0.23*
	T2 period	2.06 \pm 0.29**	2.96 \pm 0.14	3.07 \pm 0.19	2.93 \pm 0.08	3.35 \pm 0.35
	T3 period	3.32 \pm 0.24###	2.62 \pm 0.37	2.69 \pm 0.19#	2.54 \pm 0.29	2.88 \pm 0.29*
NO ($\mu\text{mol/gprot}$)	C group	4.30 \pm 0.22	3.75 \pm 0.36	4.23 \pm 0.31	4.64 \pm 0.14	3.91 \pm 0.38
	T1 period	2.87 \pm 0.36***	1.99 \pm 0.37**	2.35 \pm 0.27***	2.32 \pm 0.59**	3.48 \pm 0.43
	T2 period	2.03 \pm 0.28**	2.00 \pm 0.33**	2.83 \pm 0.12**	2.20 \pm 0.17**	2.89 \pm 0.25**
	T3 period	3.47 \pm 0.45###	2.58 \pm 0.29**	4.01 \pm 0.16###	2.86 \pm 0.38***	2.69 \pm 0.21**
cGMP (pmol/mL)	C group	30.08 \pm 4.21	35.18 \pm 2.09	36.72 \pm 4.41	38.79 \pm 2.69	43.92 \pm 3.45
	T1 period	25.59 \pm 1.87	34.02 \pm 3.59###	32.27 \pm 3.58###	33.54 \pm 4.14	37.27 \pm 2.71
	T2 period	21.58 \pm 2.16**	24.67 \pm 3.77**	21.81 \pm 4.63**	37.47 \pm 1.95	38.49 \pm 4.81
	T3 period	34.17 \pm 1.06###	31.23 \pm 1.76#	33.54 \pm 3.59###	35.46 \pm 4.07	38.24 \pm 4.28

*** Extremely significant statistical differences between C groups and other groups ($P < 0.01$). * Significant statistical differences between C groups and other groups ($P < 0.05$). ### Indicate the difference compares with T2 period is extremely significant ($P < 0.01$). # Indicate the difference compares with T2 period is significant ($P < 0.05$).

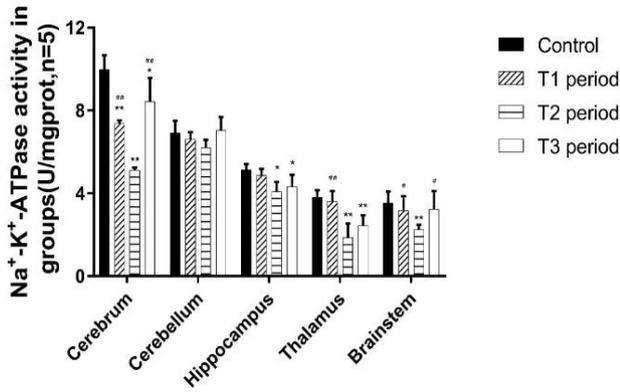


Fig. 1: Effects of ketamine on the Na⁺-K⁺-ATPase activity in different encephalic regions of miniature pigs.

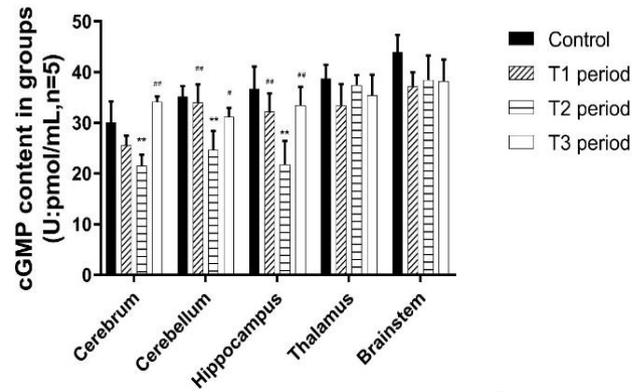


Fig. 5: Effects of ketamine on the cGMP content activities in different encephalic regions of miniature pigs.

