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ORIGINAL ARTICLE

Propofol inhibits the local activity and connectivity of nuclei in the cortico-reticulo-thalamic loop in rats

Jing Yang[1] · Wei Wang[2] · Zheng Yong[3] · Weixiu Yuan[1] · Hong Zhang[1] · Weidong Mi[1]

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Abstract

Purpose The present study aimed to investigate the effects of different dosages of propofol, that induced different depths of anesthesia, on the local activity and connectivity of nuclei within the cortico-reticulo-thalamic loops, as well as the release of amino acids in those nuclei.

Methods The nonlinear dynamics analysis of electroencephalogram, including approximate entropy (ApEn) and cross-ApEn (C-ApEn), was used to analyze the effects of different dosages of propofol on the local activity and connectivity of the important nuclei, including the primary somatosensory cortex (S1), ventroposteromedial thalamic nucleus (VPM), reticular thalamic nucleus (RTN), and oral part of the pontine reticular nucleus (PnO). The levels of glutamate (Glu), γ-aminobutyric acid (GABA), and glycine (Gly) in the S1, VPM, and RTN were detected using cerebral microdialysis.

Results ApEn was more significantly reduced in the cortex than in the subcortical nuclei from awake to deep anesthesia state induced by propofol, and C-ApEn was also more significantly reduced between cortical nucleus and subcortical nucleus than between subcortical nuclei from awake to deep anesthesia state induced by propofol. Propofol inhibited the levels of Glu in S1 and VPM, but elevated the levels in RTN. Gly level decreased in S1, and GABA level increased in S1 after infusion of propofol.

Conclusions The cortex rather than the subcortical structures, and the cortex–subcortical connectivity instead of subcortical connectivity might be the more vulnerable targets of propofol during anesthesia.

Keywords Anesthetics · Propofol · Electroencephalography · Amino acids · Microdialysis

Abbreviations

ApEn	Approximate entropy
C-ApEn	Aross-approximate entropy
S1	Primary cortex
VPM	Ventroposteromedial thalamic nucleus
RTN	Reticular thalamic nucleus
PnO	Oral part of pontine reticular nucleus
RE	Reticular neurons
TC	Thalamocortical relay cells
EEG	Electroencephalogram
GABA	γ-Aminobutyric acid
Glu	Glutamate
Gly	Glycine
S1JO	Primary somatosensory cortex, jaw region, oral surface
MAP	Mean arterial blood pressure
HR	Heart rate
rGMR	Glucose metabolism rate

Introduction

The process of anesthesia involves the suppression of local brain activity, and disruption of specific connectivity between different brain regions [1–3]. However, the exact brain nucleus and connectivity between brain nuclei are the primary target of anesthetics remain elusive. Thalamocortical connectivity and the connectivity of the thalamus with certain subcortical regions like the putamen are extensively investigated in respect of functions during anesthesia [4–6].

✉ Weidong Mi
miweidong0709@163.com

[1] Department of Anesthesiology, Chinese People's Liberation Army (PLA) General Hospital, No. 28 Fuxing Road, Beijing 100853, China

[2] Department of Orthopedics, Chinese People's Liberation Army (PLA) General Hospital, Beijing 100853, China

[3] Department of Pharmacology, Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China

However, inconsistent results were obtained under general anesthesia [7–10], which might be ascribed to the inconsistence of anesthetic dosages, anesthesia types and the depths of anesthesia.

Propofol is widely used as general anesthetic and could increase the gamma-aminobutyric acidergic neurotransmission from cortical interneurons onto cortical pyramidal cells, and from thalamic reticular neurons (RE) onto thalamocortical relay cells (TC) [11]. RE cells are important in this mechanism because they could synchronize disparate relay nuclei and enable synchrony over larger cortical regions [11]. The reticular thalamic nucleus (RTN) is a critical relay site between the neocortex and thalamus, and could control TC activities via balancing excitatory and inhibitory amino acids, indicating the important roles of RTN in various consciousness states [12]. Previous study implicated that the anesthetic-induced decrease in cortico-reticulo-thalamic signaling enhanced the inhibition in excitatory thalamic neurons, thus decreasing their output to the cortex [4]. Therefore, it is important to elucidate the changes in the local activity and functional connectivity of nuclei in the cortico-reticulo-thalamic loop during awake state and different stages of propofol-induced anesthesia.

