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Effects of anesthetic agents on in vivo axonal HCN current in normal mice

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HIGHLIGHTS

- The effects of anesthetic agents may influence axonal excitability.
- The hyperpolarization-activated cyclic nucleotide-gated (HCN) channel function was gradually suppressed in mice under isoflurane or intraperitoneal triple agents (medetomidine hydrochloride, midazolam, and butorphanol).
- Neurophysiological findings might be prone to anesthetic effects, especially in assessing the functions of ion channels including the HCN channels and axonal excitability.

ABSTRACT

Objective: The objective was to study the in vivo effects of anesthetic agents on peripheral nerve excitability.
Methods: Normal male mice were anesthetized by either isoflurane inhalation or a combination of medetomidine, midazolam, and butorphanol intraperitoneal injection ("triple agents"). Immediately after induction, the tail sensory nerve action potential was recorded and its excitability was monitored.
Results: Under both anesthetic protocols, there was an interval excitability change by long hyperpolarizing currents. There was greater threshold reduction approximately 30 min post induction, in comparison to immediately post induction. Other excitability parameters were stable over time. Modeling suggested interval suppression of internodal H conductance or leak current.
Conclusions: Anesthetic agents affected responses to long hyperpolarizing currents.
Significance: Axonal excitability during intraoperative monitoring may be affected by anesthetic agents. Interpretation of interval excitability changes under anesthesia requires caution, especially with long hyperpolarizing currents.

1. Introduction

Knowledge of potential modulators of neurophysiological functions is important for proper assessment of obtained data. Among them, pharmacological effects have been extensively studied to shed light on the precise mechanisms of neural regulation. Anesthetic agents are obvious examples to take advantage of such effects on the nervous system for clinical use. Anesthetic agents are unique because they are often an inevitable component for management of a patient, especially during intraoperative monitoring and other invasive procedures that are recorded under anesthesia. In comparison to the central nervous system, anesthetic effects on the peripheral nervous system have been less intensively studied, and the focus has mostly been placed on basic conductive parameters such as nerve conduction velocity and response amplitudes (Oh et al., 2010; Osuchowski et al., 2009). Besides these basic

Abbreviations: CI, confidence interval; HCN channel, hyperpolarization-activated cyclic nucleotide-gated channel; I_h, hyperpolarization-activated current; *I/V*, current–threshold relationship; NAP, nerve action potential; RC, recovery cycle; SDTC, strength–duration time constant; TE, threshold electrotonus.

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nerve conduction parameters, the assessment of axonal excitability has been recently paid attention, because axonal and neuronal excitability alone can manifest symptoms and signs (Krishnan et al., 2009; Moldovan et al., 2013; Nodera and Kaji, 2006). A recent study using local anesthesia confirmed dynamic changes of axonal excitability and a potential reversible structural impairment of the peripheral axolemma (Moldovan et al., 2014). Thus, the aim of the present study was to assess the effect of anesthetic agents in vivo on the axonal excitability.

2. Methods

2.1. Study protocol

The experiment was approved by the local animal facility at the Tokushima University. ICR male mice (SLC, Hamamatsu, Japan) 7–8 weeks old were tested. The following two types of anesthetic protocols were used for electrophysiological testing: (1) Isoflurane (2%) was mixed with oxygen (1.5 l/min) for induction in a plastic box. Within 3 min after induction, the mouse became sedated and was transferred to an examination table where isoflurane (1.5%) was provided through a nasal tube throughout the excitability testing. (2) "Triple-agent anesthesia" using medetomidine, midazolam, and butorphanol had been reported as alternative to a combination using ketamine due to its potential addictive effects (Kawai et al., 2011). Three agents were mixed in distilled water immediately before use: medetomidine hydrochloride (an alpha-2 adrenoceptor agonist) at 0.3 mg/kg, midazolam (a benzodiazepine derivative) at 4 mg/kg, and butorphanol (an opioid partial agonist analgesic) at 5 mg/kg. Intraperitoneal injection at 0.1 ml/10 g body weight was provided. Five days after performing electrophysiological tests under isoflurane, another test was performed under triple-agent anesthesia in half of the animals, whereas the opposite order (triple agents, followed by isoflurane) was performed in the other half.

2.2. Axonal excitability study

Electrophysiological studies were performed on the tail under either method of anesthesia with the animal warmed on a thermostat-controlled heating pad (BWT-100A, Bioresearch Center, Nagoya, Japan) to maintain the tail temperature at 33–34 °C throughout the studies. Nerve action potentials (NAPs) were recorded orthodromically by placing 30-gauge stainless steel, disposable needle electrodes as follows: the reference and active recording needles were placed 10 and 20 mm from the base of the tail, respectively; the cathode and anode were placed 40 and 50 mm, respectively, distal to the active recording electrode, and the ground electrode placed midway between the stimulating and recording electrodes. Immediately thereafter, an axonal excitability test was undertaken. It took approximately 5 min to set up the electrodes.

