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A mechanism study on propofol's action on middle latency auditory evoked potential by neurons in ventral partition of medial geniculate body in rats

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Abstract. – OBJECTIVES: To investigate the effect of propofol on the middle latency auditory evoked potentials (MLAEP) by neurons in the ventral partition of medial geniculate body (MGBv) in rats and study their mechanism.

MATERIALS AND METHODS: Sprague-Dawley (SD) rats were randomly divided into 7 groups (n = 6): group normal saline (NS), group intralipid (I), and groups of different concentrations of propofol (5.6, 16.8, 56, 168, 560 μmol/L) (P1-P5). These animals were anesthetized with ether, tracheostomized, and mechanically ventilated. After anesthesia, rats were paralyzed with vecuronium and fixed. A recording needle electrode with drugs was inserted into MGBv by means of stereotaxis. After injection of 0.2 μl propofol, normal saline or intralipid, correct insertion was verified by MLAEP response to standard sound. MLAEP including amplitudes and latencies of N0, P0, Na, Pa, and Nb waves were recorded. To identify which ion channel could be impacted by propofol, SD rats were divided into Ni^{2+} plus propofol (A1 group), Cd^{2+} plus propofol (A2 group), 4-AP plus propofol (A3 grouop), and TTX plus propofol (A4 group). The changes of MLAEP were recorded between injecting 4 ion channel blockers and propofol. Whole-cell patch clamp technique was used to confirm these variations.

RESULTS: There was no significant changes in all waves of MLAEP in MGBv after drug administration as compared with the baselins before injection in group NS, I, P1, and P2. The latency of Na, Pa, and Nb wave was significantly prolonged and the amplitude of Pa wave was decreased after injection as compared with the baseline in group P3, P4, and P5. The latency of Na, Pa, and Nb waves was significantly longer and the amplitude of Pa wave was significantly lower in group P3, P4, and P5 than in group NS and I. With TTX, amplitudes were decreased in wave Na-Pa. After given propofol, waves were unchanged in Na-Pa and latency was increased in Na, Pa, and Nb. With Cd^{2+}, latency was unchanged in Na, Pa, and Nb as well as amplitudes decreasing in Na-Pa. Nevertheless, after given Ni^{2+} and 4-AP, every wave of MLAEP had no changes. After injected propofol, amplitudes were decreased in wave Na-Pa and latency was increased in Na, Pa, and Nb. The results of patch clamp showed 56 μmol/L, 168 μmol/L and 560 μmol/L propofol inhibited the persistent sodium currents and high voltaged activated calcium currents in the brain slices of rats.

CONCLUSIONS: Propofol in 56 μmol/L, 168 μmol/L and 560 μmol/L can inhibit MLAEP in MGBv of rats in a dose-dependent manner and these changes may be caused by blocking the ion channel of persistent sodium currents and high voltaged activated calcium currents.

Key Words:
Propofol, Medial geniculate body, Middle latency, Auditory evoked, Potential, Electrophysiology, Persistent sodium, Currents, High voltaged activated, Calcium currents, Whole-cell patch clamp.

Introduction

Medial geniculate body, an important nucleus mass of auditory system in cerebral ganglion, is composed of three subgroups: ventral part, dorsal part, and medial part[1]. Ventral partition of the medial geniculate body (MGBv) is the specific auditory relay nucleus, the afferent fibers of which mainly come from ipsilateral inferior colliculus central nucleus and the efferent fibers project to primary auditory cortex[2]. It has been implicated by previous studies that MGBv is a

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key part in sound introduction and regulation, during which, sound of different characteristics is reflected by actions potentials of different modes fired by MGBv[3-8]. In alert wakefulness, sound stimulation could trigger action potential firing in "tonic" mode, while, in slow wave sleep, it could trigger the firing in "burst" mode. Different membrane potential could also induce the firing of different action potentials. The simulation could induce the firing in "tonic" mode, when membrane potential is at –56 mV, in "tonic" mode with delay of hundreds of seconds when membrane potential is at –60 mV, and in "burst" mode when membrane potential is at –75 mV. The different modes of firing is related to T type of Ca^{2+} current, high pressure activation Ca^{2+} current, A type of K^+ current and subthreshold Na^+ current, among which, T type of Ca^{2+} current induces low threshold Ca^{2+} spike potential, high pressure activation Ca^{2+} current induces high threshold Ca^{2+} spike potential. A type of K^+ current regulates the membrane excitability, and subthreshold Na^+ current increases the membrane excitability[9]. However, no previous report has covered the relationship between MLAEP and the firing mode of the specific action potential of MGBv, the important part in sound regulation and main nucleus mass in MLAEP production.

Propofol is an intravenous anesthetic currently widely employed in clinical practice, while the detailed mechanism relating to its anesthetic action has not been fully understood. It has been coincidentally indicated by some initial studies that propofol could influence the release of specific neurotransmitter in central nervous system and shares its own specific action on different areas of the brain[10-19].

However, little is known about the sensitivity of propofol on MGBv, the main nucleus mass in MLAEP production.

Therefore, in this study, we aim to establish the relationship between propofol and MLAEP via intra-nucleus mass localized injection of different concentrations of propofol and paralleled observation of changes in MLAEP.

Materials and Methods

Experimental Animals

Clean and healthy Sprague-Dawley (SD) rats with sensitive ear concha reaction, no restriction on the gender, weighing at 250-300 g, were provided by then experimental animal center of Medical College of Fudan University. The animals were housed in separate cages of relative clean environment under constant room temperature, and fed with standard feeds and freely obtained water. The rats were randomly divided into 12 groups.