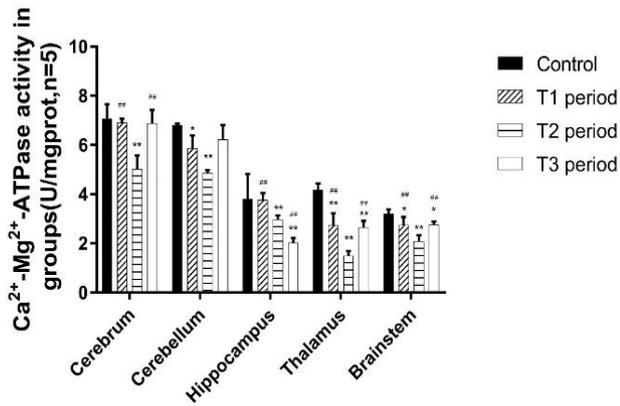


Fig. 2: Effects of ketamine on the Ca²⁺-Mg²⁺-ATPase activity in different encephalic regions of miniature pigs.

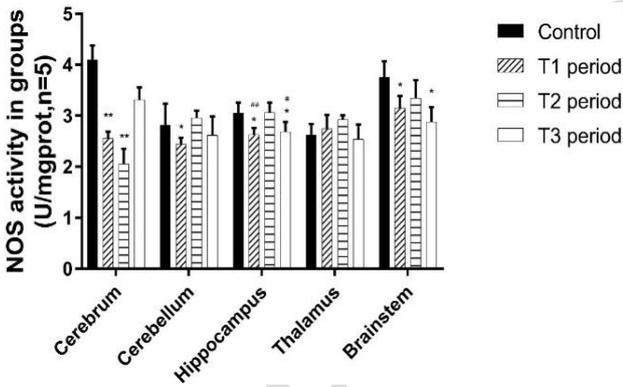


Fig. 3: Effects of ketamine on the NOS activity in different encephalic regions of miniature pigs.

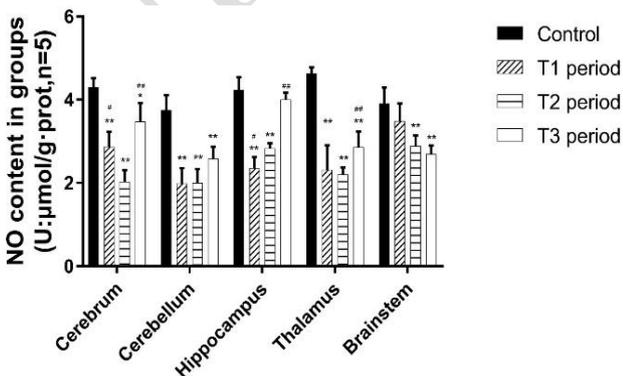


Fig. 4: Effects of ketamine on the NO content in different encephalic regions of miniature pigs.

DISCUSSION

According to the results, the activity or content of various indicators in regions of the pig brain were inhibited by ketamine as follows: 1) Na⁺-K⁺-ATPase activity was inhibited in the cerebral cortex, hippocampus, thalamus and brainstem; 2) Ca²⁺-Mg²⁺-ATPase activity was inhibited in each region; 3) NOS activity was inhibited in regions except thalamus; 4) NO content was inhibited in the cerebral cortex, cerebellum, hippocampus and thalamus and ; 5) cGMP content was inhibited in the cerebral cortex, cerebellum and hippocampus. Therefore, the effect of ketamine may be associated with inhibition of the NO/cGMP pathway in each encephalic region of minipigs.

It is well known that, ketamine is a typical NMDA antagonist. NMDA receptors are rich in the central nervous system (CNS) as excitatory amino acid receptors. The levels of Na⁺ and K⁺ inside and outside the cell membrane can be changed by the activated NMDAR, which results in a slow excitatory postsynaptic potential (Akkuratov *et al.*, 2015). In our study, ketamine also showed inhibition of Na⁺-K⁺-ATPase, but the activity recovered during T3 in all regions. It is presumed that ketamine inhibits Na⁺-K⁺-ATPase activity during anesthesia, which in turn leads to stable exchange of intracellular Na⁺, K⁺, and Ca²⁺. This may ultimately result in an anesthetic effect on neuronal excitability, transmissibility, and the release of synaptic neurotransmitters.