For neuronal excitability testing, stimulation was controlled by a PC running the QtracS program (Institute of Neurology, London, UK), connected via a digital I/O device (National Instruments, Austin, TX, USA) to a preamplifier (MEG-1200: Nihon Kohden, Tokyo, Japan) and a stimulator (DS-4: Digitimer, Letchworth, UK). Using 1-ms rectangular stimuli, the negative peak of the NAP was recorded. For excitability tests, the TRONDNF multiple excitability recording protocol was used. Stimulus–response curves, which were determined using a 1-ms-duration test stimulus increased from zero until supramaximal potentials were attained. To record threshold electrotonus (TE), the unconditioned threshold on one channel was tracked, while the threshold at discrete points was determined on three other channels as follows: (1) during and after 100 ms of hyperpolarizing and depolarizing currents, set to ±40% of the unconditioned threshold and (2) during and after 200 ms of hyperpolarizing current, set to −70% of the unconditioned threshold. For the +40% depolarizing conditioning current, the difference of threshold changes between the greatest threshold reduction and at the end of the 100-ms conditioning pulse was defined as S2 accommodation. For the −70% hyperpolarizing conditioning current, the lowest threshold reduction was defined as TE_h (peak: −70%). The difference of threshold changes between TE_h (peak: −70%) and at the end of the 200-ms conditioning pulse was defined as S3 accommodation. For the recovery cycle (RC), a supramaximal conditioning stimulus was given with delays ranging from 200 to 1.6 ms before the test stimulus was provided on another channel. The current–threshold relationship (I/V) was then recorded with a 1-ms test stimulus applied 200 ms after the onset of a long-lasting subthreshold polarizing current, the strength of which was altered in steps of 10%, from +50% (depolarizing current) to −100% (hyperpolarizing current) of the control threshold. The strength–duration time constant (SDTC) describes the stimulus strength required to excite nerves as the stimulus width is increased from a duration of 0.1–0.5 ms. A set of excitability parameters was derived from the recordings as previously described (Nodera and Rutkove, 2012). One cycle of the multiple excitability tests takes approximately 20 min.

2.3. Data analysis

Axonal excitability data at two different time points after exposure to anesthetic agents were compared by the paired t-test if normal distribution was met by the Shapiro–Wilk test, or alternatively by the Wilcoxon signed-rank test. The Mann–Whitney U test was used for comparison between two independent groups. Intraclass correlation was calculated to assess the reproducibility of the repeated measurements (SPSS version 22: IBM, New York, NY, USA). A significance was set at $P < 0.05$.

2.4. Modeling of the excitability data

The commercially available Bostock model of the human motor axon was used in the simulation of axonal excitability (MEMFit, QtracP version 17/10/2014), as previously explained in detail (Howells et al., 2012; Kiernan et al., 2005). Parameter adjustments were made to improve the fit to normal human RC, strength–duration, I/V, and TE. To reflect better the characteristic waveform changes (see Section 3), the weighting factors were set as follows: TE, 3; RC, 1; SDTC, 1; and I/V, 3. The early (initial) recordings under isoflurane and the triple protocol were first optimized, followed by identifying the best change in each parameter with floating the membrane potential to match the late (second) recording. The tested parameters were as follows: nodal and internodal resting potentials, nodal sodium permeability, percent persistent Na^+, nodal and intermodal slow K^+ conductance, nodal and internodal fast K^+ conductance, internodal H conductance, nodal and internodal leak conductance, Barrett–Barrett conductance, and total pump currents.

3. Results

Axonal excitability tests were performed and the excitability parameters were compared between the two time points, presumably comparing conditions with different tissue concentrations of anesthetic agents (see Section 4). In contrast to the early recording cycle (approximately 10–30 min after induction), the late cycle (approximately 30–50 min after induction) showed greater threshold reduction by long hyperpolarizing conditioning current (TE)

and increased S3 accommodation (Figs. 1 and 2, Table 1). To assess whether these interval changes were outside the test–retest reproducible ranges, the reproducibility of the excitability parameters between the early and late recordings was measured. As shown in Fig. 3, the excitability parameters associated with long hyperpolarizing currents were consistently deviated and the confidence intervals for the mean interval changes did not contain 0; thus, it is unlikely that the interval changes were due to random test–retest fluctuation (Tomlinson et al., 2010). These data suggested alteration of the hyperpolarization-activated current (I_h) (see Section 4).

