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Propofol reduces human TRPA1 activity in a warm environment

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ABSTRACT

Propofol, an intravenous anesthetic, has a side effect of causing vascular pain at the injection site. However, no effective method to inhibit this vascular pain has been established. Propofol-induced vascular pain is caused by activation of transient receptor potential ankyrin1 (TRPA1), which is expressed in the sensory nerve endings distributed around blood vessels. TRPA1 exhibits temperature sensitivity, and the degree of its activation has been reported to change with temperature. However, whether the temperature of propofol influences human TRPA1 (hTRPA1) activation and regulates the extent of vascular pain has not been examined. We investigated hTRPA1 activity in HEK293T cells in response to cooled or heated propofol using the patch-clamp method. We found that hTRPA1 currents were smaller in a warm environment (>35 °C) with heated propofol. Our results suggest that propofol should be kept above 35 °C to minimize hTRPA1 activation. Moreover, heating propofol decreased hTRPA1-mediated currents but did not alter activation of human $GABA_A$ receptors. This finding suggest that heated propofol can inhibit hTRPA1 activation and reduce vascular pain without losing its anesthetic function.

1. Introduction

Propofol, an intravenous anesthetic, is widely used for the induction and maintenance of anesthesia. Propofol activates type A γ-amino butyric acid (GABAA) receptors in the central nervous system and induces a loss of consciousness [1]. However, propofol has a side effect of causing local pain at the injection site [2]. Previous studies showed that the Visual Analogue Scale (VAS) score of postoperative pain and total postoperative opioid consumption are higher in patients with vascular pain than in those without it [3,4]. Therefore, vascular pain needs to be resolved because it continues to affect patients after surgery. Various treatments for propofol-induced vascular pain (vascular pain) have been investigated, including the use of premedication [5,6]. For example, pretreatment using lidocaine [5] or opioids and 5-HT3 antagonists [6] is said to be most effective. Nevertheless, these treatments do not completely prevent vascular pain. Multiple factors are said to cause vascular pain, including the rate of administration [7] and the size of the vein being injected [8]. These are indirect factors; the direct cause involves transient receptor potential ankyrin 1 (TRPA1), an ion channel [9]. One possible reason for the failure to prevent pain may be that the premedication does not inhibit the factors causing vascular pain. Additionally, the risk of side effects must be at least considered in the use of premedication. Therefore, a strategy to prevent vascular pain without the use of premedication is needed.

Propofol is emulsified in a fat emulsion because of the low water solubility of its main ingredient, 2,6-diisopropylphenol. 2,6-Diisopropylphenol has been shown to cause vascular pain by activating human TRPA1 (hTRPA1) [10]. TRPA1 is a non-selective cation channel with calcium permeability and is temperature sensitive. TRPA1 is expressed on the sensory nerve endings distributed in perivascular regions [11]. While TRPA1 was initially reported to be activated by noxious cold stimuli [12], recent studies have shown that TRPA1 is also a heat sensor. Mouse TRPA1 is a receptor required for noxious heat sensation [13]. Single-channel analysis showed that purified human TRPA1 was activated by both cold and heat stimuli, with minimal opening probability at approximately 22 °C [14]. However, agonist stimulation at a high temperature of 39 °C was reported to suppress TRPA1 activity in rats and humans [15]. Thus, TRPA1 appears to show a range of temperature sensitivity. A package insert of propofol states that it should be stored at 25 °C or below to avoid freezing [16]. Although there are differences

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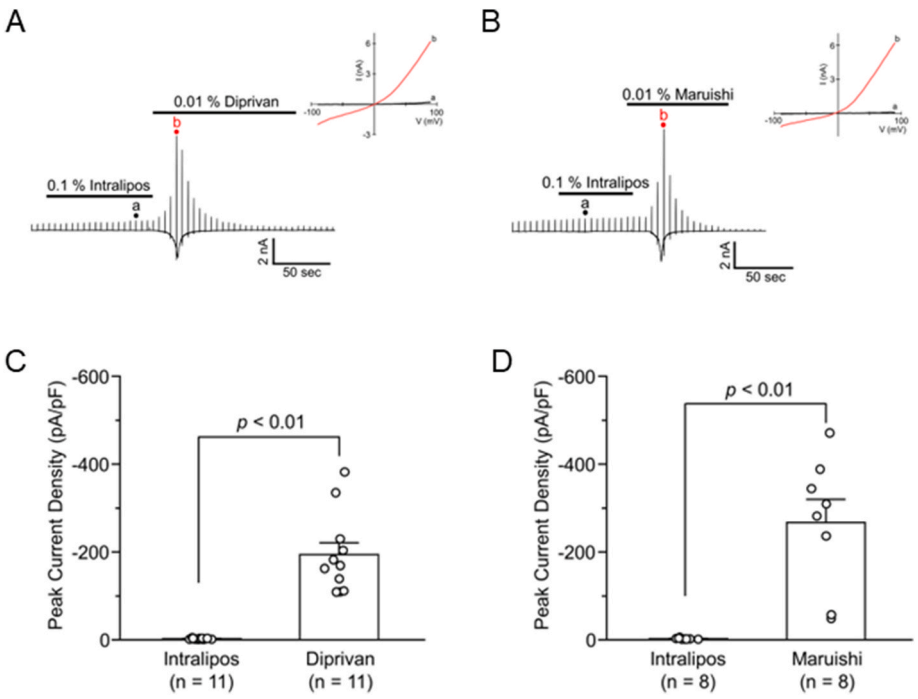

Fig. 1. Effect of hTRPA1 activation by propofol and the solvent A, B) Representative trace of Diprivan (A) or Maruishi-activated currents in HEK293T cells transfected with plasmid expressing human TRPA1 (hTRPA1). The holding potentials were −60 mV with ramp pulses (−100∼+100 mV, 300 ms). The insets show current-voltage curves at the time points of a and b. Different types of solvent compositions were used: Diprivan for long-chain triglyceride (LCT) and Maruishi for medium-chain triglyceride/long-chain triglyceride (MCT/LCT). C) Comparison of peak-current density of Intralipos and Diprivan at −60 mV (mean ± SEM, n = 11). Mann–Whitney *U* test. D) Comparison of peak-current density of Intralipos and Maruishi at −60 mV (mean ± SEM, n = 11). Mann–Whitney *U* test.

depending on hospitals, propofol is often administered to patients at room temperature. However, it is unclear whether propofol at different temperatures alters the vascular pain sensation mediated by TRPA1.

In this study, we examined the effects of propofol at different temperatures on hTRPA1 activity in HEK293T cells using the patch-clamp method. Our results suggest a simple and useful method to reduce propofol-induced vascular pain sensation.