At the same time, Na⁺-K⁺-ATPase will lead to Ca²⁺ influx after activation and, the inhibition of Ca²⁺-Mg²⁺-ATPase activity may be involved in this process. It has been reported that the activity of Ca²⁺-Mg²⁺-ATPase is inhibited by general anesthetics which results in changes of intracellular calcium concentrations ([Ca²⁺]_i), and an increase in [Ca²⁺]_i concentration forms the basis of cell signal transduction (Michard *et al.*, 2011). The activity of Ca²⁺-Mg²⁺-ATPase was significantly inhibited in each brain region during T2 but recovered or tended towards recovery during T3. Ca²⁺-Mg²⁺-ATPase may be a target of ketamine anesthesia, and inhibition of Ca²⁺-Mg²⁺-ATPase in the hippocampus during T3 still needs further study.

After that, Ca²⁺ can interact with CaM and bind to the NOS binding site. This subsequently activates NOS to generate NO, catalyzing the reaction of L-Arginine with molecular oxygen. NOS is the rate-limiting enzyme

during NO synthesis, and NOS exerts its biological effects through the formation of NO (Buchwalow *et al.*, 2017). In addition, increased intracellular Ca^{2+} concentration can also induce the release of acetylcholine and it may play a role in analgesia (Satoh and Nakazato, 1992; Bartolini *et al.*, 2011). In this study, the activity of NOS was inhibited during T1 and T2 except in the thalamus; however, the activity recovered rapidly during T3 except in the brainstem, which may be related to the anesthesia and the recovery effect of ketamine. In addition, although the increasing trend of some brain regions during T2 was not significant, it deserves attention. This may be related to the dose of the anesthetic and its mechanism of action, which needs further study.

NO plays an important role in ketamine anesthesia and analgesia. When the production of NO was reduced, excitability of nerve conduction decreased. The inhibitory conduction was then enhanced, thus presenting the anesthesia sedation state. NO can activate sGC, GTP forms cGMP under the action of sGC and divalent metal ions and regulates the function of cGMP. As the results show, the NO content was reduced in all brain regions and at first had a decreasing trend and then an increasing trend except in the brainstem. The NO change in the brainstem may be related to the change in its NOS. However, the changes of NO content were not the same as NOS, which suggests that a very small amount of NOS can significantly change the amount of NO (the enzymatic efficiency of NOS is high). Maybe there are also other ways to produce NO, which requires further study.

Moreover, during analgesia, NO and its downstream molecules are important in formation, maintenance and processing of pain signals. Therefore, reducing the NO content can relieve pain. NO is also involved in the storage, absorption and release of acetylcholine. However, it is not clear whether acetylcholine originates from the changes in Ca^{2+} , as mentioned above, or is directly affected by NO. In addition, ketamine was proven to have analgesic effects. The acute analgesic effect of ketamine less than anesthetic dose was similar to that of intravenous morphine. It also alleviates the negative emotions associated with chronic pain (Wang *et al.*, 2011). On one hand, as an NMDAR blocker, ketamine interferes with the central sensitization of chronic pain. On the other hand, it can block sodium and potassium channels in the dorsal ganglion root (DRG) and dorsal horn of the spinal cord, suggesting that a sufficient dose of ketamine can take part in analgesia (Zhou and Zhao, 2000; Schnoebel *et al.*, 2005).

cGMP is a central part of the NO-cGMP signal transduction pathway and exerts biological function by regulating PDE activity and PKG production. In this experiment, the concentration of cGMP decreased in all brain regions. Furthermore, changes in the cerebral cortex, hippocampus and cerebellum were significant or extremely significant ($P < 0.01$ or $P < 0.05$). These changes were not exactly the same as the changes in NO, especially in the cerebellum. This proves that cGMP is not only regulated by the NMDAR during the process of ketamine anesthesia. Alternatively, it is affected by NMDAR but not through the NO pathway. These results are consistent with previous reports.

