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ORIGINAL ARTICLE

Phospholipase C-related inactive protein type-1 deficiency affects anesthetic electroencephalogram activity induced by propofol and etomidate in mice

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Abstract

Purpose The general anesthetics propofol and etomidate mainly exert their anesthetic actions via GABA A receptor ($GABA_A$-R). The $GABA_A$-R activity is influenced by phospholipase C-related inactive protein type-1 (PRIP-1), which is related to trafficking and subcellular localization of $GABA_A$-R. PRIP-1 deficiency attenuates the behavioral reactions to propofol but not etomidate. However, the effect of these anesthetics and of PRIP-1 deficiency on brain activity of CNS are still unclear. In this study, we examined the effects of propofol and etomidate on the electroencephalogram (EEG).

Methods The cortical EEG activity was recorded in wild-type (WT) and PRIP-1 knockout (*PRIP-1 KO*) mice. All recorded EEG data were offline analyzed, and the power spectral density and 95% spectral edge frequency of EEG signals were compared between genotypes before and after injections of anesthetics.

Results PRIP-1 deficiency induced increases in EEG absolute powers, but did not markedly change the relative spectral powers during waking and sleep states in the absence of anesthesia. Propofol administration induced increases in low-frequency relative EEG activity and decreases in SEF95 values in WT but not in *PRIP-1 KO* mice. Following etomidate injection, low-frequency EEG power was increased in both genotype groups. At high frequency, the relative power in *PRIP-1 KO* mice was smaller than that in WT mice.

Conclusions The lack of PRIP-1 disrupted the EEG power distribution, but did not affect the depth of anesthesia after etomidate administration. Our analyses suggest that PRIP-1 is differentially involved in anesthetic EEG activity with the regulation of $GABA_A$-R activity.

Keywords Propofol · Etomidate · Phospholipase C-related inactive protein type-1 · Electroencephalogram · $GABA_A$ receptor

Introduction

Intravenous anesthetics such as propofol and etomidate induce general anesthesia. The electroencephalogram (EEG) is used to monitor the depth of anesthesia during the induction and maintenance of general anesthesia. The alpha activity of EEG

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corresponds to the levels of consciousness, and the delta activity of EEG is a typical signature of unconsciousness [1–3]. Propofol and etomidate administration increase alpha and beta activity, followed by an increase in delta activity and a decrease in beta and alpha activity in EEG [4, 5]. By the end of the infusion, propofol and etomidate decrease delta activity [4]. Several studies suggest that anesthetic effects such as sedation, neural activity, hemodynamic stability and duration of EEG seizures during electroconvulsive therapy differ between etomidate and propofol [6–9]. The neurological mechanisms underlying the anesthetic actions of these anesthetics are thought to act mainly on the $GABA_A$-R β3 subunit [8, 10–12]. Moreover, etomidate targets not only β3, but also the β2 subunit of $GABA_A$-R [13–16]. Furthermore, the activity of $GABA_A$-R is related to the β and γ bands of EEG [17]. This evidence suggests that $GABA_A$-R properties, such as membrane expression and phospho-state, could affect anesthetic effects and EEG characteristics.

Phospholipase C-related inactive protein type-1 (PRIP-1) is involved in the membrane trafficking and phosphorylation of $GABA_A$-R via the interaction with $GABA_A$-R β subunits [18–21]. Our previous studies demonstrated that PRIP-1 deficiency induces dysfunction of extrasynaptic GABAergic transmission and temporal lobe epileptic EEG discharges [22, 23]. The cell membrane expression of the $GABA_A$-R β3 subunit in *PRIP-1 KO* mice is significantly lower than that in wild-type (WT) mice and is restored by protein phosphatase inhibition [24]. *PRIP-1 KO* mice exhibit a significantly shorter duration of the loss of righting reflex (LORR) induced by propofol than do WT mice, whereas the duration of LORR induced by etomidate is unaffected by PRIP-1 deficiency [24]. This evidence suggests that PRIP-1 mediates the cell surface expression level of the $GABA_A$-R β3 subunit and GABAergic inhibitory neurotransmission, consequently modulating general anesthetic action. However, whether PRIP-1 is involved in the anesthetic effects of propofol and etomidate on brain activity remains unknown.

In the present study, we performed EEG analysis to investigate brain activity during propofol or etomidate using *PRIP-1 KO* mice after intraperitoneal (i.p.) injection of anesthetics. *PRIP-1 KO* mice did not show abnormal EEG activity without anesthesia, but EEG spectral patterns during anesthesia were significantly affected by PRIP-1 deficiency. Additionally, propofol and etomidate have different effects on anesthetic EEG activity in *PRIP-1 KO* mice. These findings illustrate the important role of PRIP-1 in producing normal EEG spectral patterns during anesthesia.

Materials and methods

Animals

Adult male WT C57BL/6J ($n = 16$) and *PRIP-1 KO* ($n = 21$) mice from 12 to 16 weeks old weighing 25–33 g were used. Animals were group-housed at 24 ± 2 °C under a 12-h light/dark cycle (lights on at 8:00 am), and food and water were available ad libitum. After EEG/electromyogram (EMG) electrode implantation, mice were housed individually throughout the experiment. All experiments were carried out in accordance with the guidelines for animal research issued by the Physiological Society of Japan and the Hirosaki University School of Medicine, and all efforts were made to minimize the number of animals used and their suffering.

Drugs

Mice were intraperitoneally (i.p.) treated with propofol (Maruishi Pharmaceuticals Co., Ltd., Tokyo, Japan) or etomidate (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) with a volume of 10 µl/g body weight. Vehicle solutions were 20% intralipid solution (Kabi-Fresenius, Tokyo, Japan). All anesthetic administrations were performed at 14:00 under a heat lamp.