2. Materials and methods

2.1. Chemicals

Two types of clinically used propofol emulsions, in different solvents, were used in this study: 1 % Diprivan® Injection (SANDOZ, Switzerland; LCT) (hereafter referred to as "Diprivan") and Propofol Intravenous 1 % (Maruishi Pharmaceutical, Japan; MCT/LCT) (hereafter referred to as "Maruishi"). The two types of propofol have the same concentration of active ingredient, but the composition of the solvent, fat emulsion, is different. Diprivan consists of only long-chain triglyceride (LCT), while Maruishi is a 1:1 mixture of medium-chain triglyceride/long-chain triglyceride (MCT/LCT). Each was diluted in bath solution to 0.01 %. Intralipos® Injection 20 % (Otsuka Pharmaceutical, Japan), which is almost identical in composition to solvent, was used as a control for propofol (as the solvent-alone treatment). Intralipos® Injection 20 % was diluted in bath solution to 0.1 %. 2,6- Diisopropylphenol and γ-Aminobutyric acid (GABA) was purchased from Sigma-Aldrich. Allyl isothiocyanate (AITC) was purchased from Tokyo Chemical Industry Co., Ltd. Stock solutions were prepared in DMSO; the final concentration of DMSO did not exceed 0.3 %.

2.2. Cell culture and transfection

HEK293T cells were cultured in DMEM (high glucose) with L-glutamine and phenol red (FUJIFILM Wako, Japan) supplemented with 10 % (vol/vol) FBS (lot #S06537S1560; Biowest, France), penicillin/streptomycin (1:100; Life Technologies, USA), and GlutaMAX (1:100; Life Technologies) at 37 °C in humidified air containing 5 % CO_2.

Cells (2×10^6 cells) were seeded in a 35 mm dish 24 h before transfection. The cells were transfected with 0.5 μg plasmid DNA (hTRPA1-pcDNA3.1, human GABA-ARα1-pcDNA3, human GABA-ARβ2-pcDNA3) using Lipofectamine (Invitrogen, USA). After incubation for 2.5–4 h, cells were reseeded on cover glasses and further incubated at 37 °C in a humidified 5 % CO_2 incubator. The cells were subjected to patch-clamp recording 20–30 h after transfection. Plasmids were generously provided by Dr. Tominaga at NIPS (Okazaki, Japan).

2.3. Electrophysiology

Transfected HEK293T cells were used for whole-cell recordings. The bath solution contained 140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM HEPES, and 10 mM glucose at pH 7.4 adjusted with NaOH. The pipette solution was composed of 140 mM KCl, 5 mM EGTA, and 10 mM HEPES, with the pH adjusted to 7.4 using KOH. Patch pipettes were made from borosilicate glass (type 8250, King Precision Glass, USA) with a four-step protocol using a P-97 (Sutter Instrument, USA). The pipette resistance was 2.5–5 MΩ. The holding potential was −60 mV, and ramp pulses from −100 to +100 mV were applied for 300 ms every 5 s. Currents were recorded using a Multiclamp 700B amplifier (Axon Instruments, USA), filtered at 1 kHz with a low-pass filter, and digitized with a Digidata 1550B (Axon Instruments). The data were acquired using pCLAMP 10 software (Axon Instruments).

Thermal and cool stimulation was applied by increasing the bath temperature using preheated or precooled solution delivered through a syringe. The bath temperature in the chamber was monitored during recordings with a thermistor (TC-344B, Warner Instruments, USA) placed within 100 μm of the patch-clamped cells. All experiments were performed at room temperature except for those involving thermal or cool stimulation. For each experiment, 4–10 trials were performed.