Establishment of Animal Model

Tracheostomy intubation was conducted under etherization, paralleled with tracheostomy mechanical ventilation. Upon termination of ether inhalation, the rats were administered with vecuronium bromide through micropump intravenous infusion at a dose of 0.1 $mg \cdot kg^{-1} \cdot h^{-1}$. Then, the rats were fixed on brain stereotaxic apparatus with hollow ear bar, and a water-cushion was placed under the animal to keep the rectal temperature at around 37°C. According to the physiological atlas of the brain (Pixinos and Watson atlas), with the anterior fontanel as the marker, the position of MGBv nucleus mass was primarily located[20]. The fur of corona capitis was removed, periost was separated with blunt dissection and a small hole was drilled with dental drill to expose dura mater membrane. A hollow stainless steel needle with an external diameter of 100 μm at the pointed end was employed as the active electrode, and as the intra-nucleus mass recording electrode simultaneously and the reference electrode was placed at prefrontal region. The groundwire was connected to an acupuncture needle, which was pricked into the tail end of the rat. The active electrode was inserted into the position 1 mm above the presumed depth, when the recording of AEP began, and the electrode was further inserted at 0.5 mm, until the multiplication of the electric potential was recorded, which was a demonstration that the pointed end of the electrode has entered into the nucleus mass. Readjust the depth of the electrode to achieve the stabilization of maximum AE. Then propofol (Batch No.: 23148F04, AstraZeneca Company, the UK, London) or propofol dissolvent (Batch No.: 7241A181, Fresenius Co. Ltd, Bad Homburg, Germany) or saline (part 1) was administered with internal cannula.

With the same method as the above, blocking agent of T type of Ca^{2+} current, Ni^{2+}, or blocking agent of high pressure activation Ca^{2+} current, Cd^{2+}, or blocking agent of A type of K^+ current, 4-AP, or blocking agent of subthreshold Na^+ current, TTX, was respectively administered with internal cannula before the administration of propofol (part 2).

Recording of Wave Form

Wave form and wave amplitude of MLAEP was correspondingly recorded about 1 min after drug administration. MLAEP is mainly composed of N_0, P_0, Na, Pa, and Nb wave. In this study, wave forms of N_0, P_0, Na, Pa, and Nb, as well as Na-Pa wave amplitude were mainly recorded. After the procedure, three rats were randomly selected from each group, and 0.1 μl of 2% pontamine sky blue was injected at the micro-injection point before the brain tissue was collected with decapitation. The brain sample was soaked in 4% decapitation solution, and coronal sections of the brain sample at a thickness of 50 μm were produced 1-2 days later with freezing microtome. The sections were dyed with neutral red, and the injection point was observed under microscope, to confirm the position of the intra-nucleus mass recording electrode.

Hearing Test

Method of the Hearing Test

The sound stimulation was imposed with version 10 MedelecSynergy evoked potential apparatus (Oxford Medical Treatment Facility Company, UK) through ear plug and hollow ear bar. The stimulation was mainly srarefaction click (click) at a main pulse width of 0.1 ms, tone burst (SPL sound) at a strength of 100 dB, and the smoothing frequency band was 30-500 Hz, the sensitivity was 2 μV, the average overlap was 100 times. MGBv intra-nucleus mass electric potential was tested pre- and post-administration, and wave latency period and amplitude of wave forms like N_0, P_0, Na, Pa, and Nb of MLAEP of MGBv intra-nucleus mass were recorded.

Preparation of Brain Slice

Artificial cerebrospinal fluid was initially prepared and the temperature was kept at 0-4 centi-degree, while constant ventilation of 95% O_2+5% CO_2 mixed air was conducted to keep the oxygen saturation state. Then, SD rats of 2-3 weeks old, after etherization, were decapitated, and the whole brain tissue was quickly collected. The obtained brain sample was then soaked in previously prepared artificial cerebrospinal fluid for 1-2 min of cooling. After that, the sample was took out, and some chipping was performed (prefrontal lobe and tissue below the cerebellum was cut off), before the sample was placed on the object stage of the section cutter. Appropriate amount of freezing cerebrospinal fluid was added so that the whole sample could be immerged. Then, with constant oxygen ventilation, the tissue sample was cut into coronal sections with the thickness of 300-400 μm by vibratome. Brain sections containing MGBv was selected and placed into oxygen-saturated cerebrospinal fluid at 30-35 centi-degree for 1 h incubation before use[21].

Whole-Cell Recording

(1) Localization under anatomical microscope, hippocampus CA3 region, external geniculate body, and lateral part of *substantia nigra* were chosen as the localization mark of MGBv[22,23]. (2) Conduct sealing and rupture of the membrane to achieve whole-cell recording mode, and place the amplifier of the patch clamp at voltage clamp mode. Get the microelectrode slowly close to the cell membrane with readjust operator, and switch the patch clamp amplifier to current clamp mode as soon as successful sealing was achieved. Impose the microelectrode with temporal negative pressure (about 100 cm water column) to break the cell membrane and achieve whole-cell recording mode. (3) Current recording at voltage clamp mode STEP was employed as the stimulation modality. The command potential was −70 mV (sodium current) or −60 mV (calcium current), and the corresponding test potential was −30 mV, and −10 mV, respectively. (4) Signal collection: Signal was amplified with Axon200B (Axon, Union City, USA) amplifier, filtered with 1 kHz low through put, and collected and storaged with Clampex procedure of Pclamp6.

Whole-cell Patch Clamp Recording

Find the ventral part of the MGBv under the microscope. Add the electrode internal fluid into the microelectrode with syringe equipped with thin needle from behind. Get the microelectrode close to the selected cell with readjust operator, and begin the sealing procedure. Switch the patch clamp amplifier to current clamp mode after successful sealing, when the observed electric potential was at about 0 mV, and then impose the microelectrode with temporal high negative pressure (about 100 cm water column) to break the cell membrane and achieve whole-cell patch clamp mode. In this condition, the electrode internal fluid could communicate with the intracellular fluid, and the potential value displayed on patch clamp amplifier was the cell membrane potential. Cells with the membrane potential at −45 mV or below were chosen for recording.