EEG and EMG recordings

Mice were implanted with cortical EEG (AP, + 1.1 mm; L, 1.45 mm; mouse brain atlas) and neck EMG electrodes (kindly gifted by Prof. Masashi Yanagisawa, International Institute for Integrative Sleep Medicine, University of Tsukuba, Japan) under isoflurane anesthesia (0.8–3%) as previously described [25]. The reference electrode was placed on the posterior cortical surface (AP, -3.5 mm; L 1.45 mm). Animals were placed in an individual EEG/EMG recording chamber (Instech Laboratories Inc., Plymouth Meeting, PA, USA) and were allowed to recover for 1–2 weeks after surgery. After recovery, their head-mounted electrode connectors were connected with a counterbalanced slip-ring commutator to allow free movement of the animals within the chamber for a > 2-day habituation period. Following the habituation period, EEG/EMG recordings were performed using a biophysical amplifier (AB-611 J; Nihon Kohden Corporation, Tokyo, Japan) and SleepWave data acquisition software (sampling rate, 128 Hz; Biosoft Studio, USA) [26].

Vigilance states of wakefulness, non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS) were identified in 10-s epochs ([27]; SleepAnalysis, Biosoft Studio, Hershey, PA, USA). Because the classification method could not distinguish between

anesthetic-induced loss of consciousness (LOC) and NREMS, LOC was included in NREMS after injection. Hypnograms were calculated using the mode state of sleep or wake states for 1-min intervals. The percentage of animals in each vigilance state was evaluated for 1 h before and 6 h after anesthetic injection. The time from drug administration to a NREMS score was considered the latency to LOC, and the duration of LOC was measured as the time between consecutive scores of NREMS. When a score of wakefulness following NREMS appeared, the animals were considered to have recovered from LOC.

EEG power spectrum (1–50 Hz) and 95% spectral edge frequency (SEF95, an index of anesthetic depth) were analyzed with the fast Fourier transform (FFT) of SleepAnalysis software (Biosoft Studio). The total absolute EEG powers of *PRIP-1 KO* mice during artifact-free sleep–wake states 1 h before anesthesia were slightly but not significantly higher than those of WT mice (Supplemental Figure S1). To compare the intergroup differences in EEG band powers, we calculated relative EEG power densities in the delta (δ, 1–4 Hz), theta (θ, 4–8 Hz), alpha (α, 8–13 Hz), beta (β, 13–30 Hz), and gamma (γ, 30–50 Hz) bands as a percentage of total power (absolute power for a given band/total absolute power × 100%). The individual and mean EEG spectrograms for 1 h before and 6 h after the injection were obtained by NeuroExplorer (ver. 5.109, Nex Technologies, Colorado Springs, CO, USA) and MATLAB (MathWorks, Inc., Natick, MA, USA) with a 0.5-Hz spectral resolution and a 60-s temporal resolution. To compare the intergroup differences in time–frequency EEG patterns after propofol or etomidate administrations, we computed relative EEG spectrograms as a percentage of total power (absolute power for each 0.5 Hz bin/total absolute power for every 60-s period).

Statistics

All data are expressed as the mean ± SEM. One-way or two-way ANOVAs with or without repeated measures were performed to determine differences between the means of EEG power densities, spectral band powers, and SEF95 values. When ANOVA revealed a significant difference, data were further analyzed with Tukey's post hoc test or Student's *t* test. We performed *t* tests to evaluate the effects of anesthetics on the latency and duration of LOC in WT and *PRIP-1 KO* mice. To compare mean relative EEG spectrograms, we implemented one-tailed Student's *t* tests at each time–frequency bin, and difference spectrograms are expressed as *t* values. For ANOVA and two-tailed *t* tests, a statistical probability value of $P < 0.05$ was considered significant. Statistical significance for one-tailed *t* tests was $P < 0.1$.

Results

Effect of PRIP-1 deficiency on EEG activity

First, to investigate whether PRIP-1 deficiency affected EEG activity in normal conditions, we analyzed the EEG signals of WT and *PRIP-1 KO* mice during a 1-h pre-anesthesia period. Although our previous study demonstrated temporal epileptiform discharges in *PRIP-1 KO* mice [23], there were no obvious epileptic EEG discharges in the frontal lobe of *PRIP-1 KO* mice under normal conditions in this study. Representative EEG/EMG signals of both genotypes during wake, NREM or REM sleep are shown in Fig. 1a–c. PRIP-1 deficiency tended to increase absolute EEG power and induced no obvious changes in the pattern of the EEG power distribution (Supplemental Figure S1). To compare the intergroup differences in the distribution pattern of EEG spectra between WT and *PRIP-1 KO* mice, we computed the spectra distributions of the relative EEG power density (Fig. 1d–f) and the mean relative EEG power density in the δ, θ, α, β, and γ wave bands in each vigilance state (Fig. 1g–i). Several frequency points showed significant differences during REMS (two-way ANOVA, group × frequency, $F_{98,\,3069} = 3.54$, $P < 0.05$; Student's *t* test, $P < 0.05$ at 5.5, 6.0, 8.0, 10, 35.0, 35.5, 37.0, 40.0, 42.0, and 42.5 Hz; Fig. 1f), but there was no significant difference in the relative band power of EEG between WT and *PRIP-1 KO* mice. These results indicated that the EEG spectral patterns of each sleep state in normal conditions were not markedly affected by PRIP-1 deficiency.