In this study, based on the recorded spontaneous local field potential activity of the cortical and subcortical regions from epidural/deep electrodes, the approximate entropy (ApEn) and cross-ApEn (C-ApEn) were used to investigate the effects of the different doses of propofol on the local activity and the network in the primary somatosensory cortex (S1), ventroposteromedial thalamic nucleus (VPM), RTN and the oral part of the pontine reticular nucleus (PnO).

Methods

Animals

Wistar rats [6 months, 280–350 g, supplied by the Medical Experimental Animal Center of the Chinese People's Liberation Army (PLA) General Hospital] were used in the current study. All animals were housed at a constant temperature of 24 ± 0.5 °C and a relative humidity of 60 ± 2% under a 12 h/12 h light–dark cycle. Experimental protocols were approved by the Animal Care and Use Committee of the PLA General Hospital, and the experiments were conducted under the guidelines for the Care and Use of Laboratory Animals, formulated by the Ministry of Science and Technology of the People's Republic of China.

A total of 48 rats were randomly divided into four groups ($n = 12$): somatosensory cortex + ventroposteromedial thalamic nucleus (S1 + VPM), somatosensory cortex + thalamic nucleus (S1 + RTN), ventroposteromedial thalamic nucleus + thalamic nucleus (VPM + RTN), and thalamic nucleus + oral part of the pontine reticular nucleus (RTN + PnO) groups. Rats were implanted with epidural electrodes (in S1) and/or deep electrodes (in VPM, RTN and PnO) bilaterally for cortical and subcortical electroencephalographic recording.

A total of 36 rats were randomly divided into 3 groups: S1, VPM, and RTN groups ($n = 12$). Rats were implanted with a microdialysis probe to measure Glu, GABA and Gly levels.

The coordinates of the required brain regions were determined according to the stereotaxic map of rat brain: S1 [anteroposterior (AP) 0.7 mm, mediolateral (ML) − 4.4 mm, dorsoventral (DV) 1.5 mm]; VPM (AP − 3.3 mm, ML ± 2.8 mm, H 6 mm); RTN (AP − 1.4 mm, ML ± 2 mm, H 6 mm); PnO (AP − 7.8 mm, ML ± 1.2 mm, H 8.2 mm). Histological verification for the stereotactic positioning of the electrode and microdialysis probe in the brain of rats were presented in Fig. 1.

Propofol administration and anesthetic state assessment

Propofol was infused continuously through a polyethylene catheter using a Graseby™ 3500 syringe pump (Smiths Medical, Watford, UK) (right internal jugular vein) following the schedule presented in Fig. 2. The infusion rates were, in order, 400 μg/(kg min), 600 μg/(kg min) and 800 μg/(kg min), and a 20-min infusion period was maintained at each infusion rate.

Before the alteration of the infusion rate to the next level, touch reflex test, reversal reflex test or tail-pinch reflex tests were conducted to assess the anesthetic states of rats. The rats which did not have a whisker or a swaying reaction were considered to be in a light anesthesia state. The rats could not actively correct the body and were supposed as in an intermediate anesthesia state. The rats which could not move head, limbs or trunk were thought to be in a deep anesthesia state.

Electroencephalographic recording

EEG was recorded with ZN16E EEG monitoring instrument (Sichuan Zhineng Electronics Industrial Company, Chengdu, China) with a sampling rate of 500 Hz, a band width of 0.53–100 Hz, and a 12-bit AD conversion resolution (digital to analog converter). The EEG signals were achieved by setting high-frequency filtering (100 Hz) and low-frequency filtering (0.53 Hz), and turning on notch filtering and < 2 kΩ impedances. Signal data were stored for further analysis.

The nonlinear information statistical parameters, ApEn and C-ApEn were automatically calculated by the EEG monitoring instrument. EEG was continuously recorded, and the ApEn and C-ApEn were calculated every minute.