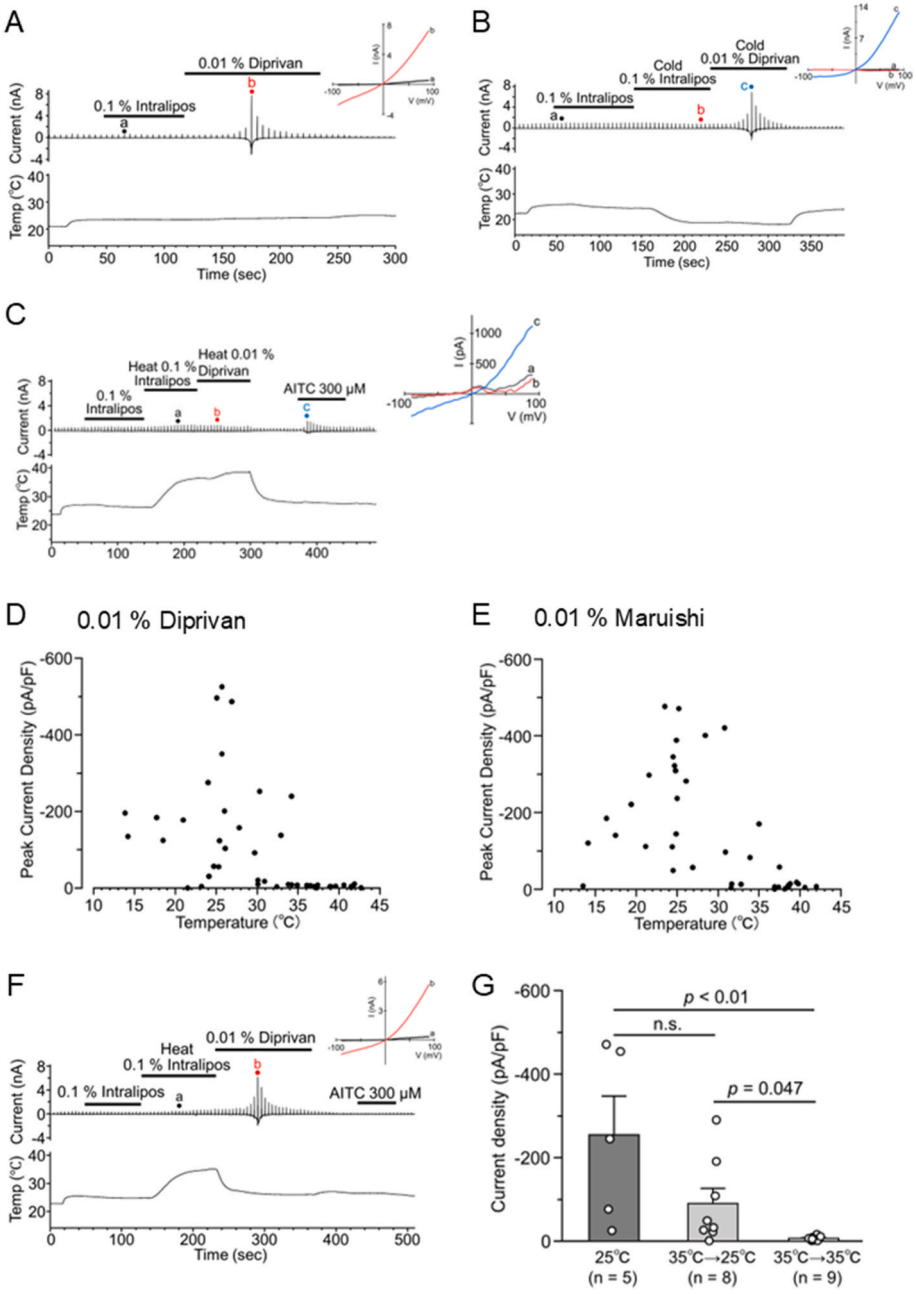

Fig. 2. Effect of propofol at various temperatures on hTRPA1 activation All experiments were performed at room temperature (approximately 25–26 °C). A, B, C) Typical traces, current-voltage curves, and graph of temperature changes of HEK293T cells expressing hTRPA1 in response to room temperature (A), cooling (B), and heating (C) Diprivan. The holding potentials were −60 mV with ramp pulses (−100~+100 mV, 300 ms). The insets show current-voltage curves at the time points of a, b, and c. D, E) The plot of hTRPA1 current density in response to Diprivan (D) and Maruishi (E) application from 10 °C to 45 °C. F) A typical trace, current-voltage curves, and graph of temperature changes of HEK293T cells expressing hTRPA1 when changing the temperature at the time of Diprivan application. The holding potentials were −60 mV with ramp pulses (−100~+100 mV, 300 ms). G) Comparison of peak-current density of Diprivan with different temperatures at −60 mV (mean ± SEM, n = 5–9). n. s., not significant, Kruskal–Wallis test (n = 5–9).

2.4. Statistical analyses

Values are presented as the mean with the standard error of the mean (SEM). Statistical analysis was performed using the Mann–Whitney *U* test or Student's t-test to calculate the significance of differences between two groups. Kruskal–Wallis test was used to calculate the significance of differences between multiple groups. $p < 0.05$ was considered significant. Statistical analyses were performed using Origin Pro2023 software (OriginLab, USA).

3. Results

3.1. Effect of propofol and solvent on hTRPA1 activity

Owing to differences in propofol concentration in the aqueous phase, MCT/LCT propofol has been reported to be associated with less pain than LCT propofol [17,18]. We examined whether there was a difference in hTRPA1-activating ability between Diprivan (a propofol emulsion in LCT) and Maruishi (a propofol emulsion in MCT/LCT). We first checked the currents in hTRPA1-expressing HEK293T cells in Diprivan and Maruishi. When 0.1 % Intralipos was applied, hTRPA1-mediated

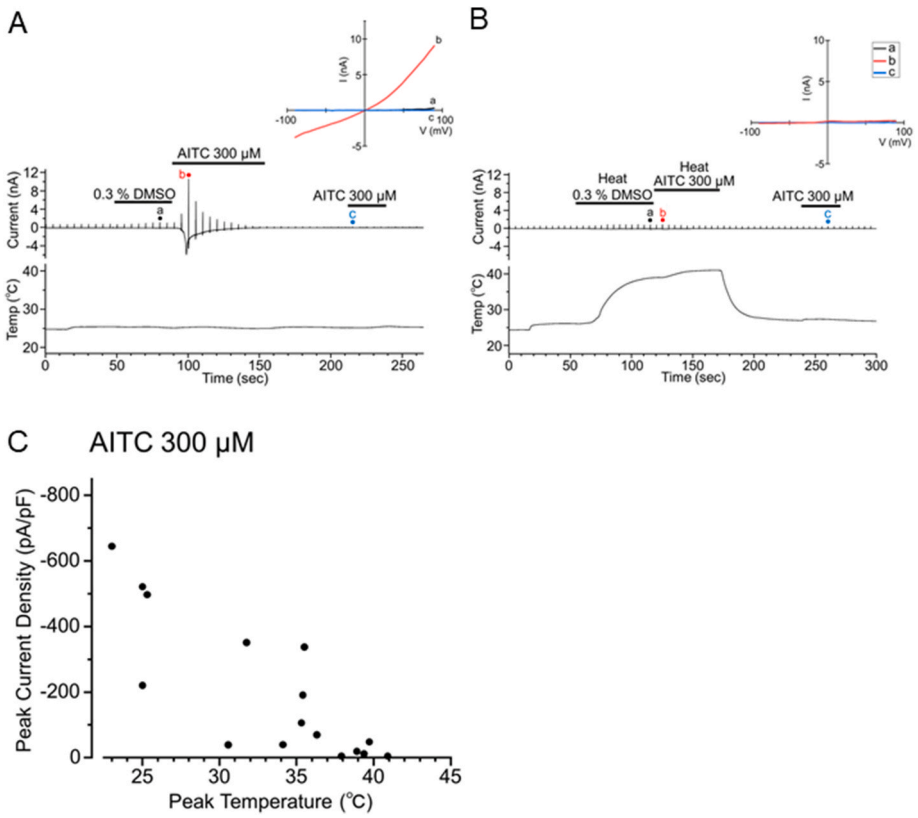

Fig. 3. Effects of Heated AITC on hTRPA1 Activity A, B) Typical traces, current-voltage curves, and graph of temperature changes of HEK293T cells expressing hTRPA1 in response to room temperature (A) and heating (B) AITC. The holding potentials were −60 mV with ramp pulses (−100~+100 mV, 300 ms). The insets show current-voltage curves at the time points of a, b, and c. C) The plot of hTRPA1 current density in response to AITC application over the range of about 25 °C–45 °C.

currents were not observed (Fig. 1A and B). In contrast, when 0.01 % Diprivan or 0.01 % Maruishi was applied, significant hTRPA1-mediated currents with outward rectification were observed (Fig. 1A–D, $p < 0.01$). These results indicated that the solvent of propofol had no effect on hTRPA1 activation. In addition, both Diprivan and Maruishi had hTRPA1-activating ability, and the degree of activation was similar.