Reduced propofol-induced EEG slowing and loss of consciousness in PRIP-1 KO mice

The contribution of PRIP-1 function to anesthetic EEG activity was assessed by recording EEG in WT and *PRIP-1 KO* mice before and after propofol injection. Representative hypnograms, SEF95 values, and EEG spectrograms acquired from both WT and *PRIP-1 KO* mice are depicted in Fig. 2a. After propofol injection, the percentage of animals in the wake state was significantly higher in *PRIP-1 KO* mice than in WT mice (Fig. 2b, top). The latency of LOC was slightly but not significantly prolonged, and the duration of LOC was significantly shortened in *PRIP-1 KO* mice (Fig. 2b, middle). The representative and mean relative EEG spectrograms showed that propofol administration transiently increased α and β band powers as well as δ and θ band powers for approximately 1 h after administration in WT mice (Fig. 2a, b, bottom). *PRIP-1 KO* mice treated with propofol did not display these time–frequency properties of EEG signals (Fig. 2b). Approximately 2 h

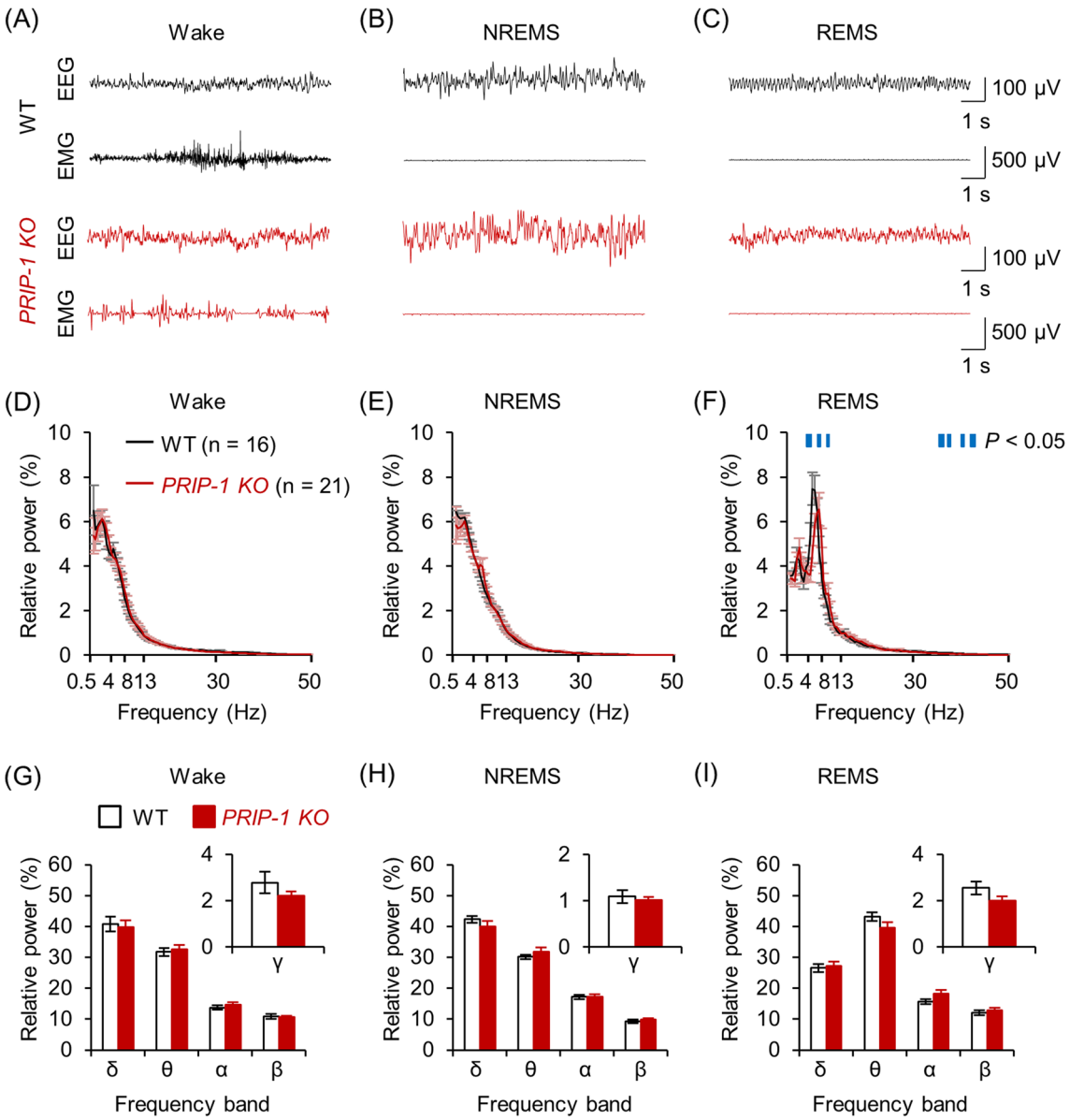


Fig. 1 Effects of PRIP-1 deficiency on EEG and EMG activity. Representative EEG and EMG activity of WT and *PRIP-1 KO* mice during wake (**a**), NREMS (**b**), and REMS (**c**) 1 h before anesthesia. Mean relative EEG power spectra and frequency bands during baseline wake (**d**, **g**), NREMS (**e**, **h**), and REMS (**f**, **i**). The following frequency bands were calculated: delta (δ, 1–4 Hz), theta (θ, 4–8 Hz), alpha (α, 8–13 Hz), beta (β, 13–30 Hz), and gamma (γ, 30–50 Hz). During REMS, several significant changes were observed in the power spectrum of *PRIP-1 KO* mice (**f**; blue lines indicate significant differences between WT and *PRIP-1 KO* mice). Group $n \geq 7$ mice. Data are presented as the mean ± SEM

after the propofol injection, the β and γ band powers of WT mice were significantly lower than those of *PRIP-1 KO* mice (Fig. 2c). Furthermore, the α band power of WT mice, observed from approximately 2–3.5 h after propofol injection, was significantly lower than that of *PRIP-1 KO* mice. The SEF95 values of WT mice decreased for approximately 2 h and increased from 2 to 4 h after propofol injection (Fig. 2d). These changes in SEF95 observed in WT mice were not present in *PRIP-1 KO* mice (one-way ANOVA with repeated measures, group × time interaction, $F_{2519,\ 30,240} = 1.92$, $P < 0.05$). These results indicated that a lack of PRIP-1 decreased the effects of propofol on EEG activity and consciousness.