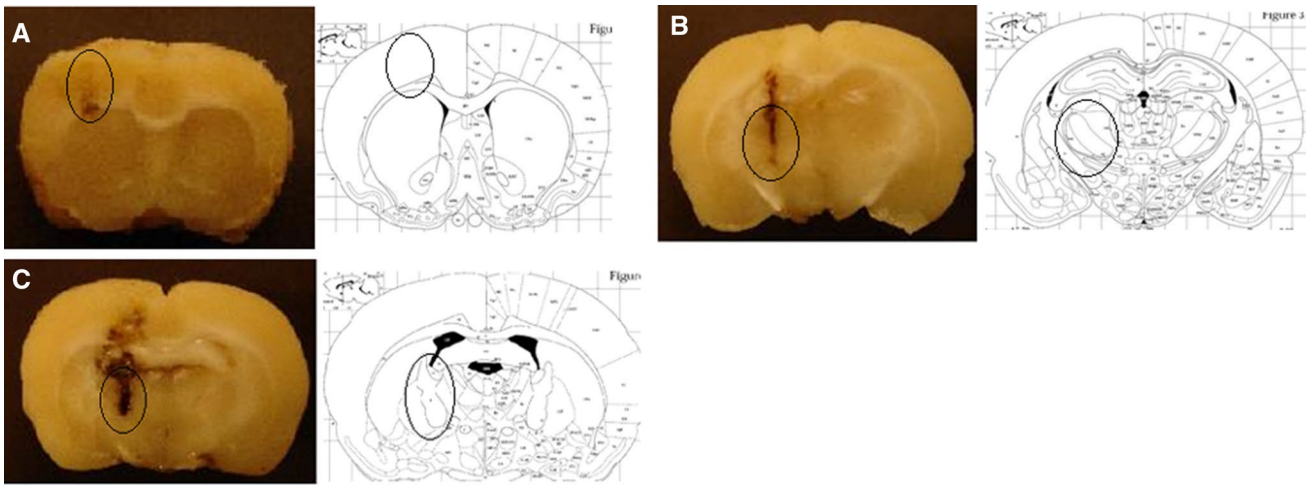

Fig. 1 Representative histological verification for the stereotactic positioning of the electrode and microdialysis probe in the brain of rats. **a** S1. **b** VPM. **c** RTN. The circles indicate the tip and the trace of the electrode/probe

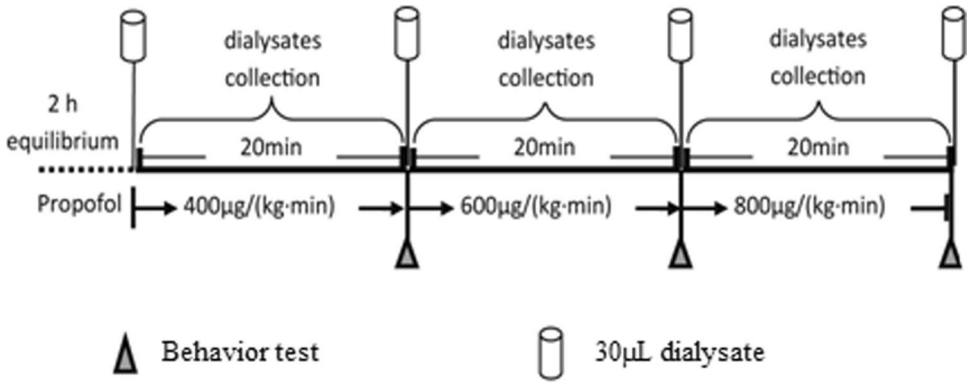

Fig. 2 Protocol of propofol administration and collection of dialysate

However, only the ApEn and C-ApEn determined from a continuous of 2-min recording were used in the analysis. The program used to calculate ApEn and C-ApEn was described in a previous study [13].

Detection of amino acids in the dialysate

Microdialysis probes (Harvard Apparatus, USA) were placed as above described. Dialysate samples (30 μl) were collected for 20 min, and stored immediately to a − 80 °C freezer prior to amino acid analysis. The concentrations of Glu, GABA, and Gly in the dialysates were measured, respectively, by OPA-β-mercaptoethanol precolumn derivatization, reversed phase gradient elution and fluorescence detection using high-performance liquid chromatography (HPLC) (Agilent, USA).

Measurement of hemodynamic parameters

The arterial blood pressure and heart rate were recorded before propofol infusion and 2 min prior to each change of infusion rate to the next level. For blood gas analysis, 0.5 ml arterial blood was collected before and after the propofol infusion to detect PaO_2 and $PaCO_2$.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and analyzed using SPSS 13.0 (SPSS, Inc.). Statistical significance for a difference in EEG, amino acid and hemodynamic parameters was tested using repeated measures of analysis of variance, followed by LSD for multiple comparisons. Hemodynamic parameters were analyzed using one-way analysis of variance. A $P < 0.05$ was considered as statistically significant.