STDC and RC were similar between the two cycles. The stimulus–response curves were similar under triple-agent anesthesia, but slightly shifted to the right (greater stimulus) under isoflurane suggesting decreased excitability. The amplitudes and the latencies of the NAP showed no significant interval changes in either anesthetic group ($P = 0.3–0.9$). The waveforms between the types of anesthetic agents showed differences: greater superexcitability ($P < 0.01$), greater TE_h (90–100 ms) ($P < 0.01$), and TE_h (peak: −70%) ($P < 0.05$) by triple-agent anesthesia than isoflurane at the late recording cycle.

To identify the excitability parameter to explain the interval excitability changes the most, mathematical modeling was performed (Table 2). As expected by the most notable interval changes identified by a long hyperpolarizing current (Table 1), optimizing the following parameters could reproduce the interval excitability change from the early (initial) recordings to the late (second) recordings under both anesthetic protocols: the leak conductance (G_{Lk}), the Barrett–Barrett conductance, and the internodal H conductance. By arbitrarily changing these parameters, the observed excitability changes were well reproduced (Fig. 4). Because these modeled waveforms were similar to each other, further identification of the single responsible parameter to explain the interval change might not be possible (see Section 4).

4. Discussion

In this study, we recorded axonal excitability in normal mice under two protocols to investigate the effects of anesthetic agents on axonal excitability. There was interval excitability change along the course of anesthesia, which showed less hyperpolarization-activated currents with a longer duration of anesthesia. The present study suggests that the effects of anesthetic agents should be taken into account when interpreting neurophysiological parameters that are recorded under anesthesia.

4.1. Anesthetic agents and ion channels

4.1.1. Isoflurane

Isoflurane is one of the most commonly used inhaled anesthetic agents for human and rodents (Stokes et al., 2009), which enhances GABA-mediated inhibition in the central nervous system (Kotani and Akaike, 2013). Isoflurane also acts on other receptors such as voltage-gated Na^+ channels, acetylcholine, TWIK-related K^+ channels-1, voltage-gated Ca^{2+} channels, and the potassium ATP channel (Fanchaouy et al., 2013; Kakinohana, 2014; Mowrey et al., 2013; OuYang and Hemmings, 2007). The neuronal and ionic mechanisms underlying the actions of isoflurane remain elusive, but modulation of ionotropic receptors, background K^+ channels, and hyperpolarization-activated cyclic-nucleotide gated (HCN) channels are considered to play a major role (Chen et al., 2009).

HCN channels are activated by membrane hyperpolarization (I_h: hyperpolarization-activated current), are permeable to Na^+ and K^+, and are constitutively open at voltages near the resting membrane