PRIP-1 deficiency affected etomidate-induced EEG activity but not the anesthetic phenotype

To investigate whether PRIP-1 affects the etomidate-induced alteration of EEG, we analyzed EEG activity during etomidate anesthesia in *PRIP-1 KO* mice. The vigilance state during etomidate anesthesia was not different between WT and *PRIP-1 KO* mice (Fig. 3a, b, top). Accordingly, animals displayed comparable latency and duration of LOC induced by etomidate (Fig. 3b, middle). A transient δ band shift of relative EEG power was observed immediately after etomidate injection in both genotypes (Fig. 3a, b, bottom). Approximately 0–0.5 h after etomidate injection, a sudden increase in EEG power in the low-frequency (δ and θ) range occurred in *PRIP-1 KO* mice (Fig. 3c). *PRIP-1 KO* mice also displayed prolonged potentiation of low-frequency EEG activity approximately 3 h after etomidate injection. The SEF95 values after etomidate injection were not altered by PRIP-1 deficiency (one-way ANOVA with repeated measures, group × time interaction, $F_{2519,\ 52,920} = 1.00$, $P > 0.05$; Fig. 3d). These results indicated that PRIP-1 deficiency induced a steep decline in EEG spectral activity after etomidate administration without any effects on the vigilance state transition and SEF95.

Differences in anesthetic effects between propofol and etomidate in wild-type and PRIP-1 KO mice

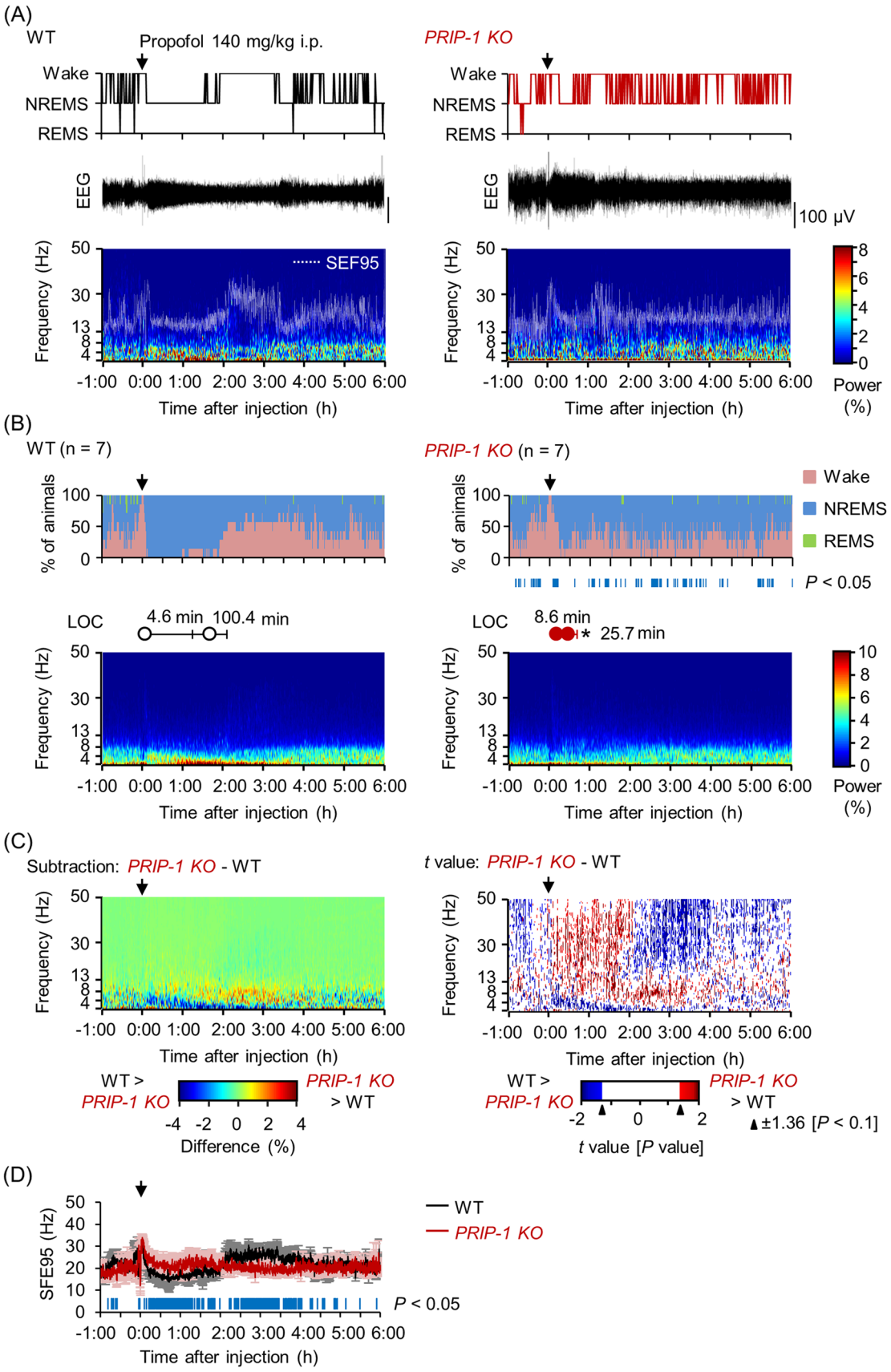
Finally, we compared the anesthetic effects of propofol and etomidate on the EEG time–frequency distribution between both genotypes. Immediately after anesthetic injections, the lower frequency shift of relative EEG power induced by propofol was greater than that induced by etomidate in WT mice (Fig. 4a). Etomidate produced spanning theta wave activity. Before emergence from LOC (propofol, 100.4 min; etomidate, 134.7 min; Student's *t* test, $P > 0.05$), WT mice injected with propofol showed a more potent high-frequency shift than did mice injected with etomidate. Because of the reduction in the anesthetic action of propofol on *PRIP-1 KO* mice, propofol failed to induce more potent slow wave activity than etomidate in *PRIP-1 KO* mice (Fig. 4b). Prolonged theta wave activation and reduced high-frequency activity occurred in *PRIP-1 KO* mice injected with etomidate. These results suggested differences in the anesthetic effects of propofol and etomidate on EEG activity and that PRIP-1 played an important role in producing a normal EEG response to anesthetics.