Results

Behavioral observations

Four rats showing criteria behaviors were excluded from the experiment, including one rat still showing touch reflex after infusion of 400 μg/(kg min) propofol (from S1 + VPM EEG group), two rats still showing reversal reflex after infusion of 600 μg/(kg min) propofol (one from the S1 + RTN EEG group; one from the RTN microdialysis group), and one rat still showing tail-pinch reflex after infusion of propofol at 800 μg/(kg min) (from the S1 microdialysis group).

Electrophysiological modifications

As shown in Fig. 3, in the infusion rate of 400 μg/(kg min), only the ApEns of bilateral S1 and right VPM was significantly decreased compared to the corresponding dose of 0 μg/(kg min); in the infusion rate of 600 and 800 μg/(kg min), except the ApEn of right PnO in 800 μg/(kg min), all the other ApEns of S1, VPM, RTN, and PnO showed significantly declined than those in the corresponding dose of 0 μg/(kg min). Importantly, the reduced extent of ApEn in the cortical nucleus (S1: left 0.23, right 0.22) was more significant than that in the subcortical nuclei (VPM: left 0.15, right 0.15; RNT: left 0.08, right 0.16; PnO: left 0.16, right 0.08) from awake to deep anesthesia state.

As shown in Fig. 4, in the infusion rate of 400 μg/(kg min), all the bilateral C-ApEns did not change significantly; in the infusion rate of 600 μg/(kg min), the C-ApEn of bilateral S1-VPM, right PnO-RTN, right S1-RTN were significantly decreased compared to the corresponding dose of 0 μg/(kg min); in the infusion rate of 800 μg/(kg min), all the bilateral C-ApEn showed markedly declined. Importantly, the reduced extent of C-ApEn in the connection between cortical nucleus and subcortical nuclei (S1-VPM: left 0.12, right 0.23; S1-RTN: left 0.18, right, 0.28) was more significant than that in connection between subcortical nucleus (RNT-VPM: left 0.09, right 0.12; PnO-RTN: left 0.09, right 0.14) from awake to deep anesthesia state.

Amino acid release

The catheter of one rat from the RTN group was not placed in the intended areas, so this rat was excluded from the experiment. The Glu efflux was significantly decreased in S1 under the infusion rate of 400 μg/(kg min), and in VPM under the infusion rate of 600 and 800 μg/(kg min), but markedly increased in RTN under the infusion rate of