3.2. Effect of propofol at various temperatures on hTRPA1 activity

Next, we examined the effect of propofol at 10 °C–45 °C on hTRPA1 activity with room temperature propofol as the control. Bath solutions were precooled or preheated to the desired temperatures before the application of propofol. Since Diprivan and Maruishi showed similar degrees of TRPA1 activation in Result 1, Diprivan was used as the main reagent here. Diprivan-activated hTRPA1 currents were observed both at room temperature (Fig. 2A) and cooled conditions (Fig. 2B), but not in heated condition (Fig. 2C). hTRPA1-mediated currents were very small with Diprivan or Maruishi at temperatures above 35 °C (Fig. 2D and E). To examine the effect of preheating on hTRPA1 activation, we applied room temperature Diprivan after preheating it to 35 °C (Fig. 2F). A clear Diprivan-evoked hTRPA1 currents were observed, and although the current density was not significantly different compared with that under room temperature conditions, but half of the measured hTRPA1 currents had smaller current values (Fig. 2G). AITC, a TRPA1 activator, did not cause hTRPA1 activation after activation by Diprivan (Fig. 2F), which may be because of desensitization. In addition, preheating to 35 °C and applying Diprivan at 35 °C resulted in the lowest current density due to hTRPA1. These results suggest that propofol should be kept above 35 °C to minimize hTRPA1 activation.

3.3. Effects of heated AITC on hTRPA1 activity

Among the compounds that activate TRPA1, AITC has been shown to covalently modify cysteine residues in the N-terminal cytoplasmic ankyrin repeat domain of TRPA1 [19]. Thus, we examined whether the compound that binds to a different site of TRPA1 (AITC) had different results from propofol shown in Fig. 2D and E. As a result, the AITC-activated hTRPA1 currents were observed at room temperature (Fig. 3A), but when heated to 35 °C or higher, the currents were very small (Fig. 3B). As shown in Fig. 3C, although some AITC-mediated hTRPA1 currents were observed around 35 °C, the currents were smaller at temperatures above 35 °C.

3.4. Effects of heated propofol on hTRPA1 activity

To examine the effects of heated propofol, we examined whether heated Diprivan affected hTRPA1-activating ability. In this study, in order to put the solution into the syringe and pass it through the tube to raise the temperature inside the chamber to 35 °C or higher at room temperature (25–26 °C), the solution had to be heated to around 60 °C. Therefore, propofol was heated to 60 °C for 1 h and then returned to room temperature before use. Compared with the currents evoked by room temperature Diprivan (Fig. 4A), the hTRPA1-mediated currents evoked by once heated Diprivan were small (Fig. 4B). The peak-current density was significantly lower in once heated Diprivan than in Diprivan (Fig. 4C, $p = 0.013$). AITC-activated hTRPA1 currents were observed after exposure to once heated Diprivan (Fig. 4B).

3.5. Effects of heated 2,6-diisopropylphenol on hTRPA1 activity

To determine the effect of heated on 2,6-diisopropylphenol, the

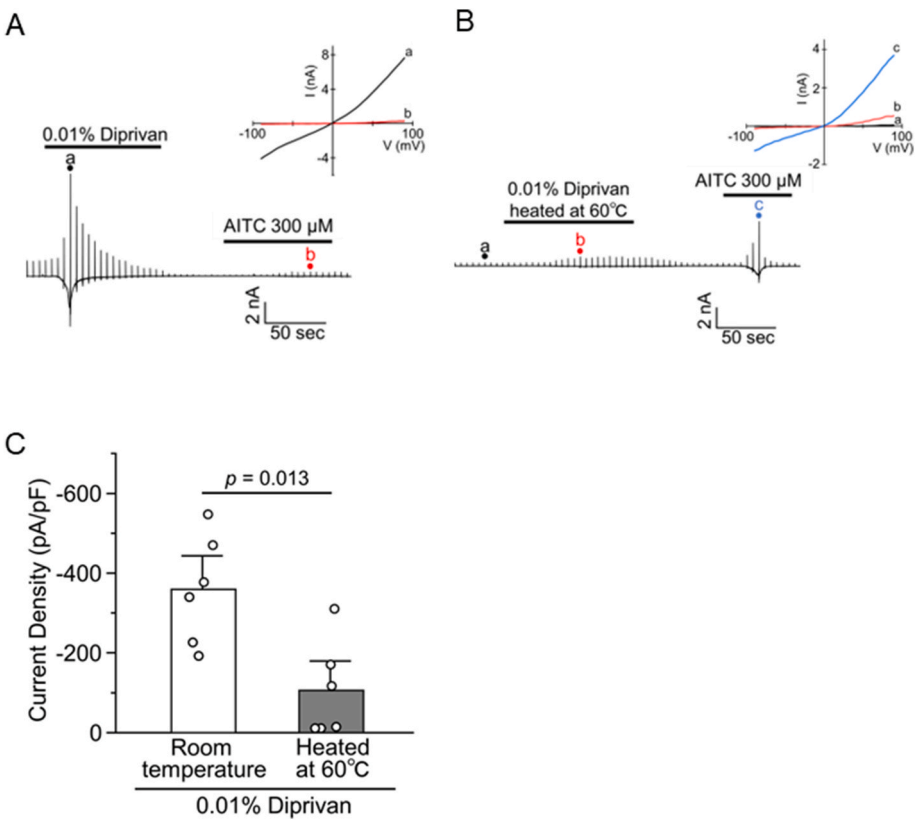

Fig. 4. Effects of hTRPA1 activation by heated propofol Reagents at room temperature and reagents heated to 1 h and then retuern to room temperature were used. A, B) Representative trace of 0.01 % Diprivan– (A) or once heated Diprivan– (B) activated hTRPA1 currents in HEK293T cells. The holding potentials were −60 mV with ramp pulses (−100~+100 mV, 300 ms). The insets show current-voltage curves at the time points of a, b, and c. C) Comparison of hTRPA1 current density at −60 mV in room temperature Diprivan and once heated Diprivan. The values were expressed as mean ± SEM, with n = 6. Student's t-test.

active ingredient in the propofol, we examined whether heated 2,6-diisopropylphenol affected hTRPA1-activating ability. As in Result 3, 2.6-diisopropylphenol was heated to 60 °C for 1 h and then returned to room temperature before use. Compared with the hTRPA1 current induced by room temperature 2,6-disopropylphenol (Fig. 5A), the hTRPA1-evoked currents by once heated 2,6-disopropylphenol were small (Fig. 5B). AITC-activated hTRPA1 currents were also observed after washout of once heated 2,6-diisopropylphenol (Fig. 5B). The peak-current density was significantly lower in once heated 2,6-diisopropylphenol than in room temperature 2,6-diisopropylphenol (Fig. 5C, $p = 0.008$)

