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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)

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