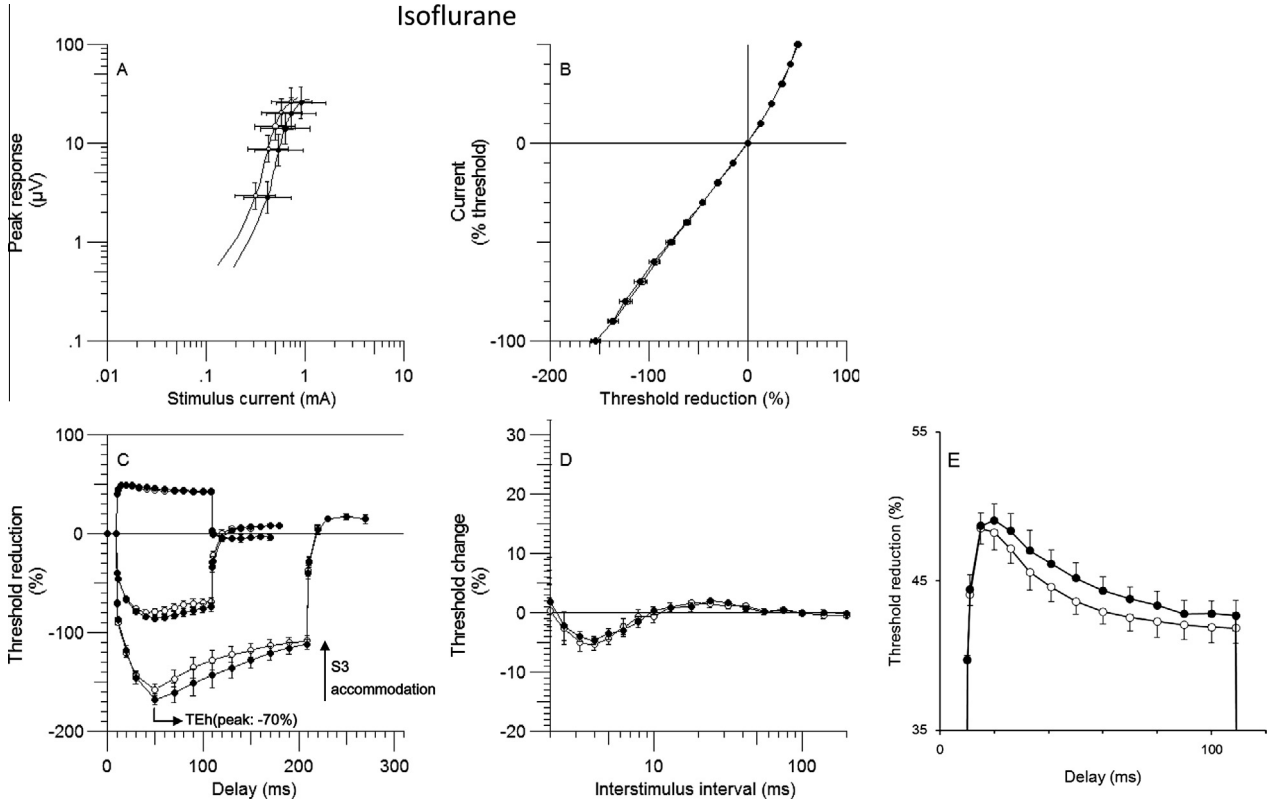

Fig. 1. Axonal excitability testing under isoflurane anesthesia, immediately after induction (early phase: open circles, approximately 10–30 min after induction) and 30–50 min after induction (late phase: closed circles). The threshold current in the late phase was greater than the early phase by the long hyperpolarizing conditioning current (threshold electrotonus, panel C).

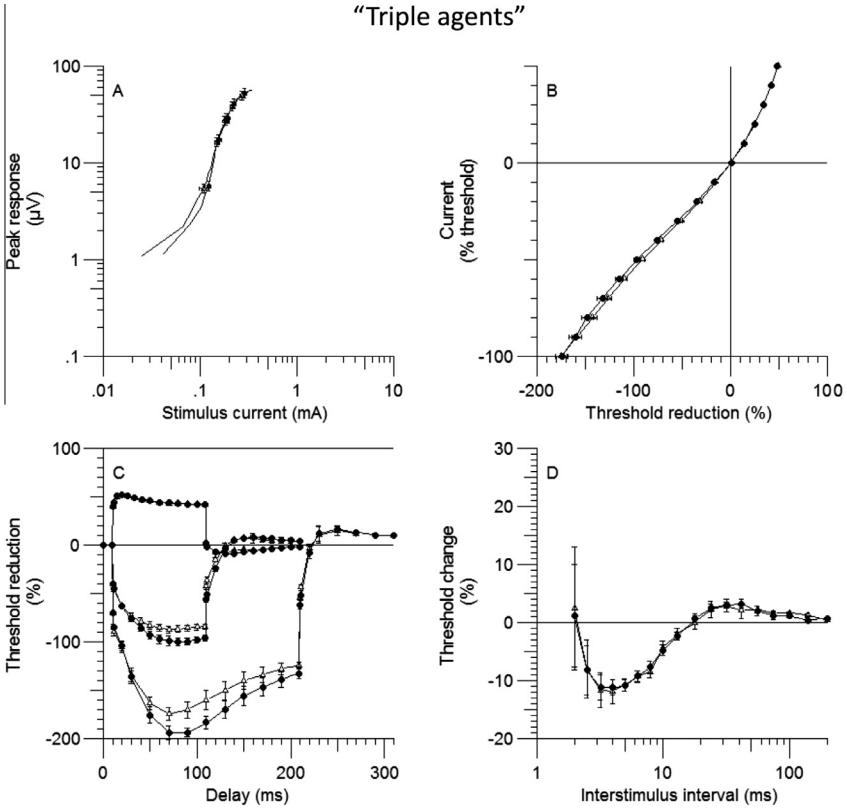

Fig. 2. Axonal excitability testing under triple-agent anesthesia, immediately after induction (early phase: open circles) and 30–50 min after induction (late phase: closed circles). Similar to isoflurane (Fig. 1), the threshold current in the late phase was greater than the early phase by the long hyperpolarizing conditioning current (threshold electrotonus, panel C).

Table 1
Nerve excitability parameters under two anesthetic protocols.