3.6. Human $GABA_A$-activating ability of heated propofol

Because heated propofol and 2,6-diisopropylphenol lost the ability to activate hTRPA1 to some extent (Figs. 4 and 5), we examined whether heated propofol maintained its anesthetic ability using HEK293T cells expressing human $GABA_A$ receptors. $GABA_A$ receptors consist of a pentamer of two α subunits, two β subunits, and one γ subunit [20–22]. The most common combination of $GABA_A$ receptor subunits expressed in the central nervous system is α1β2γ2 [23], and the anesthetic effects of propofol are thought to result from its binding to the α1β2 subunit of the $GABA_A$ receptor [24]. Therefore, we used transfected cells with human GABA-ARα1-pcDNA3 and human GABA-ARβ2-pcDNA3 plasmids. First, GABA, a positive control, was applied to determine whether $GABA_A$ receptors were activated, and the results confirmed GABA-activated human α1β2 $GABA_A$–mediated currents (Fig. 6A). Similar human α1β2 $GABA_A$–mediated currents were observed by application of Diprivan (0.01 %) or once heated Diprivan at room temperature (Fig. 6B and C). Although the mechanism is unclear, some current activation was observed upon Diprivan washout (Fig. 6B and C). The peak-current density was not significantly different at room temperature Diprivan and once heated Diprivan (Fig. 6D).

3.7. Human $GABA_A$-activating ability of heated 2,6-diisopropylphenol

To confirm whether heated 2,6-diisopropylphenol, the active ingredient of propofol, maintains its anesthetic ability, we measured human α1β2 $GABA_A$ receptor-mediated current using HEK293T cells expressing the human α1β2 $GABA_A$ receptor. Once heated 2,6-disopropylphenol currents were observed compared to human α1β2 $GABA_A$ mediated currents with 2,6-disopropylphenol at room temperature (Fig. 7A and B). Similar to Result 5, the mechanism is unclear, but some current activation was observed after washing 2,6-diisopropylphenol (Fig. 7B). The peak current densities were similar for room temperature 2,6-diisopropylphenol and once heated 2,6-diisopropylphenol, there were not significantly different (Fig. 7C).

4. Discussion

In the present study, we explored strategies to reduce propofol-induced vascular pain sensation. As previous reports showed that TRPA1 has temperature sensitivity, we examined whether hTRPA1 activation is affected by propofol at different temperatures in HEK293T cells. No studies have examined temperature-dependent effects of propofol in patch-clamp experiments. In an environment above 35 °C, propofol-induced hTRPA1 currents were smaller. The results from the pre-heating experiments indicated that propofol should be kept at temperatures above 35 °C to minimize TRPA1 activation.

Propofol has been reported to interact with methionine residues M912 and M953 at sites within the S5–S6 pore region of TRPA1 [25,26]. It has also been suggested that the S5–S6 pore region is involved in the

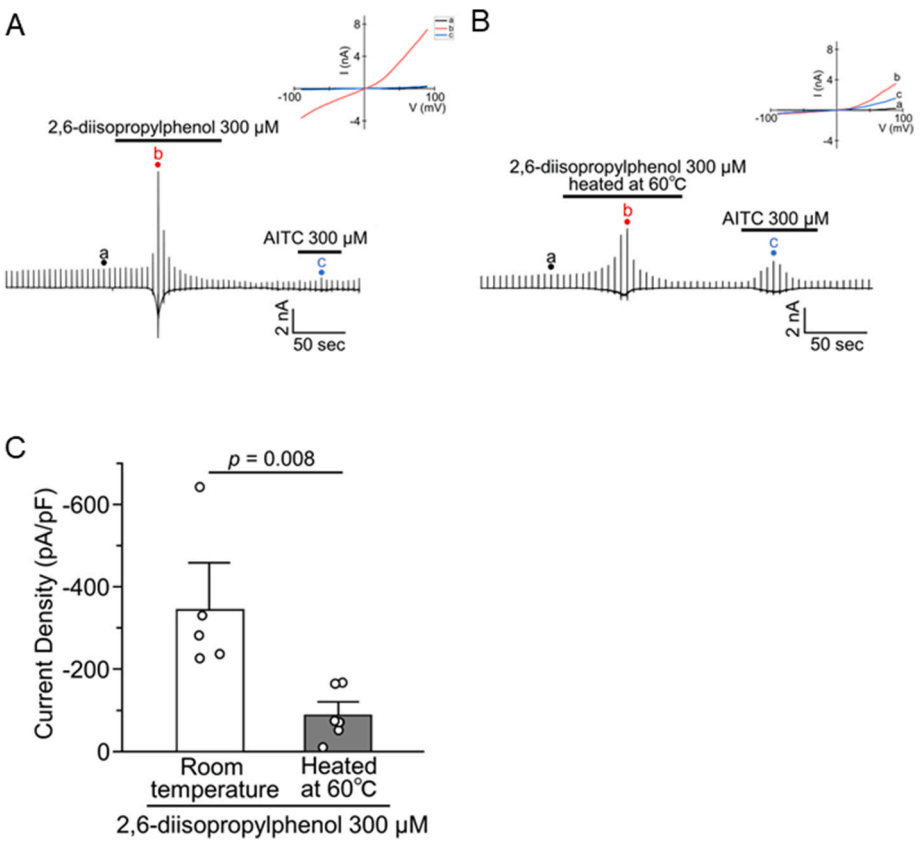

Fig. 5. Effects of hTRPA1 activation by heated 2,6-diisopropylphenol Reagents at room temperature and reagents heated to 1 h and then retuern to room temperature were used. A, B) Representative trace of 2,6-diisopropylphenol 300 μM– (A) or once heated 2,6-diisopropylphenol 300 μM– (B) activated hTRPA1 currents in HEK293T cells. The holding potentials were −60 mV with ramp pulses (−100∼+100 mV, 300 ms). The insets show current-voltage curves at the time points of a, b, and c. C) Comparison of hTRPA1 current density at −60 mV in room temperature 2,6-diisopropylphenol and once heated 2,6-diisopropylphenol. The values were expressed as mean ± SEM, with n = 5–6. Mann–Whitney *U* test.

thermal stimulation-sensitive of TRPA1 [14]. These suggests that propofol and heat are activated in relation to the same pore area, and therefore pre-heating before TRPA1 agonists application may have some effect on the pore area. As shown in Fig. 3, not only propofol but also AITC-mediated hTRPA1 currents were small at the thermal environment above 35 °C. Therefore, heating to 35 °C is thought to have an inhibitory effect on TRPA1 gating.

hTRPA1-mediated currents were not observed during pre-cooling but were observed during pre-heating (Fig. 2), indicating that hTRPA1 is activated by thermal stimulation. However, as shown in Fig. 2F and G, hTRPA1 currents were observed when the temperature was lowered during the application of propofol after pre-heating. It is possible that some hTRPA1 that was not sensitized by thermal stimulation was activated by Diprivan. This suggests that hTRPA1 is not completely desensitized by thermal stimulation at approximately 35 °C. In a previous report, co-application of heat and agonist resulted in suppression and desensitization of TRPA1 activation at 39 °C, but TRPA1 activation was still observed at 34 °C [15]. Therefore, it is likely that continuous heating above 35 °C would make TRPA1 less activated by chemical agonists. Consequently, it is important to apply propofol while keeping the temperature around hTRPA1 above 35 °C from pre-heating, which may suppress propofol-induced hTRPA1 activation at temperatures closer to body temperature.