Discussion

In this study, we analyzed and compared EEG recordings between WT and *PRIP-1 KO* mice. As *PRIP-1 KO* mice showed slightly enhanced absolute EEG power, their relative EEG spectra were compared with those of WT mice. PRIP-1 deficiency blocked propofol-induced EEG slowing and reduced the duration of LOC. In addition, *PRIP-1 KO* mice treated with etomidate displayed steep and prolonged enhancement of slow wave EEG activity, but the induction, duration and depth of etomidate-induced anesthesia were unaffected by PRIP-1 deficiency. These results suggested that the influence of propofol and etomidate on EEG activity was differentially affected by PRIP-1 deficiency.

$GABA_A$-Rs containing the β3 subunit are considered pathogenetic factors in some psychiatric and developmental disorders [28, 29]. β3 subunit knockout mice ($\beta3^{-/-}$ mice) have an epilepsy phenotype and the characteristics of Angelman syndrome [30, 31]. $\beta3^{-/-}$ mice also display a reduction in GABA-evoked currents in hippocampal neurons [32]. In addition, $\beta3^{-/-}$ and other Angelman syndrome model mice display high-amplitude slow wave activity [33, 34]. In this study, we found an increased overall absolute EEG power of *PRIP-1 KO* mice during NREMS and REMS. Nonetheless, these mice exhibited little seizure-like behavior, epileptiform discharges and an altered spectral distribution of the frontal cortex. This might be because of the differences of EEG recording methodology (selected 7-h vs. continuous 48-h recording periods; 12–16 vs. 8 weeks old; frontal vs. temporal cortices) between our present and previous studies [23]. In our previous studies, the membrane expression levels of the cortical and hippocampal β3 subunit were downregulated in *PRIP-1 KO* mice, probably resulting in a reduction in extrasynaptic GABAergic currents and temporal lobe epileptic EEG abnormalities [23]. Furthermore, *PRIP-1 KO* mice show impaired motor coordination in the rotarod test and tend to walk more in the open field test [35]. This evidence suggests that the abnormal EEG and behavioral phenotypes of *PRIP-1 KO* mice might be partly caused by downregulated expression of the $GABA_A$-R β3 subunit. Because there is no clinical and pathological evidence at this point, further study is needed to investigate the role of PRIP-1 in the pathology of epilepsy and Angelman syndrome.

According to some studies, propofol induces general anesthesia with characteristic EEG and local field potential (LFP) activity in humans and rats [2, 36]. In EEG and LFP, transient β band activity is induced by propofol, followed by stable α band activation during LOC induction. These changes reverse during recovery from LOC. In our WT mice, propofol produced transient α and β band activation, followed by prolonged δ and θ band activation during induction. During the emergence from propofol anesthesia, these EEG dynamics reversed. The EEG slowing of WT mice was more potent than that of humans and rats after propofol administration, but slowing and reversal transitions similar to those of humans and rats were observed. These findings confirm that propofol-induced EEG slowing is common to both humans and rodents.


(A)
WT
Propofol 140 mg/kg i.p.
PRIP-1 KO
Wake
NREMS
REMS
EEG
100 μV
SEF95
Frequency (Hz)
50
30
13
8
4
-1:00 0:00 1:00 2:00 3:00 4:00 5:00 6:00
Time after injection (h)
8
6
4
2
0
Power (%)
(B)
WT (n = 7)
PRIP-1 KO (n = 7)
% of animals
100
50
0
Wake
NREMS
REMS
P < 0.05
LOC
4.6 min
100.4 min
8.6 min
25.7 min
10
(C)
Subtraction: PRIP-1 KO - WT
t value: PRIP-1 KO - WT
WT > PRIP-1 KO
PRIP-1 KO > WT
-4 -2 0 2 4
Difference (%)
-2 0 2
t value [P value]
±1.36 [P < 0.1]
(D)
SFE95 (Hz)
40
20
10
WT
PRIP-1 KO
P < 0.05