After the cell membrane potential was stabilized, when recording constant sodium current, place the cell clamp at −70 mv and impose a voltage change from −70 mv to −30 mv with jump step mode, at a time course of 1 s, an interval of 20 s, and remove the leak current with P/4 procedure. During the recording of calcium current, the operation was basically the same with that of sodium current recording, while the command potential was −60 mv and a voltage change from −60 mv to −10 mv was imposed, at a time course of 300 ms. The voltage recording range of I-V curve was from −60 mv to 40 mv.

Figure 1. The shadow indicated the localization of intra-nucleus mass recording electrode of MGBv.

Statistical Analysis

SPSS12.0 package was employed for data treatment. All measurement data were expressed as mean ± standard deviation (mean ± SD). Paired *t*-test was employed for intra-group comparison, and one-way ANOVA was used for group comparison. Chi-square test was used for numeration data comparison. $p < 0.05$ was recognized as with statistical significance.

Results

Localization of intra-nucleus mass recording electrode (Figure 1).

No statistical significance was determined during the group comparison of weight and gender ($p > 0.05$) (Table I).

Compared with that of pre-administration, no significant changes were detected in MLAEP waves inside MGBv among 5.6 μmol/L (P1 group), 16.8 μmol/L (P2 group) propofol, saline group (NS group), and propofol dissolvent group (I group) ($p > 0.05$). Drug administration all induced latency period extension of MLAEP Na, Pa, and Nb waves and decrease of Na-Pa wave amplitude of MGBv intra-nucleus mass in 56 μmol/L (P3 group), 168 μmol/L (P4 group) and 560 μmol/L (P5 group) propofol group ($p < 0.05$). Compared with NS group, the effect of propofol in P3, P4, and P5 group displayed certain degree of dose-dependent inhibition on MLAEP (Figure 2).

Administration of blocking agent of T type of Ca^{2+} current, Ni^{2+}, induced no significant influence on MLAEP. After propofol medication, Na-Pa wave amplitude was decreased in A1 group, which was paralleled with latency period extension of Na, Pa, and Nb waves. After administration of blocking agent of high pressure activation Ca^{2+} current, Cd^{2+}, significant latency period extension of MLAEP Na, Pa, and Nb waves was induced. Then, after propofol medication, no latency period changes were detected in Na, Pa, and Nb in B1 group, paralleled with wave amplitude decrease of Na-Pa. After administration of blocking agent of A type of K^+ current, 4-AP, no significant changes was detected in rat MLAEP. After propofol medication, Na-Pa wave amplitude decrease was detected in B1 group, paralleled with latency period extension of Na, Pa, and Nb waves. After administration of blocking agent of subthreshold Na^+ current, TTX, significant decrease of rat MLAEP Na-Pa wave amplitude was detected. After propofol medication, no Na-Pa wave amplitude changes was detected in D1 group, paralleled with latency period extension of Na, Pa, and Nb waves (Figure 3).

Table I. Comparison of weight and gender in different groups (n = 6).

	NS group (NS)	I group (I)	P1 group (P1)	P2 group (P2)	P3 group (P3)	P4 group (P4)	P5 group (P5)
Weight (g)	259 ± 9	268 ± 9	270 ± 15	260 ± 12	267 ± 10	256 ± 11	260 ± 14
Gender (M/F)	3/3	3/3	4/2	2/4	3/3	3/3	3/3