We also observed that hTRPA1-mediated currents activated by once heated propofol and 2,6-diisopropylphenol were smaller (Figs. 4 and 5), Human $GABA_A$-mediated currents were caused by once heated propofol and 2,6-diisopropylphenol to a similar extent as at room temperature (Figs. 6 and 7). These indicate that heated propofol, with or without fat emulsion, causes a change in its hTRPA1-activating ability, but propofol activity on human $GABA_A$ receptors was not lost with heating. In this study, heating propofol at 60 °C had some effect on hTRPA1 activating ability. Therefore, once heated propofol at 60 °C may have changed the way TRPA1 is activated or the propofol itself chemical or structural modification. However, we have not been able to confirm any modifications caused by heating, so we will be examined in a future study. In addition, this study examined only the combination of α1β2 subunits of $GABA_A$ receptors. It has been reported that propofol also acts on β3 subunits [27,28], but the effects on expression systems, including β3 subunits, have not been explored in this study. Furthermore, $GABA_A$ receptors also contain gamma subunits in vivo. To clarify the effects of heated propofol on $GABA_A$ receptors, it is necessary to investigate other subunit sets of $GABA_A$ receptors, such as the β3 subunits, and GABA responses in isolated neurons.

In order to apply these results to clinical practice, it is important to consider the local temperature of human peripheral veins. In the peripheral veins where the infusion is administered, the blood temperature is similar to the tissue temperature, which is 32–33 °C [27]. Thus, it is necessary for propofol to be heated to 37–39 °C before administration to raise the local area where propofol is administered above 35 °C. Although we were unable to conform any chemical changes in heated propofol at 60 °C, the clinical usage guidelines for propofol [28] state that “no significant changes were observed and the quality can be determined to be stable” even after heating at 40 °C for four weeks in the following stress test items: product description, pH, purity test (free fatty acid), particle number test, mean particle diameter, quantitative test. Therefore, since there is thought to be no problem with the stability of propofol heated to 37–39 °C, it is believed that it can be administered to humans even if heated to 39 °C. In fact, when propofol is administered to

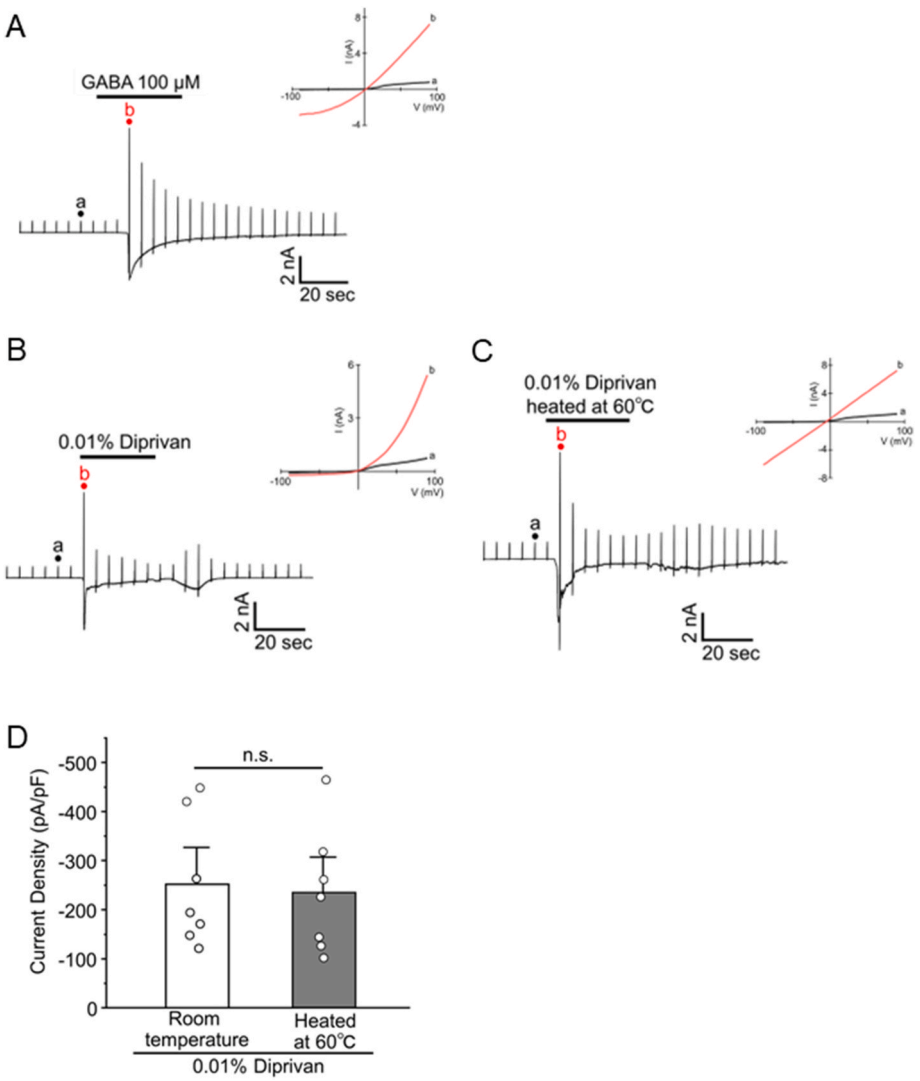

Fig. 6. Effects of human GABAA activation by heated propofol Reagents at room temperature and reagents heated to 1 h and then retuern to room temperature were used. Representative trace of GABA- (A), Diprivan- (B) or once heated Diprivan- (C) activated human α1β2 $GABA_A$ currents in HEK293T cells. The holding potentials were −60 mV with ramp pulses (−100~+100 mV, 300 ms). The insets show current-voltage curves at the time points of a and b. D) Each bar graph indicates the average value of the peak-current density of Diprivan and once heated Diprivan at −60 mV. n. s., not significant, Student's t-test (n = 6–7).

humans, the pain experienced varies from person to person. We believe that future clinical trials are needed in humans to determine whether administering propofol heated to 37–39 °C will result in any changes to vascular pain.