	Isoflurane (N = 10)			Triple agents (N = 5)		
	Early	Late	P-values	Early	Late	P-values
Amplitude (μV)	41.6 ± 14.5	44.8 ± 14.4	0.3	60.2 ± 15.2	61.7 ± 14.2	0.6
Peak latency (ms)	1.7 ± 0.1	1.8 ± 0.2	0.6	1.7 ± 0.2	1.7 ± 0.3	0.9
Threshold electrotonus						
TE_d (10–20 ms)	47.3 ± 1.1	48.4 ± 1.2	0.1	50.6 ± 1.3	50.8 ± 1.1	0.2
TE_d (40–60 ms)	43.0 ± 0.8	44.4 ± 0.9	**0.03**	43.8 ± 0.6	44.2 ± 0.9	0.9
TE_d (90–100 ms)	41.9 ± 1.0	42.8 ± 0.9	0.25	41.7 ± 0.6	41.8 ± 0.4	0.2
TE_h (10–20 ms)	−71.1 ± 1.6	−73.2 ± 1.8	**0.03**	−68.2 ± 2.3	−70.2 ± 2.1	0.2
TE_h (20–40 ms)	−78.9 ± 2.6	−83.2 ± 2.3	**0.02**	−78.8 ± 3.6	−85.5 ± 3.4	**0.01**
TE_h (90–100 ms)	−69.8 ± 4.3	−75.3 ± 4.5	**0.04**	−84.7 ± 2.9	−97.3 ± 3.0	**0.02**
S2 accommodation	5.6 ± 0.9	5.7 ± 0.9	0.7	8.3 ± 1.4	8.6 ± 1.0	0.9
TE_h (peak: −70%)	−161.4 ± 6.3	−176.7 ± 5.9	**<0.01**	−176.7 ± 6.7	−197.3 ± 4.2	**0.01**
S3 accommodation (−70%)	51.7 ± 3.9	62.7 ± 3.2	**<0.01**	50.2 ± 7.4	61.5 ± 8.8	**0.03**
Recovery cycle						
Refractoriness at 2 ms	0.3 ± 4.3	1.8 ± 4.1	0.3	2.3 ± 10.5	1.2 ± 8.8	0.5
Superexcitability (%)	−6.8 ± 0.5	−5.8 ± 0.6	0.4	−12.6 ± 1.8	−11.8 ± 1.4	0.2
Late subexcitability (%)	1.8 ± 0.4	1.8 ± 0.2	0.8	2.8 ± 1.1	3.1 ± 0.5	0.8
Current/threshold relationship						
Resting I/V slope	0.74 ± 0.04	0.72 ± 0.02	0.32	0.69 ± 0.02	0.64 ± 0.01	0.1
Minimum I/V slope	0.58 ± 0.02	0.55 ± 0.02	**0.04**	0.47 ± 0.01	0.45 ± 0.02	**<0.01**
Hyperpol. I/V slope	0.72 ± 0.04	0.84 ± 0.09	0.17	0.66 ± 0.08	0.79 ± 0.07	0.6
Strength–duration time constant	0.22 ± 0.02	0.23 ± 0.03	0.5	0.29 ± 0.04	0.28 ± 0.03	0.4

potential; thus, HCN channels are one of the important mechanisms of regulating axonal excitability (Benarroch, 2013; Howells et al., 2012). Genetic mutations in the HCN genes can upregulate I_h, which result in neuronal hyperexcitability and underlie human epilepsy (Reid et al., 2012). Dysfunction of HCN channels has been associated with neuromuscular diseases such as intermittent porphyria and neuropathic pain (Emery et al., 2011; Lin et al., 2008; Postea and Biel, 2011). Of interest, the late recording of the hyperpolarizing TE showed downward waveform shift by weak (−40%) and strong (−70%) stimuli; at the same time, the S3 accommodation values (−70%) were increased by both anesthetic methods. The exact interpretation for these seemingly