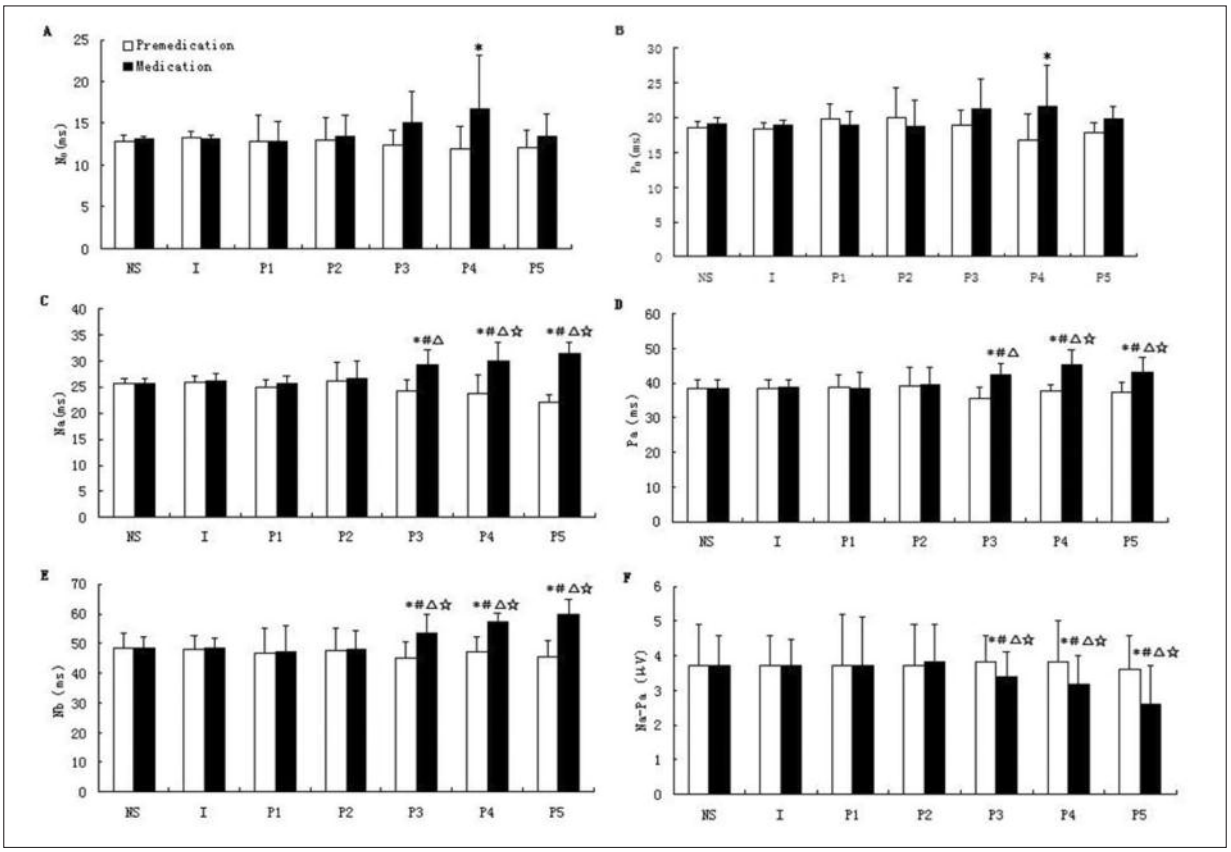

Figure 2. Comparison of MLAEP to different dose of propofol in rats MGBv. ***A,*** The change of N0 waves caused by different dose of propofol. ***B,*** The change of P0 waves caused by different dose of propofol. ***C,*** The change of Na waves caused by different dose of propofol. ***D,*** The change of Pa waves caused by different dose of propofol. ***E,*** The change of Nb waves caused by different dose of propofol. ***F,*** The change of Na-Pa waves caused by different dose of propofol. Dose of propofol was 5.6 μmol/L, 16.8 μmol/L, 56 μmol/L, 168 μmol/L, 560 μmol/L respectively in P1, P2, P3, P4, and P5 group. NS and I group injected normal saline (NS) or propofol dissolvent. Significance was considered at $p < 0.05$. $^{*}p < 0.05$ indicated medication compare to premedication; $^{\#}p$ versus NS group; $^{\Delta}p < 0.05$ versus I group. $^{\star}p < 0.05$ versus P1 group.

Test of propofol's inhibition effect on MGBv cell constant sodium current by whole-cell patch clamp technique. Under normal condition, the employed stimulation modality of orbicular-ovate cells in brain slices was jump step mode (command potential at −70 mV, test potential at −30 mV, stimulation time course of 1 s, stimulation interval of 20 s), which was able to record constant sodium current, and the stabilization time course could reach 400 s. After 120 s reaching the current stabilization (as the control of pre-administration), perfuse the slice with ACSF containing 56 μmol/L propofol. The inhibition effect of propofol on constant sodium current could be observed within 400 s and the average value of constant sodium current was decreased from 1.000 ± 0.0222 pA to 0.5558 ± 0.0277 pA after administration ($n = 6$, Figure 4 A and B); in the time course figure, compared with the control group (simultaneously perfused with ACSF containing no drug), significant inhibition effect was detected 280 s after administration in the drug group (control group $n = 6$, propofol 56 μmol/L group $n = 6$, Figure 4C). In order to further investigate the character of propofol's inhibition effect on constant sodium current, the above experimental procedure was repeated under different drug concentrations. It was shown that administration with concentration above 16.8 μmol/L all induced certain degree of inhibition on constant sodium current, and the maximal effect was obtained at 560 μmol/L (cell population of each group $n = 6$, Figure 4D). Therefore, it was concluded that propofol produced dose-dependent inhibition on sodium current.

Similar effect was observed on propofol's action on high pressure activation calcium potential of rat MGBv ventral neurons. After 120 s reaching the current stabilization (as the control of

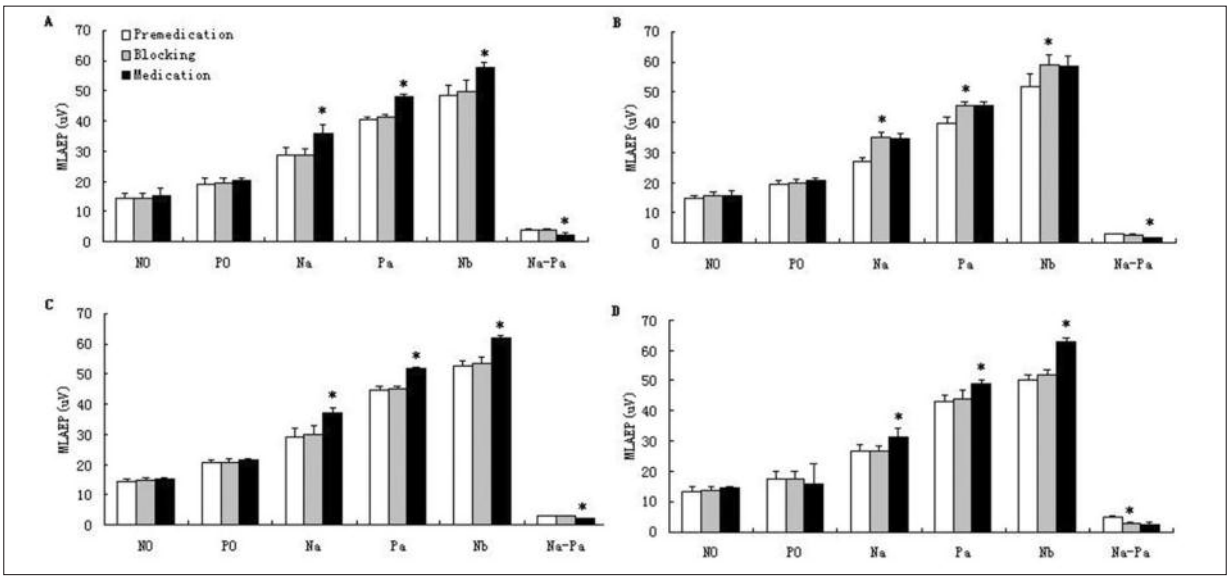

Figure 3. The effect on MLAEP of MGBv with different channel blockers. The changes of No, Po, Na, Pa Nb, and Na-Pa waves caused by 56 μmol/l propofol were recorded after treating with ***(A)*** Ni^{2+}, blocking agent of T type of Ca^{2+} current, ***(B)*** cd^{2+}, blocking agent of high pressure activation Ca^{2+} current, ***(C)*** 4-AP, blocking agent of A type of K^+ current or TTX, blocking agent of subthreshold Na^+ current. Significance was considered at $p < 0.05$. *$p < 0.05$ versus premedication.