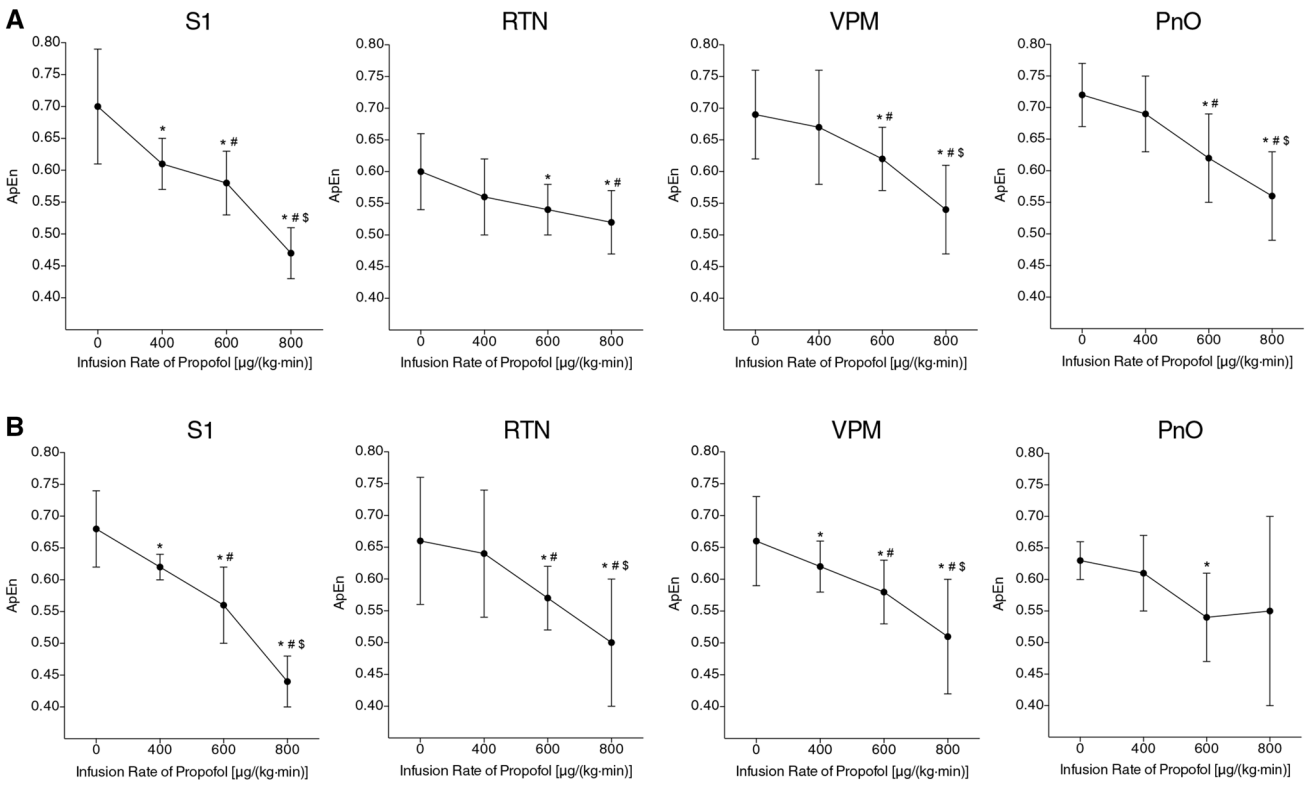

Fig. 3 Changes of the bilateral ApEns of S1, VPM, RTN, and PnO under increasing dosages of propofol. **a** Left side of ApEns. **b** Right side of ApEns. $^{*}P < 0.05$ vs. baseline; $^{\#}P < 0.05$ vs. 400 μg/(kg min) propofol infusion; $^{\$}P < 0.05$ vs. 600 μg/(kg min) propofol infusion