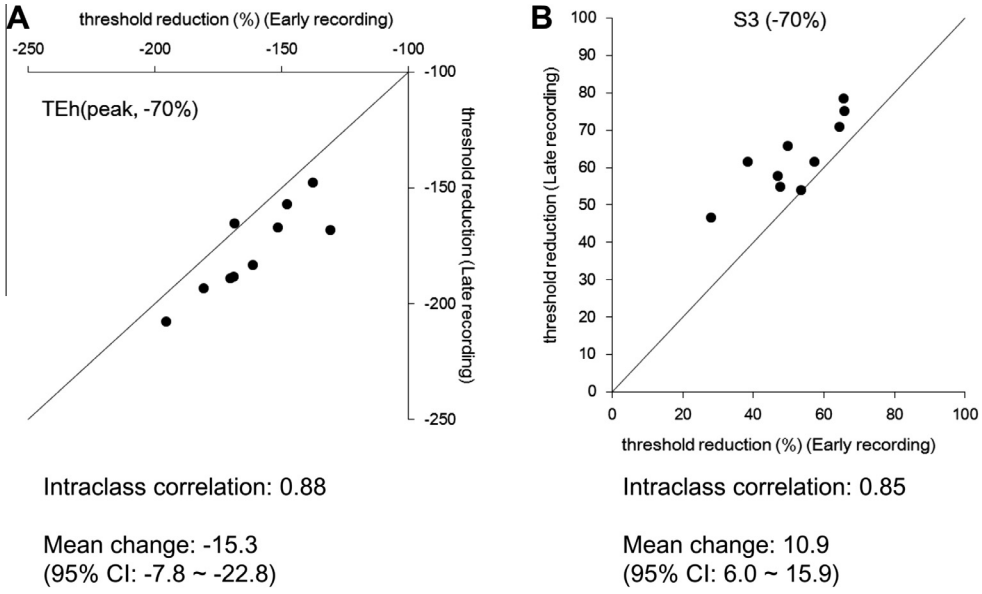

Fig. 3. Interval excitability changes at the two time points show greater threshold reduction by the long hyperpolarizing currents (TE_h (peak, −70%): panel A) and greater accommodation thereafter (S3: panel B) at the late recording than at the early time point. The high intraclass correlations indicate high reliability of repeated measurements. The 95% confidence intervals (CIs) for the mean changes did not contain 0, arguing against random test–retest fluctuation as an explanation for the interval changes.

Table 2
The list of the best change of each parameter to fit the interval difference of the excitability data (original value: optimized parameter for the early recording).

Isoflurane				"Triple anesthesia"			
Parameter	Final value	Original value	% Reduction of error	Parameter	Final value	Original value	% Reduction of error
G_{Lk}	37	42	−20.9	G_{Lk}	24.5	27.5	−12.9
G_{BB}	76.7	73.4	−12.5	G_{BB}	61.8	62	−4.9
G_H	107	122	−12.3	G_H	86.5	94.5	−4.7
G_{LkN}	0.028	0.59	−6.0	G_{LkN}	0.54	0.59	−3.8
G_{KfN}	25	29	−3.4	$P_{NaP(\%)}$	0.121	0.132	−1.6

G_{Lk} = leak conductance, G_{BB} = Barrett–Barrett conductance, G_H = internodal H conductance, G_{LkN} = nodal leak conductance, G_{KfN} = nodal fast K^+ conductance, $P_{NaP(\%)}$ = percent persistent Na^+.

contradictory changes is elusive; however, it could reflect either (1) a slower time course of the HCN channels to react to hyperpolarization or (2) different responses for multiple HCN subunits with different channel kinetics (e.g., HCN1 vs. HCN2) (Benarroch, 2013; Howells et al., 2012; Koncz et al., 2011).

In addition, TE_d (40–60 ms) became greater with time in the isoflurane group ($P = 0.03$) (Fig. 1E). However, its significance is uncertain, because there was no significant interval change in other related parameters (e.g., TE_d (10–20%), TE_d (90–100 ms), S2 accommodation, and late subexcitability), which suggest changes in the membrane potential or reduced slow K^+ current. Possibly, the change might reflect impaired fast K^+ current (Nodera and Kaji, 2006).

4.1.2. Which mechanism is responsible for excitability changes by isoflurane?

As shown in Fig. 4, the simulated effects of decreased hyperpolarization-activated current, decreased leak conductance, and increased Barrett–Barrett conductance are similar; thus, further identification of the responsible mechanism could not be possible. However, Patel et al. actually reported an "increase" of the leak current in the central nervous system by isoflurane, which was the opposite of the simulated result in this study (Patel et al., 1999); thus, the possibility of decreased leak current is unlikely to explain the excitability changes. Furthermore, although the Barrett–Barrett conductance could explain the interval excitability changes, it is related to the function of the axo-glial junction as seen in the excitability changes with maturation (Farrar et al., 2013). Besides apoptosis of glial cells by isoflurane (which is not directly associated with the present study because of the short study period) (Sun et al., 2014), we are not aware of any study of isoflurane interference with the axo-glial junction. In conclusion, the excitability changes in the present study indicate progressive suppression of I_h.