There were no differences in hTRPA1 activity between Diprivan and Maruishi (Fig. 1), suggesting there may not be differences in the extent of pain sensation between the two types of propofol. The vascular pain sensation evaluated in most of previous clinical study was a subjective patient assessment obtained from the time of propofol administration before loss of consciousness. Although Maruishi dissolved in MCT/LCT has been reported to be associated with less pain than Diprivan dissolved in LCT in the clinic [29], hTRPA1-activating ability was not different between the two propofol types in vitro (Fig. 1). Therefore, the differences in pain sensation in humans in response to different propofol types may be from factors other than hTRPA1 activity.

5. Conclusions

Our results showed that hTRPA1 activation by propofol was smaller in warm environments above 35 °C and suggest that heated propofol may reduce vascular pain. Our findings also suggest that heated propofol inhibits hTRPA1 activation and reduces vascular pain without losing its anesthetic function.

CRediT authorship contribution statement

Chihiro Suda: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Yasunori Takayama:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis. **Makoto Tominaga:** Writing – review & editing, Resources, Methodology, Investigation. **Tomoko Akase:** Supervision, Project administration, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

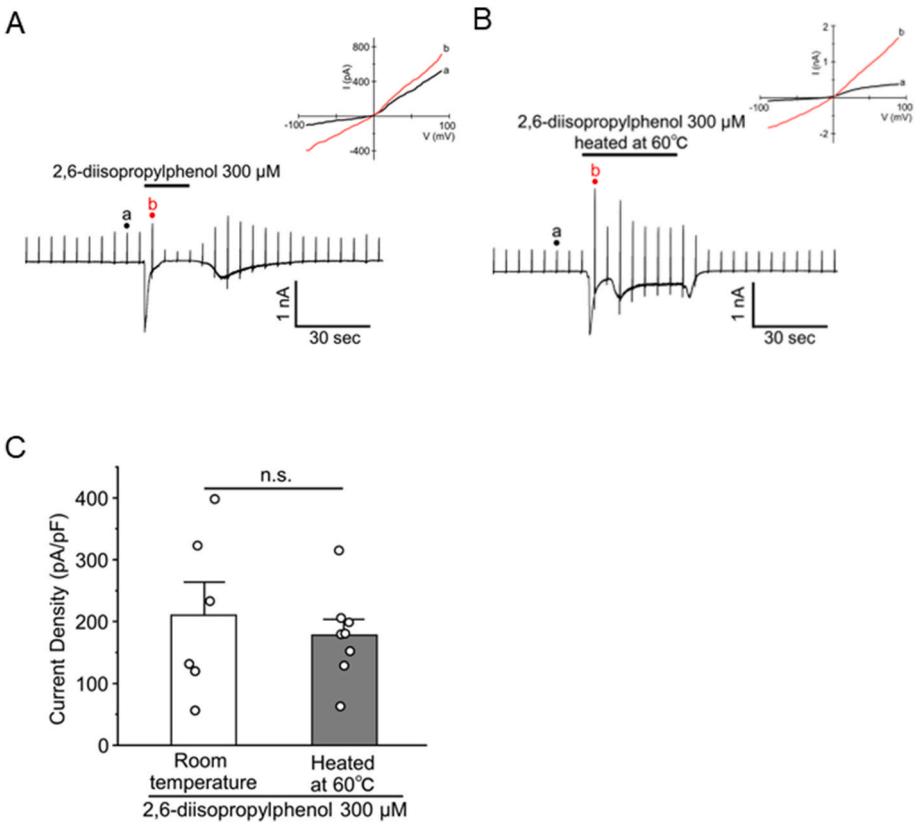

Fig. 7. Effects of human GABAA activation by heated 2,6-diisopropylphenol Reagents at room temperature and reagents heated to 1 h and then retuern to room temperature were used. Representative trace of 2,6-diisopropylphenol - (A) or once heated 2,6-diisopropylphenol - (B) activated human α1β2 $GABA_A$ currents in HEK293T cells. The holding potentials were −60 mV with ramp pulses (−100~+100 mV, 300 ms). The insets show current-voltage curves at the time points of a and b. D) Each bar graph indicates the average value of the peak-current density of 2,6-diisopropylphenol and once heated 2,6-diisopropylphenol at −60 mV. n. s., not significant, Student's t-test (n = 6–8).

the work reported in this paper.