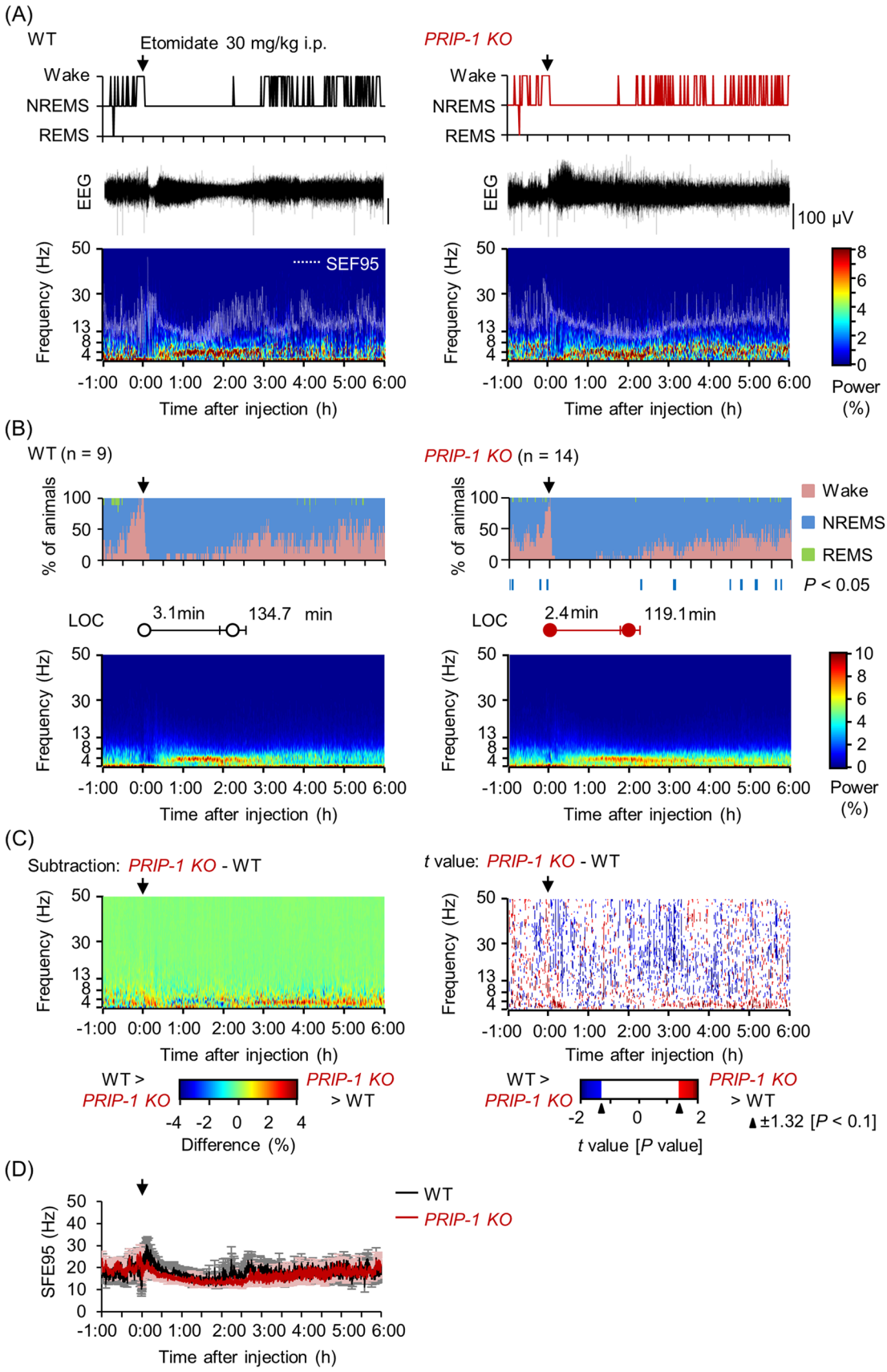
◂**Fig. 2** Effects of propofol on vigilance states and EEG activity of WT and *PRIP-1 KO* mice. Representative hypnograms, EEG traces, SEF95 values, and spectrograms of WT and *PRIP-1 KO* mice 1 h before and 6 h after propofol administration (**a**). Mean percentage of animals in sleep–wake states (top), latency and duration of LOC (middle) and spectrograms (bottom) of WT and *PRIP-1 KO* mice (**b**). *PRIP-1 KO* mice showed a reduction in propofol-induced sleep (top; blue lines indicate significant differences between genotypes) and LOC (middle; $^*P < 0.05$ vs. WT mice). Subtracted and *t* value spectrograms are shown in **c**. Red and blue signals indicate significant differences (one-tailed *t* values = ±1.36, $P < 0.1$). A lack of PRIP-1 blocked the enhancement of slow wave EEG activity produced by propofol. Decreases in SEF95 values induced by propofol were also prevented by PRIP-1 deficiency (**d**). Blue lines indicate significant differences between WT and *PRIP-1 KO* mice. Arrows indicate the timing of propofol injection. Group $n = 7$ mice. Data are presented as the mean ± SEM

In this study, the EEG activity of *PRIP-1 KO* mice after propofol injection was quite different from that of WT mice. *PRIP-1 KO* mice displayed little or no propofol-induced EEG slowing and LOC, consistent with our previous study demonstrating that *PRIP-1 KO* mice display attenuation of hypnosis and immobilization induced by propofol [24]. These attenuations might be attributed to reductions in the membrane expression and phosphorylation level of the $GABA_A$-R β3 subunit, resulting in suppression of extrasynaptic GABAergic transmission [23, 24]. Our results are in accordance with some previous studies suggesting that the $GABA_A$-R β3 subunit is a key molecule involved in propofol anesthesia [8, 10, 11, 15]. PRIP-1 might contribute to the anesthetic response to propofol by regulating the functional membrane expression of the $GABA_A$-R-containing β3 subunit.

β3-containing $GABA_A$-Rs fully mediate the immobilizing actions of both etomidate and propofol [10, 37, 38]. In addition, etomidate potentiates β2-containing $GABA_A$-Rs [10, 39]. Several electrophysiological studies revealed that etomidate reportedly enhances low-frequency LFP power spectral density in WT mice, but the low-frequency power spectral density is depressed in β3 mutant mice [40, 41]. These authors concluded that β2- and β3-containing $GABA_A$-Rs exhibit opposing actions on low-frequency EEG activity. The previous finding of increased low-frequency power density by etomidate application is consistent with our results from WT mice. However, the low-frequency EEG activity of etomidate-injected *PRIP-1 KO* mice was more enhanced and prolonged than that of WT mice immediately and 3–6 h after administration, although the expression and function of β3-containing $GABA_A$-Rs in the membrane are considered to be reduced in *PRIP-1 KO* mice. These results indicate that the function of β3-containing $GABA_A$-Rs is not completely restricted by PRIP-1 deficiency and that etomidate-induced EEG activity of *PRIP-1 KO* mice exhibits different properties from that of β3 mutant mice. Because PRIP-2 is expressed ubiquitously and is functionally equivalent to PRIP-1, reduced β3-containing $GABA_A$-R function might be partly relieved [42]. Our previous studies suggest that PRIP-2 does not fully compensate PRIP-1-mediated GABAergic neurotransmission in *PRIP-1 KO* mice [18, 23, 24]. The amount and/or function of PRIP-2 alone might not be sufficient to retain the β3-containing $GABA_A$-R activity. However, the mechanism by which PRIP-1 deficiency enhances etomidate-induced low-frequency EEG activity could not be explained by our results. Since the function of PRIP-1 is involved in extrasynaptic $GABA_A$-R membrane stability, etomidate-induced β2-containing $GABA_A$-R activation and the cell-specific localization of β2- and β3-containing $GABA_A$-Rs might be responsible for the enhancement of etomidate-induced low-frequency EEG activity in *PRIP-1 KO* mice [19, 20, 43, 44]. Additionally, PRIP-1 may have an unknown role in β2-containing $GABA_A$-R stability and/or activity. In any case, the anesthetic effect of etomidate on low-frequency EEG activity is likely influenced by PRIP-1 deficiency. Further research is needed to reveal the correlation between EEG activity during etomidate anesthesia and the function of PRIP-1.