pre-administration), perfuse the slice with ACSF containing 56 μmol/L propofol (Figure 5A). The inhibition effect of propofol on high pressure activation calcium current could be observed within 400 s, and the average value of calcium current was decreased from 0.9223 ± 0.0267 pA to 0.7795 ± 0.0314 pA after administration ($n = 6$, $p < 0.05$, Figure 5B); in the time course figure, compared with the control group (simultaneously perfused with ACSF containing no drug), significant inhibition effect was detected in the drug group (control group $n = 6$, DHEAS 1μmol/L group $n = 6$, Figure 5C). The above experimental procedure was repeated under different drug concentrations. It was shown that administration with concentration from 5.6 μmol/L to 560 μmol/L induced certain degree of inhibition on high pressure activation calcium current and the maximal effect was obtained at 560 μmol/L (cell population of each group $n = 6$, $p < 0.05$, Figure 5D). Therefore, it was concluded that propofol also produced inhibition on calcium current.

Discussion

In this study, via central nucleus mass microinjection method, the effect of propofol under different concentration combined with related ion channel blocking agent on the wave amplitude and middle latency period auditory-evoked potential (MLAEP) of rat MGBv ventral neurons was observed and the corresponding mechanism was investigated. Finally, via *in vitro* patch clam technique, the influence of propofol on related ion channels of rat MGBv ventral nucleus mass was tested.

Anesthesia depth monitoring is one of the key points in current anesthesiology studies. In clinical practice, indexes like blood pressure, heart rate, and sweating were used to determine the anesthesia depth, while all of the above lacked the sensitivity and specificity, which is unable to avoid over deep or over superficial anesthesia. Auditory evoked potential (AEP) is the mainly employed brain electric technique of clinical anesthesia depth monitoring. Central nucleus mass direct micro-injection method is a direct method to observe the effect of drug on cell, to explore the action site of the drug, and to avoid the influences caused by factors like metabolism during injection through routine routes (intravenous, intramuscular injection, etc.)[24]. In this study, this technique was employed to directly observe the effect of intravenous anesthetic, propofol, on MGBv ventral neurons producing MlAEP and to record MLAEP of MGBv. Meanwhile, referring to related reports, different concentration of propofol was administered to determine the existence of dose-dependent manner.