Ketamine can significantly counteract the increased effect of AMPA, Glu and NMDA on cGMP, but help sodium nitrate increase cGMP significantly (Miyawaki *et al.*, 1997). Acetylcholine could also directly induce an increase in cGMP content, as the cGMP inhibition of ketamine was not completely mediated by NMDAR and NOS (Galley and Webster, 1996). The above results reveal that cGMP inhibition may be associated with other factors, not the results of ketamine suppression by NOS and NMDAR. For instance, GABA can be closely related to the NO-cGMP pathway in that NMDAR and NOS can modulate hippocampal GABAergic inhibition via NO-cGMP signaling (Gasulla and Calvo, 2015). Furthermore, the activation of GABA also inhibits the NO-cGMP signaling pathway (Sagi *et al.*, 2014).

In summary, the anesthesia and analgesic effects of ketamine are related to the activities or contents of the Na^+ - K^+ -ATPase, Ca^{2+} - Mg^{2+} -ATPase, NOS, NO, and cGMP. The changes of each index were consistent with the physiological responses of pigs when they were anesthetized. However, it is interesting to note that the changes of each indicator were different in the brain regions and anesthesia period. Some brain regions changed later or recovered slower than others, which may be related to the pharmacological effects of ketamine, the physiological functions of brain regions, and the information interaction among brain regions. And in the awakening period, the depression of the minipigs may be due to that some indicators had not returned to normal.

Many factors can influence the anesthetic effect of ketamine. While in our study, only the effect of ketamine on NO-cGMP signaling pathway was involved. More needs to be studied, such as its effect on GABA, acetylcholine and pharmacokinetics. In addition, porcine anesthetic protocols require more anesthetics than a single ketamine, and more anesthetics are required to make the balanced anesthetic program that best meets the practice.

Conclusions: The results of this study suggest that ketamine is a suitable drug for pig anesthesia and analgesic. And the anesthetic and analgesic effects produced by ketamine may be related to the NO-cGMP signaling pathway. While more about ketamine need to be studied, such as its effect on GABA, acetylcholine and its pharmacokinetics. Besides, more drugs need to be studied for the balanced anesthetic protocol for pigs.

Acknowledgements: This study was supported by the National Natural Science Foundation of China (31572580 and 31372491)

Authors contribution: WHL, YZ and LG conceived and designed the study. YZ, YC, XRL and YNL executed the experiment and collected the samples. WHL and JHZ analyzed the data. WHL wrote and edited the manuscript. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

REFERENCES

- Akkuratov EE, Lopacheva OM, Kruusmagi M, *et al.*, 2015. Functional interaction between Na^+ / K^+ -ATPase and NMDA receptor in cerebellar neurons. *Mol Neurobiol* 52:1726-34.