Propofol- and etomidate-induced EEG activity revealed different anesthetic properties. Beta, δ and θ band EEG activity during and after anesthesia was high in propofol-injected WT mice, whereas θ band EEG activity was high in etomidate-injected WT mice. Although both propofol and etomidate might facilitate the activity of $GABA_A$-Rs containing the β3 subunit, the effects of these anesthetics on EEG activity are quite different. This difference might be caused by the individual property of etomidate binding to the β2 subunit of $GABA_A$-Rs. In addition, the low-frequency (δ and θ band) EEG activity differences between propofol- and etomidate-treated WT mice partially disappeared in *PRIP-1 KO* mice, suggesting that PRIP-1 contributes to the function of β2 subunit-containing $GABA_A$-Rs associated with slow wave EEG activity. Furthermore, propofol application can reportedly inhibit the internalization of the AMPA receptor GluR2 subunit, and both propofol and etomidate facilitate glycine receptor activation [45, 46]. This evidence implies that EEG activity during and after anesthesia might be regulated by a complicated mechanism.

In addition, many studies have been reported about the effect of propofol on NMDA receptor. For example, the expression level and phosphorylation state of NMDA receptor were regulated by propofol treatment [47–49]. It was reported that NMDA receptor-mediated neurotoxicity was reduced by propofol treatment; however, such effect was not observed when etomidate was treated [50, 51]. These evidences suggest that the distinct effect of propofol and etomidate on NMDA receptor might contribute to the difference of EEG activities between propofol- and etomidate-injected mice. There is a possibility of a relation of PRIP-1 to NMDA receptor; however, we are not aware of any study concluding


(A)
WT
Etomidate 30 mg/kg i.p.
PRIP-1 KO
Wake
NREMS
REMS
EEG
100 μV
Frequency (Hz)
SEF95
Time after injection (h)
Power (%)
(B)
WT (n = 9)
PRIP-1 KO (n = 14)
% of animals
Wake
NREMS
REMS
P < 0.05
LOC
3.1min
134.7 min
2.4min
119.1min
(C)
Subtraction: PRIP-1 KO - WT
t value: PRIP-1 KO - WT
WT > PRIP-1 KO
PRIP-1 KO > WT
Difference (%)
t value [P value]
±1.32 [P < 0.1]
(D)
SFE95 (Hz)
WT
PRIP-1 KO

◂**Fig. 3** Effect of etomidate on sleep–wake states and EEG frequency components of WT and *PRIP-1 KO* mice. Representative hypnograms, EEG traces, and spectrograms of WT and *PRIP-1 KO* mice (**a**). Mean percentage of animals in sleep or waking (top), LOC (middle) and spectrograms (bottom) of WT and *PRIP-1 KO* mice (**b**). WT and *PRIP-1 KO* mice showed comparable etomidate-induced sleep (top; blue lines indicate significant differences between genotypes) and LOC (middle). Subtracted and difference spectrograms are shown in **c**. Red and blue signals indicate significant differences (one-tailed t values $= \pm 1.32$, $P < 0.1$). A lack of PRIP-1-induced steep enhancement of δ and θ band activity and prolonged these slow wave activities after etomidate administration. SEF95 values of WT and *PRIP-1 KO* mice were parallel (**d**). Arrows indicate the timing of etomidate administration. Group $n \geq 9$ mice. Data are presented as the mean ± SEM

that NMDA receptor is one of the direct binding partners of PRIP-1. Additionally, the effects of intravenous anesthetics on background two-pore potassium (K2P) channels are well-studied. Propofol has no effect on K2P channel family (TASK-1 and TASK-3), while etomidate inhibits these K2P channels [52, 53]. Although K2P channels are thought to not play essential roles for intravenous general anesthesia [54], there is a possibility that excitability of neurons in *PRIP-1 KO* mice might be activated by K2P channels because of PRIP-1 deficiency-induced protein phosphatases activation [18, 55]. Taken together, the NMDA receptor, other receptors and channels might indirectly relate to PRIP-1-associated pathway. Further study is needed to explore the complicated molecular mechanisms underlying the alterations in EEG frequency properties of propofol or etomidate-injected *PRIP-1 KO* mice.