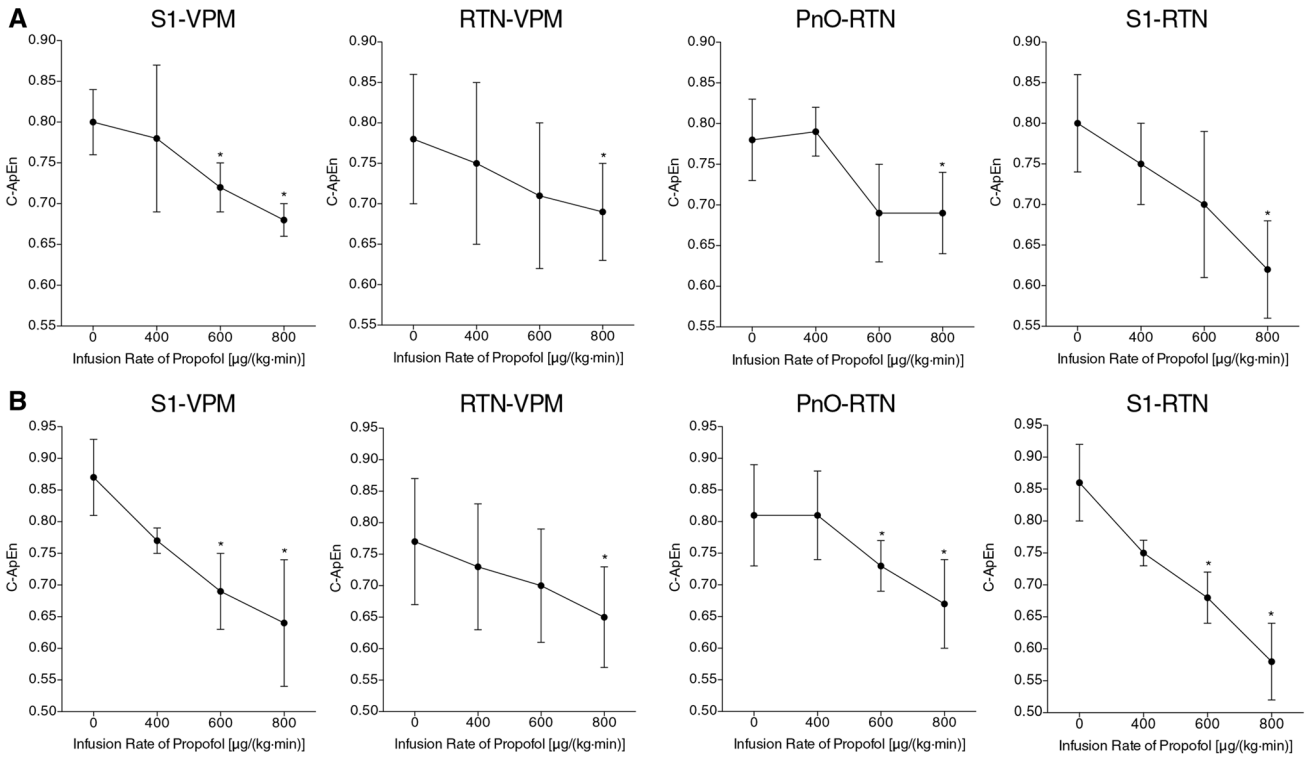

Fig. 4 Changes of the bilateral C-ApEns of S1-VPM, S1-RTN, RTN-VPM, and PnO-VPM under increasing dosage of propofol. **a** Left side of ApEns. **b** Right side of ApEns. *$P < 0.05$ vs. baseline

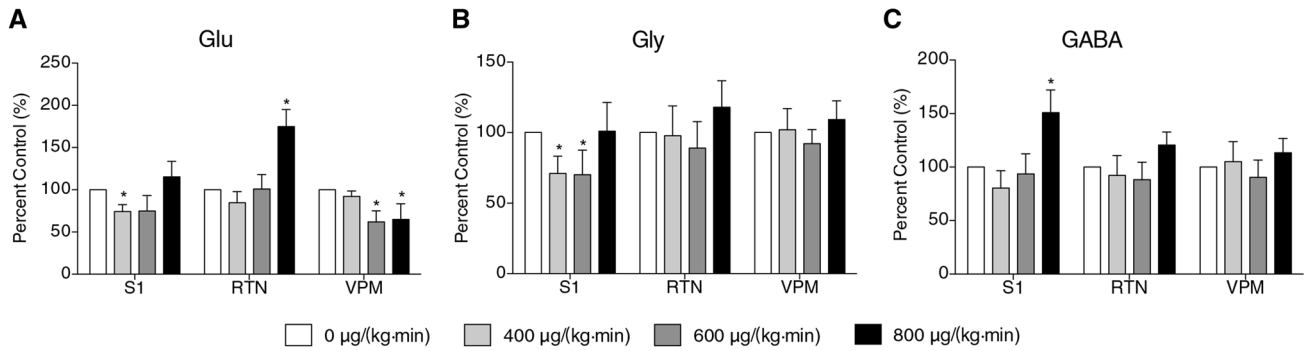

Fig. 5 Effects of increasing doses of propofol on the releases of Glu (**a**), Gly (**b**), and GABA (**c**) and in S1, RTN, and VPM of rats. *$P < 0.05$ vs. baseline

800 μg/(kg min) (Fig. 5a). The Gly efflux was significantly decreased only in S1 under the infusion rate of 400 and 600 μg/(kg min) (Fig. 5b). In addition, the GABA efflux was significantly elevated only in S1 under the infusion rate of 800 μg/(kg min) (Fig. 5c).

Hemodynamic parameters

As presented in Table 1, HR was significantly decreased under the infusion rate of 600 and 800 μg/(kg min). MAP was significantly decreased under the infusion rate of

Table 1 Effects of increasing dosages of propofol on systemic hemodynamics in the rats

	Baseline	400 μg/(kg min)	600 μg/(kg min)	800 μg/(kg min)
HR (unit)	367 ± 47	374 ± 37	329 ± 39[a]	323 ± 35[a]
MAP	121 ± 8	120 ± 7	110 ± 5	99 ± 4[a]
PaO_2	104 ± 9			98 ± 5
$PaCO_2$	31 ± 1			35 ± 1[a]

HR heart rate, *MAP* mean arterial blood pressure

[a]$P < 0.05$ compared with baseline

800 μg/(kg min). PaO_2 remained at the normal level, but $PaCO_2$ was significantly increased.