4.1.3. Combination of medetomidine, midazolam, and butorphanol

For animal surgery and minor procedures, the use of injectable anesthesia is simple and practical. Among many anesthetic agents, barbiturate and ketamine have been popular among animal researchers and veterinarians. However, the addictive properties of barbiturate and ketamine have prompted alternative anesthetic agents for animal research (Morgan and Curran, 2012). As one example, the combination of medetomidine (an alpha-2 adrenoceptor agonist), midazolam (a benzodiazepine derivative), and butorphanol (an opioid partial agonist analgesic) was reported to be effective for longer than 45 min in multiple strains of mice without a notable adverse effect (Kirihara et al., 2013). However, the neurophysiological effects of the combination protocol ("triple agents") have not been fully elucidated. The present study suggested progressive suppression of axonal I_h, as similarly shown with isoflurane. Because of the use of a combination regimen, the

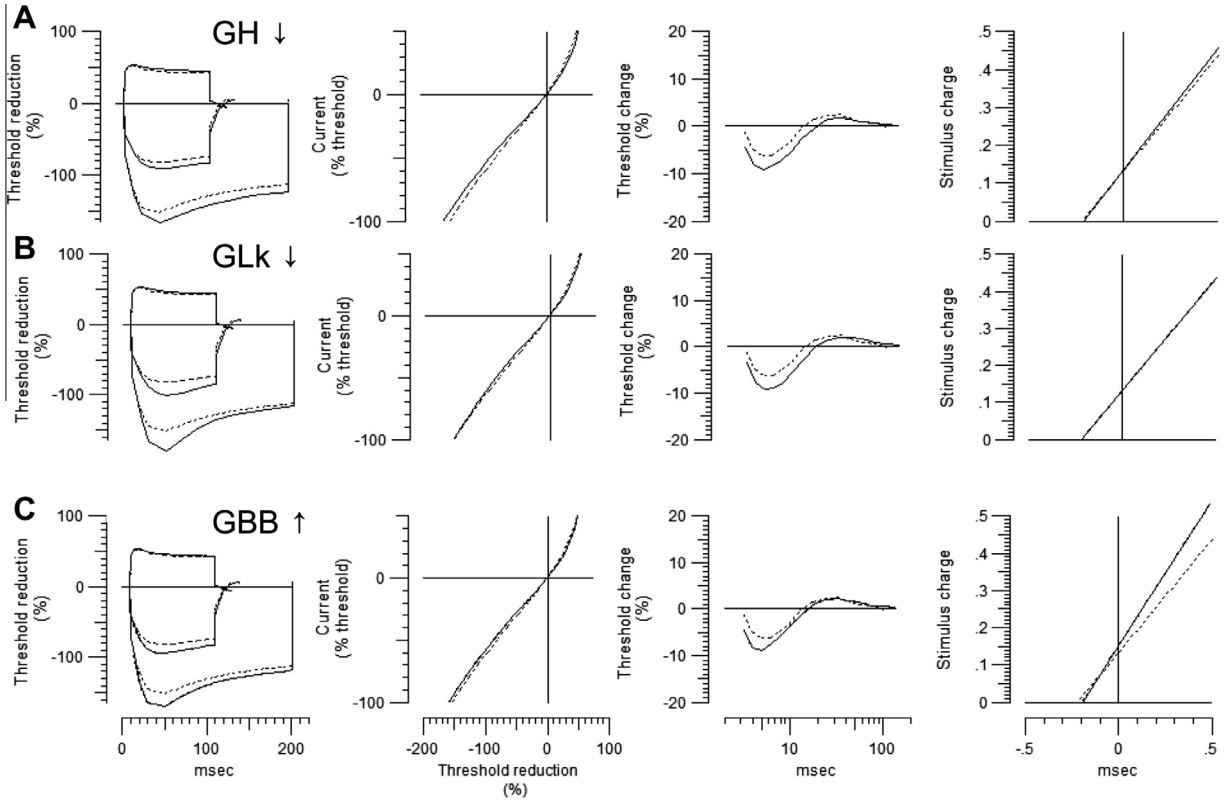

Fig. 4. Modeled changes of the excitability waveforms by altering the three parameters that best matched the interval waveform changes (Table 2). The dotted lines are the simulated waveforms by optimizing the parameters in the early phase of the isoflurane anesthesia and the solid lines are the results of changing one excitability parameter: (A) decreasing the internodal H conductance (G_H) to 65% of the original value, (B) decreasing the leak conductance (G_{Lk}) to 50% of the original, and (C) increasing the Barrett–Barrett conductance (G_{BB}) to 122% of the original. Note the greater threshold changes by the long hyperpolarizing current (threshold electrotonus) and greater superexcitability (recovery cycle) as the notable changes in these three simulations and they are indistinguishable. The degrees of the parameter changes were selected arbitrarily.

pharmacological agent responsible for suppression of I_h cannot be specified (see Section 4.2).