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References

[1] J.W. Dundee, New I.V. anaesthetics, Br. J. Anaesth. 51 (7) (1979) 641–648, https://doi.org/10.1093/bja/51.7.641.
[2] W. Klement, J.O. Arndt, Pain on injection of propofol: effects of concentration and diluent, Br. J. Anaesth. 67 (3) (1991) 281–284, https://doi.org/10.1093/bja/67.3.281.
[3] C. Lee, H. Yim, H. Ham, Effect of injection pain and withdrawal movement of propofol and rocuronium in the induction of anaesthesia on postoperative pain outcomes in gynaecological laparoscopic surgery: a prospective observational study, Singap. Med. J. 65 (6) (2022) 326–331, https://doi.org/10.11622/smedj.2022040.
[4] A.K. Persson, F.D. Pettersson, L.E. Dyrehag, J. Åkeson, Prediction of postoperative pain from assessment of pain induced by venous cannulation and propofol infusion, Acta Anaesthesiol. Scand. 60 (2) (2016) 166–176, https://doi.org/10.1111/aas.1263.
[5] L. Jalota, V. Kalira, E. George, Y.Y. Shi, C. Hornuss, O. Radke, L.N. Pace, C.C. Apfel, Prevention of pain on injection of propofol: systematic review and meta-analysis, Br. Med. J. 342 (2011) d1110, https://doi.org/10.1136/bmj.d1110.
[6] E. Bakhtiari, S.H. Mousavi, M. Gharavi Fard, Pharmacological control of pain during propofol injection: a systematic review and meta-analysis, Expet Rev. Clin. Pharmacol. 14 (7) (2021) 889–899, https://doi.org/10.1080/17512433.2021.1919084.
[7] C.L. Huang, Y.P. Wang, Y.J. Cheng, L. Susetio, C.C. Liu, The effect of carrier intravenous fluid speed on the injection pain of propofol, Anesth. Analg. 81 (5) (1995) 1087–1088, https://doi.org/10.1097/00000539-199511000-00033.
[8] M.J. McCulloch, N.W. Lees, Assessment and modification of pain on induction with propofol (Diprivan), Anaesthesia 40 (11) (1985) 1117–1120, https://doi.org/10.1111/j.1365-2044.1985.tb10615.x.
[9] J.A. Matta, P.M. Cornett, R.L. Miyares, K. Abe, N. Sahibzada, G.P. Ahern, General anesthetics activate a nociceptive ion channel to enhance pain and inflammation, Proc. Natl. Acad. Sci. U. S. A. 105 (25) (2008) 8784–8789, https://doi.org/10.1073/pnas.0711038105.
[10] R. Nishimoto, M. Kashio, M. Tominaga, Propofol-induced pain sensation involves multiple mechanisms in sensory neurons, Pflugers Arch. Eur. J. Physiol. 467 (9) (2015) 2011–2020, https://doi.org/10.1007/s00424-014-1620-1.
[11] U. Anand, W.R. Otto, P. Facer, N. Zebda, I. Selmer, M.J. Gunthorpe, I.P. Chessell, M. Sinisi, R. Birch, P. Anand, TRPA1 receptor localisation in the human peripheral nervous system and functional studies in cultured human and rat sensory neurons, Neurosci. Lett. 438 (2) (2008) 221–227, https://doi.org/10.1016/j.neulet.2008.04.007.
[12] G.M. Story, A.M. Peier, A.J. Reeve, S.R. Eid, J. Mosbacher, T.R. Hricik, T.J. Earley, A.C. Hergarden, D.A. Andersson, S.W. Hwang, P. McIntyre, T. Jegla, S. Bevan, A. Patapoutian, ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures, Cell 112 (6) (2003) 819–829, https://doi.org/10.1016/s0092-8674(03)00158-2.
[13] I. Vandewauw, K.D. Clercq, M. Mulier, K. Held, S. Pinto, N.V. Ranst, A. Segal, T. Voet, R. Vennekens, K. Zimmermann, J. Vriens, T. Voets, A TRP channel trio mediates acute noxious heat sensing, Nature 555 (7698) (2018) 662–666, https://doi.org/10.1038/nature26137.
[14] L. Moparthi, V. Sinica, V.K. Moparthi, et al., The human TRPA1 intrinsic cold and heat sensitivity involves separate channel structures beyond the N-ARD domain, Nat. Commun. 13 (1) (2022) 6113, https://doi.org/10.1038/s41467-022-33876-8.
[15] S. Wang, J. Lee, J.Y. Ro, M.-K. Chung, Warmth suppresses and desensitizes damage-sensing ion channel TRPA1, Mol. Pain 8 (2012) 22, https://doi.org/10.1186/1744-8069-8-22.
[16] 1% Diprivan® Injection Interview Form, Sept, eighteenth ed., 2021 revision.
[17] A.W. Doenicke, M.F. Roizen, J. Rau, W. Kellermann, J. Babl, Reducing pain during propofol injection: the role of the solvent, Anesth. Analg. 82 (3) (1996) 472–474, https://doi.org/10.1097/00000539-199603000-00007.
[18] H. Ohmizo, S. Obara, H. Iwama, Mechanism of injection pain with long and long-medium chain triglyceride emulsive propofol, Can. J. Anaesth. 52 (6) (2005) 595–599, https://doi.org/10.1007/BF03015768.
[19] A. Hinman, H.H. Chuang, D.M. Bautista, D. Julius, TRP channel activation by reversible covalent modification, Proc. Natl. Acad. Sci. U. S. A. 103 (51) (2006) 19564–19568, https://doi.org/10.1073/pnas.0609598103.

[20] Y. Chang, R. Wang, S. Barot, D.S. Weiss, Stoichiometry of a recombinant GABAA receptor, J. Neurosci. 16 (17) (1996) 5415–5424, https://doi.org/10.1523/JNEUROSCI.16-17-05415.1996.
[21] V. Tretter, N. Ehya, K. Fuchs, W. Sieghart, Stoichiometry and assembly of a recombinant GABAA receptor subtype, J. Neurosci. 17 (8) (1997) 2728–2737, https://doi.org/10.1523/JNEUROSCI.17-08-02728.1997.
[22] S.J. Farrar, P.J. Whiting, T.P. Bonnert, R.M. McKernan, Stoichiometry of a ligand-gated ion channel determined by fluorescence energy transfer, J. Biol. Chem. 274 (15) (1999) 10100–10104, https://doi.org/10.1074/jbc.274.15.10100.
[23] R.M. McKernan, P.J. Whiting, Which GABAA-receptor subtypes really occur in the brain? Trends Neurosci. 19 (4) (1996) 139–143, https://doi.org/10.1016/S0166-2236(96)80023-3.
[24] D.W. Lam, J.N. Reynolds, Modulatory and direct effects of propofol on recombinant GABAA receptors expressed in xenopus oocytes: influence of alpha- and gamma2-subunits, Brain Res. 784 (1–2) (1998) 179–187, https://doi.org/10.1016/s0006-8993(97)01334-6.
[25] H.T. Ton, T.X. Phan, A.M. Abramyan, L. Shi, G.P. Ahern, Identification of a putative binding site critical for general anesthetic activation of TRPA1, Proc. Natl. Acad. Sci. U.S.A. 114 (14) (2017) 3762–3767, https://doi.org/10.1073/pnas.1618144114.
[26] K.A. Woll, K.A. Skinner, E. Gianti, N.V. Bhanu, B.A. Garcia, V. Carnevale, R. G. Eckenhoff, R. Gaudet, Sites contributing to TRPA1 activation by the anesthetic propofol identified by photoaffinity labeling, Biophys. J. 113 (10) (2017) 2168–2172, https://doi.org/10.1016/j.bpj.2017.08.040.
[27] S.S. Jayakar, X. Zhou, D.C. Chiara, Z. Dostalova, P.Y. Savechenkov, K.S. Bruzik, W. P. Dailey, K.W. Miller, R.G. Eckenhoff, J.B. Cohen, Multiple propofol-binding sites in a γ-aminobutyric acid type A receptor (GABAAR) identified using a photoreactive propofol analog, J. Biol. Chem. 289 (40) (2014) 27456–27468, https://doi.org/10.1074/jbc.M114.581728.
[28] R. Jurd, M. Arras, S. Lambert, B. Drexler, R. Siegwart, F. Crestani, M. Zaugg, K. E. Vogt, B. Ledermann, B. Antkowiak, U. Rudolph, General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit, Faseb. J. 17 (2) (2003) 250–252, https://doi.org/10.1096/fj.02-0611fje.
[29] H. Barcroft, O.G. Edholm, Temperature and blood flow in the human forearm, J. Physiol. 104 (4) (1946) 366–376, https://doi.org/10.1113/jphysiol.1946.sp004129.