Discussion

ApEn and C-ApEn are nonlinear information statistical parameters, which can be used to characterize the dynamics of the neural networks underlying the electroencephalogram (EEG) [14]. ApEn of EEG quantifies the complexity of EEG data in a time series. High levels of ApEn during anesthesia demonstrate that the subject is awake, whereas low levels of ApEn correlate with deeper unconsciousness. C-ApEn is very similar to ApEn in design, but analyzes two related time series to measure the degree of their asynchrony. While the single-channel ApEn measures the temporal complexity of the EEG, the two-channel C-ApEn reflects the spatial decorrelation of cortical potentials from two remote sites [15, 16]. It was still unclear whether the thalamus or cortex is responsible for anesthesia-induced effects [4, 17–24]. In this study, we used the nonlinear dynamics analysis of EEG and ApEn to investigate the effect of propofol on the local activity in the cortex and subcortical nuclei. Our data indicated that the activities of the cortex and subcortical structures were suppressed by propofol. However, the reduced extent of ApEn in the cortical nucleus (S1) was more significant than that in the subcortical nuclei (VPM, RNT, and PnO) from awake to deep anesthesia state induced by propofol, implying that the spontaneous EEG activity in the cortical nucleus was inhibited more than that in the subcortical nuclei under the same anesthesia depth.

Similarly, the results from a previous imaging study indicated that the regional cerebral glucose metabolism rate (rGMR) in the occipital, temporal and frontal lobes was markedly decreased at a sedative dosage of propofol, but the change in the thalamus was not significant [25]. Therefore, these studies, including ours, indicated that the cortex rather than the subcortical structures might be a more vulnerable target for anesthesia by propofol. It is worthy to point out that the actual concentration of propofol was not detected in different regions, thus it was unclear whether the inhibitory action of propofol on cortical activity resulted from the preferential distribution of propofol in the cortex or the higher vulnerability of the cortex to propofol.

The disconnection over cortico-cortical and thalamocortical networks may partly explain the mechanisms underlying anesthetic-induced unconsciousness [26, 27]. A review of recent imaging studies on the possible targets of propofol in the human brain suggested that propofol-induced unconsciousness was associated with a global metabolic and vascular depression in the human brain, particularly in the nonspecific thalamocortical and frontoparietal networks [27]. In this study, we used the nonlinear dynamics analysis of EEG and C-ApEn, to demonstrate the effect of propofol on the connectivity between the cortex and subcortical nuclei. Our data indicated that connectivity between the cortex and subcortical structures was inhibited by propofol. However, the reduced extent of C-ApEn in S1-VPM or S1-RTN was more marked than that in RTN-VPM or PnO-RTN from awake to deep anesthesia state induced by propofol, implying that the EEG connection between the cortex and the subcortical nucleus was inhibited to a greater extent than that between the subcortical nuclei under the same anesthesia depth. According to the small-world network theory, the thalamocortical system may be especially vulnerable to anesthetics as the drugs only need to disrupt a few long-distance connections to produce a set of disconnected components [19].

The exploration of the effects of general anesthetics on the release of neurotransmitter could facilitate the understanding of the neurophysiological consequences of anesthetics. During anesthesia, EEG activity was suppressed in cortex, which involves inhibition of glutamate-mediated excitatory synaptic transmission and facilitation of GABA-mediated inhibitory synaptic transmission [28]. It has been demonstrated that propofol promotes GABA and inhibits *N*-methyl-D-aspartate neurotransmission [17]. In this study, changes in EEG were well consistent with the depth of anesthesia in rats, but not with changes in extracellular excitatory and inhibitory amino acid levels in the corresponding brain regions. In general, the inhibitory amino acids in the cortex increased significantly relative to excitatory amino acids, consistent with the continuous decline of cortical EEG activity. In addition, the excitatory amino acids in the VPM declined obviously relative to inhibitory amino acids, also consistent with the continuous reduced EEG activity in VPM. Therefore, the changes of excitatory and inhibitory amino acid almost reflected the changes of EEG activity. However, the increase of excitatory amino acids in RTN appeared to be contradicted with the decline of EEG activity. Neurons in the RTN lacked the consistency of cells in the VPM, so the changes of EEG activity shown in this study might not reflect the activity of entire RTN. Furthermore, Glu could also exert an inhibitory effect on certain RTN cells via the metabolophilic glutamate receptor [29].

Conclusion

Propofol possibly targets the cortex rather than the subcortical structures, and the cortex-subcortical connectivity instead of subcortical connectivity to exert the anesthesia activity.

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