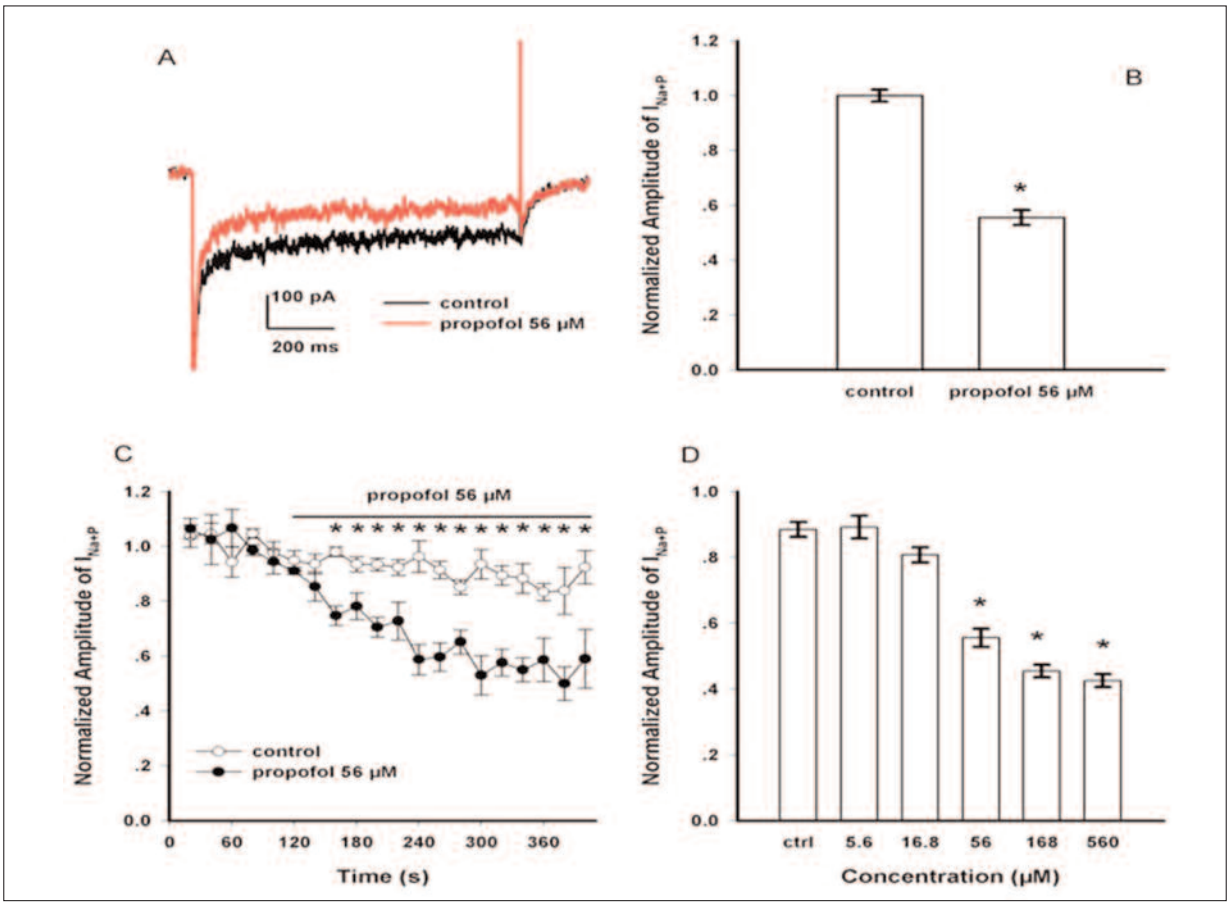

Figure 4. The effect of propofol on sodium current. ***A,*** The sodium current was respectively recorded in 4.5 min interval during the premedication and medication of propofol in 56 μmol/L command potential at –70 mV, test potential at –30 mV, stimulation time course of 1 s, stimulation interval of 20 s). ***B,*** The changes of sodium current was illustrated in 280 ms interval between the premedication and medication of 56 μmol/L propofol (n = 6, $p < 0.05$). ***C,*** The process of propofol in 56 μmol/L affecting on the sodium current (n = 6, $*p < 0.05$ versus control group). ***D,*** Propofol produced dose-dependent inhibition on sodium current (n = 6, $*p < 0.05$ versus control group).

It was shown that propofol with concentration above 56 μmol/L could produce influence on MLAEP wave amplitude and latency period of some rat MGBv ventral neurons, with main demonstration on Na, Pa, and Nb waves. This result indicated that propofol with the concentration of complete anesthesia could induce changes of wave amplitude and latency period of the rat latency period auditory-evoked potential, and with the increase of concentration, latency period of Na, Pa, and Nb waves was extended while wave amplitude of Na-Pa was decreased. Results of this study were in accordance with the many previous reports like Schwender et al[25], which was that intravenous anesthetic-induced changes of MLAEP wave form mostly involved the latency period and wave amplitude of Na, Pa, and Nb waves. The possible reason could be that Na, Pa, and Nb waves were relatively stable, while N_0 and P_0 waves mainly reflected early electric potential changes of MGBv nucleus mass, which was not so stable in caudal colliculus that so significant change was detected after drug injection. Furthermore, it was demonstrated by many studies that wave forms of MLAEP related to ambiguous memory were mainly Na, Pa, and Nb waves[26]. Therefore, through the study of Na, Pa, and Nb waves, deeper investigation of MLAEP mechanism could be performed.

The different modes of action potentials were mainly associated with the relevant currents, that is the different variations of the main waveform were mainly associated with voltage-gated ion channels. Therefore, we then use different types of ion channel blockers via central nucleus mass micro-injection method to clarify the relationship between propofol and ion channels.