4.2. Limitations of the study

This study has limitations. First, it is possible that anesthetic effects on peripheral axons may differ among different species and possibly different mouse strains; thus, the present results may not be directly applicable to human recordings. Maurer et al. recorded sensory excitability in patients before and after the induction of general anesthesia (propofol or sevoflurane). There were some nonsignificant interval excitability changes, and the authors attributed the changes to altered body temperature (Maurer et al., 2010). However, their recording protocol did not include strong and long hyperpolarization (−70% TE) that is suitable for the assessment of HCN current. Besides, their study showed decreased overshoot after hyperpolarizing current (TE_h (overshoot)) that might indicate an impaired HCN current in their study. Further study in human subjects using the present recording protocol would clarify the issue.

Second, the inhibitory effect of I_h by the triple-agent protocol cannot be specified to a single agent. When we tried to induce anesthesia by using one or two of the triple agents, adequate depth of anesthesia was not achieved and proper excitability recording was impossible due to body movement (data not shown). Among the three agents in the "triple-agent protocol," our literature search did not identify evidence to convincingly show an effect on HCN channels by either midazolam (including other benzodiazepines) or butorphanol. However, elucidation of the mechanisms of action of general anesthetics in the nervous system is currently under way; many regulatory mechanisms including HCN channels have been suggested (Hemmings et al., 2005). Of note, Barth et al. reported that the activation of alpha-2 adrenergic receptors suppressed HCN channels in the apical dendrite of the prefrontal cortex; thus, medetomidine could be responsible for the observed alteration of I_h, but its effect on the peripheral axons is elusive (Barth et al., 2008).

Third, the comparison was not against a control condition (i.e., without anesthesia) because it is practically impossible to record the excitability study under no anesthesia because of motion artifact. Recording in vitro using an extracted nerve tissue is possibly considered as control; however, an in vitro condition does not necessarily reflect conditions in a living organism. Interval progression of I_h by both protocols is likely due to gradual anesthetic effects on the peripheral nervous system.

Fourth, although it is possible that the ion channel functions other than HCN channels are altered, the small numbers of animals might have limited the observation of a subtle difference in excitability measures.

Fifth, the exact reasons for the interval excitability changes are only elusive. Given the relatively slow pharmacokinetics, submaximal distribution of the anesthetic agents in the early recordings could explain the difference over the two time points (Kastner et al., 2003; Knych et al., 2013; Lockhart et al., 1991; Yasuda et al., 1991). Furthermore, similar results of simulated excitability waveforms by modifying the leak conductance, Barrett–Barrett conductance, or internodal H conductance (Fig. 4) limit further assessment for a specific mechanism. In addition, a neurophysio-

logical study performed in vivo is prone to systemic effects of pharmacological agents, such as changes in vascular tones, cardiac output, and glucose metabolism (Berthoud and Reilly, 1992; Freo et al., 2008).

4.3. Clinical significance

This study has clinical significance. On the one hand, this study showed that the majority of axonal excitability measures are constant under anesthesia; thus, their fluctuation should suggest the presence of "real" insulting factors such as tissue ischemia, electrolyte changes, and temperature changes. On the other hand, because axonal responses to long hyperpolarizing current were shown to be prone to anesthetic effects, the parameters obtained from hyperpolarizing TE and *I*/*V* should be cautiously interpreted.

Recently, Nowicki et al. reported the effects of isoflurane anesthesia on *F* waves in adult rat (Nowicki et al., 2014). They used different concentrations of isoflurane and showed reduced persistence of *F* waves with the use of high concentration (3.5%), but marginal difference at lower concentration (2.5%). In combination with the present data, reduction of axonal and neuronal excitability, presumably due to impaired HCN current, is responsible for the altered appearance of *F* responses. If *F* waves are to be used for intraoperative monitoring, the effects of isoflurane anesthesia need to be taken into account.

Lastly, in vivo assessment of axonal excitability by the present technique is achievable in human and animal subjects under various methods of anesthesia. Because alteration of HCN channel functions have been reported with a number of anesthetic agents (e.g., propofol, ketamine, halothane, pentobarbital, etomidate, loperamide, lidocaine, and isoflurane) (Hemmings et al., 2005), threshold tracking could be a useful method to sensitively detect the neuroregulatory effects of HCN channels intraoperatively.

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Conflict of interest statement: None of the authors has a conflict of interest.

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