In this study, we had administered propofol and etomidate to mice by i.p. bolus injection; however, these anesthetics are usually injected by intravenous bolus injection or continuous infusion in humans and large animals. Thus, the results of our present study should be interpreted with caution. A recent study has revealed transient increase and exponential decrease of actual propofol concentration in mouse brain, which are induced by intravenous bolus injection of propofol [56]. This novel propofol pharmacokinetic model would be useful for investigation of the functional effects of PRIP-1 on anesthetic EEG activity at desired level of propofol and/or etomidate in mouse brain. In addition, our present study could not reveal the mechanism of EEG slowing produced by propofol and etomidate. The thalamic reticular and thalamocortical neurons are thought to play important roles in generation of EEG oscillation during general anesthesia [54]. A previous immunocytochemical study reveals that the reticular neurons appear to express $GABA_A$-R β3 subunit and other thalamic nuclei are likely to express $GABA_A$-R β2 subunit [57], implying that the β3 subunit downregulation induced by PRIP-1 deficiency alters inhibitory tone of both the reticular neurons and the thalamocortical relay neurons. Further electrophysiological studies are required to examine the effects of PRIP-1 deficiency on anesthetic-dependent neural oscillation produced by the reticular and thalamocortical neurons.

In summary, our present study shows that PRIP-1 plays important roles in the induction and retention of anesthetic EEG activity induced by both propofol and etomidate. Although the methodology of this study is not entirely consistent in regard to general anesthesia in humans and the results should be interpreted with caution, PRIP-1-dependent $GABA_A$-R modulation might be a regulatory factor to control general anesthesia to preclude adverse events such as unexpected awakening during surgery and delayed emergence after general anesthesia.

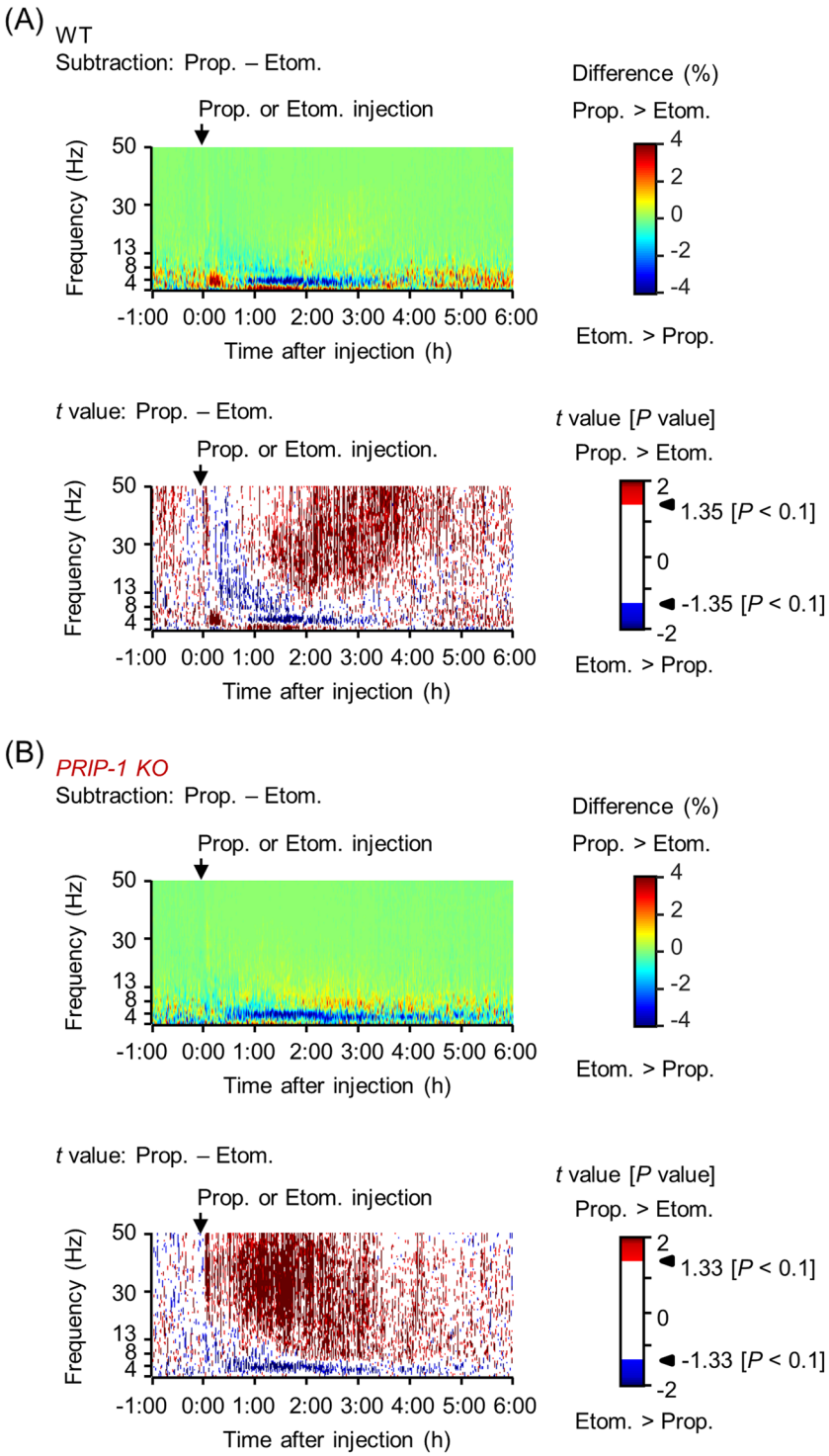


Fig. 4 Comparisons of the effects of propofol and etomidate on EEG spectrograms in WT and *PRIP-1 KO* mice. Subtracted spectrograms from the mean relative EEG spectrogram of propofol to that of etomidate (top) and t value spectrograms (bottom) are shown in **a** WT mice and **b** *PRIP-1 KO* mice. Red and blue signals indicate significant differences (**a** one-tailed t values = ± 1.35, $P < 0.1$; **b** t values = ± 1.33, $P < 0.1$). Compared with etomidate, propofol induced potent slow wave activity in WT mice but not in *PRIP-1 KO* mice. In both genotypes, the high-frequency activity observed after propofol injection was significantly higher than that after etomidate injection. Group $n \geq 7$ mice

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