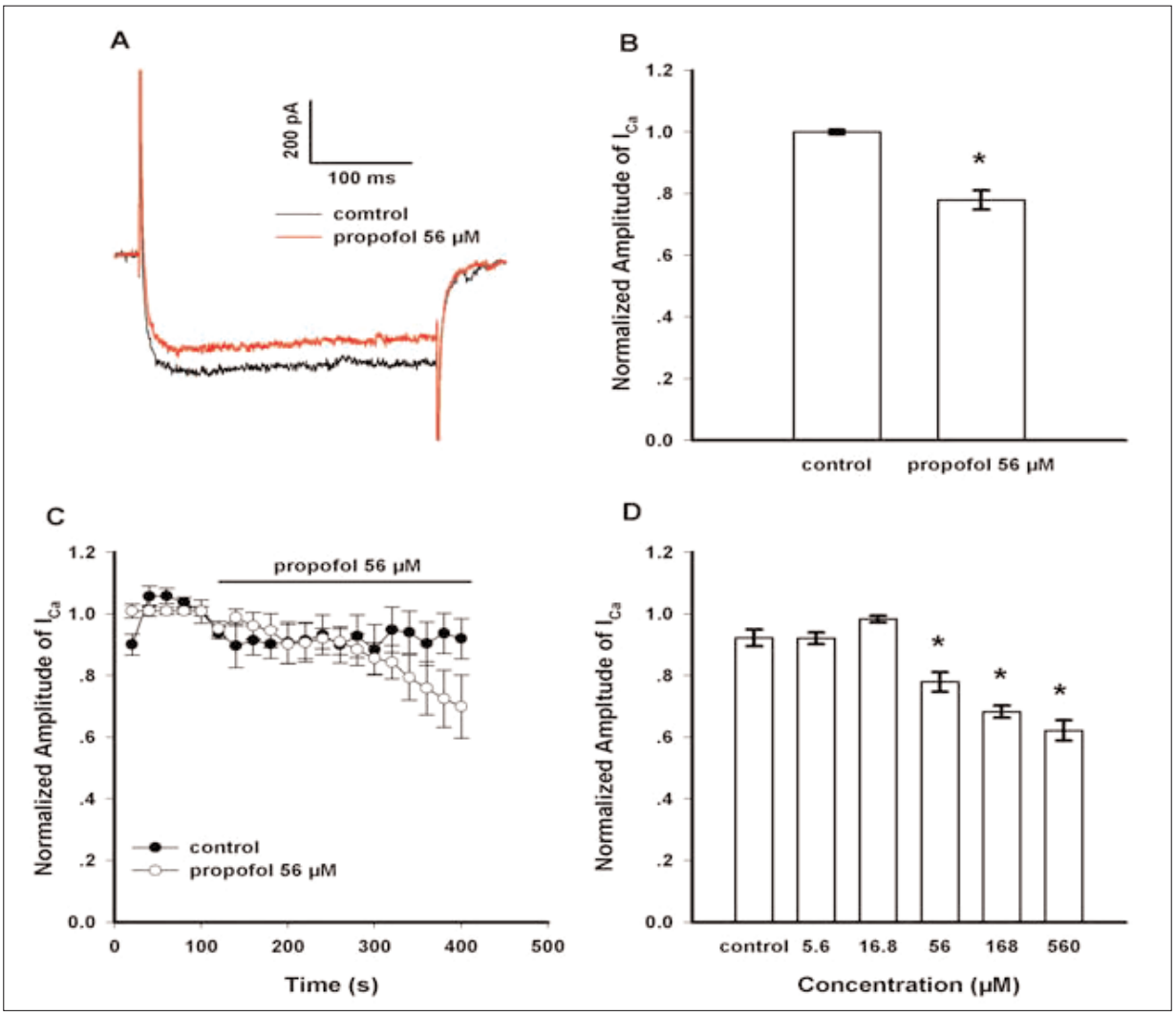

Figure 5. The effect of propofol on high pressure activation calcium potential. ***A,*** The high pressure activation calcium potential was respectively recorded in 4.5 min interval during the premedication and medication of propofol in 56 μmol/L (command potential at –60 mV, test potential at –10 mV, stimulation time course of 300 ms, stimulation interval of 20 s). ***B,*** The changes of high pressure activation calcium current were illustrated in a 280 ms interval between the premedication and medication of 56 μmol/L propofol (n = 6, $p < 0.05$). ***C,*** The process of propofol in 56 μmol/L affecting on the high pressure activation calcium current (n = 6, $*p < 0.05$ versus control group). ***D,*** Propofol produced dose-dependent inhibition on high pressure activation calcium current (n = 6, $*p < 0.05$ versus control group).

After the administration of blocking agent of T type of Ca^{2+} current and A type of K^+ current, no significant influence on rat MLAEP was observed. Then, after the medication with propofol, amplitude of MLAEP Na-Pa wave was decreased in A1 and C1 group, paralleled with the latency period extension of Na, Pa, and Nb waves. After the administration of blocking agent of subthreshold Na^+ current, TTX, significant amplitude decrease of MLAEP Na-Pa wave was observed. Then after the medication with propofol, no changes of Na-Pa wave amplitude were decreased in D1 group, paralleled with the latency period extension of Na, Pa, and Nb waves. After the administration of blocking agent of high pressure activation Ca^{2+} current, Cd^{2+}, significant latency period extension of MLAEP Na, Pa, and Nb waves was detected, while after the medication with propofol, paralleled with the decrease of Na-Pa wave amplitude. Therefore, it was considered that the mechanism of propofol's action on MlLAEP was mainly through blocking of Na^+ and Ca^{2+} ion channel and production of related potential, which imposed influence on MLAEP

(the mainly involved wave forms were Na, Nb, and Pa waves). During this process, the inhibition on Na^+ channel was mainly demonstrated by the decrease of Na-Pa wave amplitude, and the inhibition on high pressure activation Ca^{2+} channel was mainly demonstrated by the latency period extension of Na, Nb, and Pa waves. These results indicated that changes of the MLAEP produced by ventral partition of MGBv were related to voltage-gated ion channels. Meanwhile, it was shown that propofol's effect on MLAEP might be through the action on voltage-gated ion channel, and the mainly involved ion channels were Na^+ and Ca^{2+} channel[27-29].

Since the effect of propofol on MLAEP may be affected by Na^+, Ca^{2+} ion channels, we use the brain slices whole-cell patch clamp technique to further validate the effect of propofol on persistent sodium currents and activation of calcium currents of ventral neurons of medical geniculate body in rats. The results showed that propofol inhabited persistent sodium currents and activation of calcium currents on ventral neurons of medical geniculate body in rats.

Recently, the theory that general anesthesia effect of propofol was through multiple sites has been validated in many studies. Propofol was shown to not only share a close relationship with central neurotransmitter, GABA, but also enjoy relative sensitivity to central voltage-gated sodium channels and some calcium channels[17,30-37]. Therefore, it could be explained that propofol, via the effect on the ion channels of MGBv (crucial nucleus mass of brain auditory conduction pathway), produced corresponding complete anesthesia action, and also brought about changes to MLAEP of MGBv. In clinical practice, appropriated anesthesia depth monitoring was achieved by observation of wave form changes.

Conclusions

In this study, via *in vivo* and *in vitro* methods, it was primarily demonstrated that propofol produced the influence on MLAEP of MGBv probably through inhibition on constant sodium channel and high threshold calcium channel.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Morest DK. The neuronal architecture of the medial geniculate body of the cat. J Anant (Lond) 1964; 99: 143-160.
2) Winer AJ, Kelly JB, Larne DT. Neural architecture of the rat geniculate body. Hear Res 1999; 130: 19-41.
3) Tang J, Yang W, Suga N. Modulation of thalamic auditory neurons by the primary auditory cortex. J Neurophysiol 2012; 108: 935-942.
4) Xiong Y, Yu YQ, Kenji F. An *in vivo* intracellular study of auditory thalamic neurons. Thal Rel Sys 2003; 2: 253-260.
5) Yu YQ, Xiong Y, Chan YS, He JF. Corticofugal gating of auditory information in the thalamus: an *in vivo* intracellular recording study. J Nuerosci 2004; 24: 3060-3069.
6) Storace DA, Higgins NC, Read HL. Thalamocortical pathway specialization for sound frequency resolution. J Comparative Neurol 2011; 519: 177-193.
7) Ma X, Suga N. Specific and nonspecific plasticity of the primary auditory cortex elicited by thalamic auditory neurons. J Neurosci 2009; 29: 4888-4896.
8) Read HL, Miller LM, Schreiner CE, Winer JA. Two thalamic pathways to primary auditory cortex. Neuroscience 2008; 152: 151-159.
9) Tennigkeit F, Puil E, Schwarz DW. Firing modes and memberane properties in lemniscal auditory thalamus. Acta Otolaryngol 1997; 117: 254-257.
10) Grasshoff C, Drexler B, Rudolph U, Antkowiak B. Anaesthetic drugs: linking molecular actions to clinical effects. Curr Pharm Des 2006; 12: 3665-3679.
11) Cheng VY, Martin L, Elliott EM, Kim JH, Mount HT, Taverna FA, Roder JC, Macdonald JF, Bhambri A, Collinson N, Wafford KA, Orser BA. Alpha5GABAA receptors mediate the amnestic but not sedative-hypotic effects of the general anesthetic etomidate. J Neurosci 2006; 26: 3713-3720.
12) Solt K, Eger El 2nd, Raines DE. Differential modulation of human N-methyl-o-aspartate receports by structurally diverse general anesthetics. Anesth Analg 2006; 102: 1407-1411.
13) Bali M, Akabas MH. Defining the propofol binding site location on the GABAA receptor. Mol Pharmacol 2004; 65: 68-76.
14) Liu Y-W, Zuo W, Ye J-H. Propofol stimulates noradrenalin-inhibited neurons in the ventrolateral preoptic nucleus by reducing GABAergic inhibition. Anesth Analg 2013; 117: 358-362.
15) Lian Q, Wang B, Zhou, W. Self-administration of propofol is mediated by dopamine D1 receptors in nucleus accumbens in rats. Neuroscience 2013; 231: 373-383.
16) Andrada J, Livingston P, Lee BJ. Propofol and etomidate depress cortical, thalamic, and reticular