- Bartolini A, Di Cesare Mannelli L and Ghelardini C, 2011. Analgesic and antineuropathic drugs acting through central cholinergic mechanisms. *Recent Patents CNS Drug Discov* 6:119-40.
- Bermejo MK, Milenkovic M, Salahpour A, et al., 2014. Preparation of synaptic plasma membrane and postsynaptic density proteins using a discontinuous sucrose gradient. *J Visual Experim JoVE*:e51896.
- Buchwalow I, Schneckeburger J, Atiakshin D, et al., 2017. Oxidative stress and NO generation in the rat pancreatitis induced by pancreatic duct ligation. *Acta Histochemica* 119:252-6.
- Chen X, Shu S and Bayliss DA, 2009. HCN1 channel subunits are a molecular substrate for hypnotic actions of ketamine. *J Neurosci* 29:600-9.
- Craven R, 2007. Ketamine. *Anaesthesia* 62:48-53.
- Daş G, Vernunft A, Gors S, et al., 2016. Effects of general anesthesia with ketamine in combination with the neuroleptic sedatives xylazine or azaperone on plasma metabolites and hormones in pigs. *J Anim Sci* 94:3229.
- Ding Y, Yao P, Hong T, et al., 2017. The NO-cGMP-PKG signal transduction pathway is involved in the analgesic effect of early hyperbaric oxygen treatment of neuropathic pain. *J Headache Pain* 18:51.
- Enz A, Schupbach-Regula G, Bettschart R, et al., 2013. Experiences with pain control during piglet castration in Switzerland Part 1: Inhalation anesthesia. *Schweizer Archiv fur Tierheilkunde* 155:651-9.
- Galley HF and Webster NR, 1996. Brain nitric oxide synthase activity is decreased by intravenous anesthetics. *Anesth Analg* 83:591-4.
- Gasulla J and Calvo DJ, 2015. Enhancement of tonic and phasic GABAergic currents following nitric oxide synthase inhibition in hippocampal CA1 pyramidal neurons. *Neurosci Lett* 590:29-34.
- Hao L, Wei X, Guo P, et al., 2016. Neuroprotective effects of inhibiting fyn-nitrosylation on cerebral ischemia/reperfusion-induced damage to CA1 hippocampal neurons. *Int J Mol Sci* 17:1100.
- Heid A and Hamm U, 2013. Animal welfare versus food quality: factors influencing organic consumers' preferences for alternatives to piglet castration without anaesthesia. *Meat Sci* 95:203-11.
- Karaçay B and Bonthius DJ, 2015. The neuronal nitric oxide synthase (nNOS) gene and neuroprotection against alcohol toxicity. *Cell Mol Neurobiol* 35:1-13.
- Michard E, Lima PT, Borges F, et al., 2011. Glutamate receptor-like genes form Ca²⁺ channels in pollen tubes and are regulated by pistil D-serine. *Science* 332:434-7.
- Miyawaki I, Nakamura K, Yokubol B, et al., 1997. Suppression of cyclic guanosine monophosphate formation in rat cerebellar slices by propofol, ketamine and midazolam. *Can J Anaesth* 44:1301-7.
- Morgan CJ and Curran HV, 2012. Independent Scientific Committee on Drugs (ISCD) Ketamine use: a review. *Addiction* 107:27-38.
- Neitz A, Mergia E, Imbrosci B, et al., 2014. Postsynaptic NO/cGMP increases NMDA receptor currents via hyperpolarization-activated cyclic nucleotide-gated channels in the hippocampus. *Cereb Cortex* 24:1923-36.
- Russwurm M, Russwurm C, Koesling D, et al., 2013. NO/cGMP: the past, the present, and the future. *Methods Mol Biol* 1020:1-16.
- Sagi Y, Heiman M, Peterson JD, et al., 2014. Nitric oxide regulates synaptic transmission between spiny projection neurons. *Proceedings of the National Academy of Sciences of the United States of America* 111:17636-41.
- Santos GM, Bt BDL and Tendillo Cortijo FJ, 2013. Effects of intramuscular alfaxalone alone or in combination with diazepam in swine. *Vet Anaesth Analg* 40:399-402.
- Satoh E and Nakazato Y, 1992. On the mechanism of ouabain-induced release of acetylcholine from synaptosomes. *J Neurochem* 58:1038-44.
- Schnoebel R, Wolff M, Peters SC, et al., 2005. Ketamine impairs excitability in superficial dorsal horn neurones by blocking sodium and voltage-gated potassium currents. *British J Pharmacol* 146:826-33.
- Shen K, Johnson DW and Gobe GC, 2016. The role of cGMP and its signaling pathways in kidney disease. *American journal of physiology. Renal Physiol* 311:F671-81.
- Wang J, Goffer Y, Xu D, et al., 2011. A single subanesthetic dose of ketamine relieves depression-like behaviors induced by neuropathic pain in rats. *Anesthesiology* 115:812-21.
- Wang Y, Jia B, Li X, et al., 2017. The effect of xylazine anesthesia on goats central NO/cGMP Pathway. *Pak Vet J* 37:415-20.
- Zacharioudaki A, Lelovas P, Sergentanis TN, et al., 2017. Induction of anaesthesia with remifentanyl after bolus midazolam administration in Landrace/Large White swine. *Vet Anaesth Analg* 44.
- Zhou C, Liang P, Liu J, et al., 2015. HCN1 channels contribute to the effects of amnesia and hypnosis but not immobility of volatile anesthetics. *Anesth Analg* 121:661-6.
- Zhou ZS and Zhao ZQ, 2000. Ketamine blockage of both tetrodotoxin (TTX)-sensitive and TTX-resistant sodium channels of rat dorsal root ganglion neurons. *Brain Res Bull* 52:427-33.