formation neurons during anesthetic-induced unconsciousness. Anesth Analg 2012; 114: 661-669.

17) Jin Z, Choi MJ, Park CS Jin YH. Propofol facilitated excitatory postsynaptic currents frequency on nucleus tractus solitarii (NTS) neurons. Brain Res 2012; 1432: 1-6.

18) Li KY, Guan YZ, Krnjevi K, Ye JH. Propofol facilitates glutamatergic transmission to neurons of the ventrolateral preoptic nucleus. Anesthesiology 2009; 111: 1271-1278.

19) Wang JP, Guo Z. Propofol suppresses activation of the nociception specific neuron in the parafascicular nucleus of the thalamus evoked by coronary artery occlusion in rats. Eur J Anaesthesiol 2009; 26: 60-65.

20) Chu X, Xu N, Li P Mao L, Wang JQ. Inhibition of cardiovascular activity following microinjection of novel opioid-like neuropeptide nociceptin (orphanin FQ) into the rat rostral ventrolateral medulla. Brain Res 1999; 829: 134-142.

21) Kitakaze M, Node K, Komamura K, Minamino T, Inoue M, Hori M, Kamada T. Evidence for nitric oxide generation in the cardiomyocytes: its augmentation by hypoxia. J Mol Cell Cardiol 1995; 127: 2149-2154.

22) Clerici WJ, Coleman JR. Postnatal cytoarchitecture of the rat medial geniculate body. J Comp Neurol 1998; 399: 110-124.

23) Yao XH, Wan ZB, Xiong Y. Alteration of membrance properties of MGBv neurons and the effect of GABA receptor agonist on them during postnatal development in rats. Neurosci Bullet 2005; 21: 182-188.

24) Smith C, McEwan AI, Jhaveri R, Wilkinson M, Goodman D, Smith LR, Canada AT, Glass PS. The interaction of fentanyl on the Cp50 of propofol for loss of consciousness and skin incision. Anesthesiology 1994; 81: 820-828.

25) Thornton C. Evoked potentials in anaesthesia. Eur J Anaesthesiol 1991; 8: 89-107.

26) Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron 2000; 26: 13-25.

27) Rehberg B, Duch DS. Suppression of central nervous system sodium channels by propofol. Anesthesiology 1999; 91: 512-520.

28) Yamakura T, Lewohl JM, Harris RA. Differential effects of general anesthetics on G protein-coupled inwardly rectifying and other potassium channels. Anesthesiology 2001; 95: 144-153.

29) Dreixler JC, Jenkins A, Cao YJ, Roizen JD, Houamed KM. Patch-clamp analysis of anesthetic interactions with recombinant SK2 subtype neuronal calcium-activated potassium channels. Anesth Analg 2000; 90: 727-732.

30) Bali M, Akabas MH. Defining the propofol binding site location on the GABAA receptor. Mol Pharmacol 2004; 65: 68-67.

31) Franks NP, Honore E. The TREK K2P channels and their role in general anaesthesia and neuroprotection. Trends Pharmacol Sci 2004; 25: 601-608.

32) Grasshoff C, Rudolph U, Antkowiak B. Molecular and systemic mechanisms of general anaesthesia: the "multisite and multiple mechanisms" concept. Curr Opin Anaesthesiol 2005; 18: 386-391.

33) Martella G, Persis CD, Bonsi P, Natoli S, Cuomo D, Bernardi G, Calabresi P, Pisani A. Inhibition of persistent sodium current fraction and voltage-gated L-type calcium current by propofol in cortical neurons: implication for its antiepileptic activity. Epilepsia 2005; 46: 624-635.

34) Sato Y, Kobayashi E, Hakamata Y, Kobahashi M, Wainai T, Murayama T, Mishina M, Seo N. Chronopharmacological studies of ketamine in normal and NMDA epsilon 1 receptor knockout mice. Br J Anaesth 2004; 92: 859-864.

35) Brown ML, Eidam HA, Paige M, Jones PJ, Patel MK. Comparative molecular field analysis and synthetic validation of a hydroxyamide-propofol binding and functional block of neuronal voltage-dependent sodium channels. Bioorg Med Chem 2009; 17: 7056-7063.

36) Jones PJ, Wang Y, Smith MD, Hargus NJ, Eidam HS, White HS, Kapur J, Brown ML, Patel MK. Hydroxyamide analogs of propofol exhibit state-dependent block of sodium channels in hippocampal neurons: Implications for anticonvulsant activity. J Pharmacol Expe Ther 2007; 320: 828-836.

37) Xie Z, McMillan K, Pike CM, Cahill AL, Herring BE, Wang Q, Fox AP. Interaction of anesthetics with neurotransmitter release machinery proteins. J Neurophysiol 2013; 109: 758-767.