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ORIGINAL ARTICLE

EFFECTS OF ISOFLURANE INHALATION ANESTHESIA ON MOUSE ECG

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Abstract General anesthesia is important for pharmacological studies in laboratory animals. Although a number of transgenic animals have been reported, basic analyses concerning mouse ECG and heart rate variability (HRV) have not been rigorously examined.

In the present study, we analyzed the effects of isoflurane as an inhalational anesthetic agent on ECG in C57BL6 mice, which have highly homogeneous genetic background. With 2% isoflurane anesthesia, the heart rate was acceptable (~500 bpm). Interestingly, C57BL6 mice showed relatively large standard deviation (57.3 bpm). Our data indicates that C57BL6 mice have various heart rates, although its genetic background is homogeneous. Higher concentrations of isoflurane resulted in a decreased heart rate, in a dose-dependent manner, and resulted in a marginal response to the sympathetic β-blocker, propranolol. Furthermore, we evaluated HRV as well as ECG parameters in mice. Our results indicate the importance of selecting an anesthesia for pharmacological studies using standard ECG parameters and HRV in C57BL6 mice. We provide basic ECG parameters from a large population of C57BL6 mice, which are fundamental baseline data for cardiovascular physiology.

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Key words: Inhalation anesthesia; isoflurane; mice; ECG; arrhythmia.

Introduction

General anesthesia in laboratory animals is an important issue for the reliability of pharmacological studies. Recently, many transgenic mice have become popular models for human inherited cardiac disease. Electrophysiological studies including electrocardiograms (ECGs), have been performed on such transgenic mice to characterize the electrical phenotype of the heart. However, little is known regarding the impact of experimental conditions on the outcome of electrophysiological studies in these mice. Additionally, the lack of basic analyses of mice ECG recordings from large populations has prevented the interpretation of some physiological and pharmacological experiments.

The type of anesthetic used varies with the type of experiment. Sodium pentobarbital and ketamine/xylazine have been used widely in mice[1)], while inhalation anesthetic agents, such as isoflurane, have significant advantages in invasive studies[2)]. Isoflurane has a slight effect on the hemodynamic status of mice, compared with injectable anesthetics, such as pentobarbital[2, 3)].

The use of injectable anesthetic combinations such as ketamine/xylazine has increased[4)]. However, since ketamine was classified as a narcotic drug in 2006, the use of ketamine has decreased significantly in Japan.

In the present study, we examined the effects of isoflurane inhalation anesthesia, evaluating the basal ECG parameters of 146

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mice for a cardiovascular study. We believe that our data can provide baseline ECG information for assessment of anesthetic regimens in future pharmacological studies.

Materials and Methods

This study was performed in accordance with the institutional guidelines of Hirosaki University and was approved by the Animal Care and Use Committee. Eight-week-old male C57BL6 mice (Japan SLC Inc., Japan) were purchased. The animals were housed under standard laboratory conditions.

General Anesthesia

Mice (12-16 weeks old), weighing 32 ± 3 g, were used. Anesthesia was induced by placing the mice in an anesthesia induction chamber (15 × 15 × 7 cm) containing 1-4% isoflurane (Forane; Abbott Japan Co., Ltd., Japan) and room air (airflow rate 2 l/min). Subsequently, the anesthesia was maintained for a 30-min period (anesthetic maintenance state). In preliminary experiments, 4% isoflurane anesthesia often resulted in sick sinus syndrome and these experiments were terminated (detailed information in the Results).

Loss of the righting reflex was used to investigate the hypnotic properties of inhalation anesthetics in mice as a behavioral endpoint. All experiments were conducted between 1:00 and 4:00 pm.

Evaluating Heart Rate

The ECG recording and calculation of heart rate (HR), R-R interval, and other ECG-related parameters were measured simultaneously (ML846 Power Lab system, AD Instruments, Dunedin, New Zealand). Heart rate variability (HRV) is considered an indicator of cardiac vagal activity[5, 6]. HRV analysis was distinguished into two analysis: time domain analysis, based on calculation of deviation form R-R interval average (standard deviation of R-R intervals: SDNN), and spectral analysis, that is based on the HRV power spectrum. SDNN represents total variability. HRV power spectrum is composed of four components (TP – Total Power, VLF – Very Low frequency, LF – Low frequency – and HF – High frequency) assessment. Generally, the total HRV power (TP) reflects global autonomic tension, LF – sympathetic/ parasympathetic tone.

The authors set the range for the respective spectral components at: very low frequency (VLF)<0.15<low frequency (LF)<1.5<high frequency (HF)<5, according to manufacture's protocol. For a pharmacological analysis, mice were administered propranolol (0.2 mg/kg) for sympathetic blockade.

Statistical Analysis

Results are expressed as means ± the standard error of the mean (SEM). They were analyzed with *post hoc* mean comparisons using the Newman-Keuls multiple-range test. Statistical significance was determined by analysis of variance (ANOVA) followed by the Dunnet test. P values < 0.05 were considered to indicate statistical significance.

Results

Figure 1Ai shows representative ECG traces with 2% isoflurane anesthesia. With 2% isoflurane, the heart rate (HR) was acceptable (～500 bpm).

The averaging view of ECG showed conserved P, Q, R, S, and T waves (Fig. 1Aii). ECG parameters of 71 mice were analyzed statistically (Table 1). The calculated parameters were: PR interval (35.7 ± 0.9 ms), P duration (18.5 ± 1.0

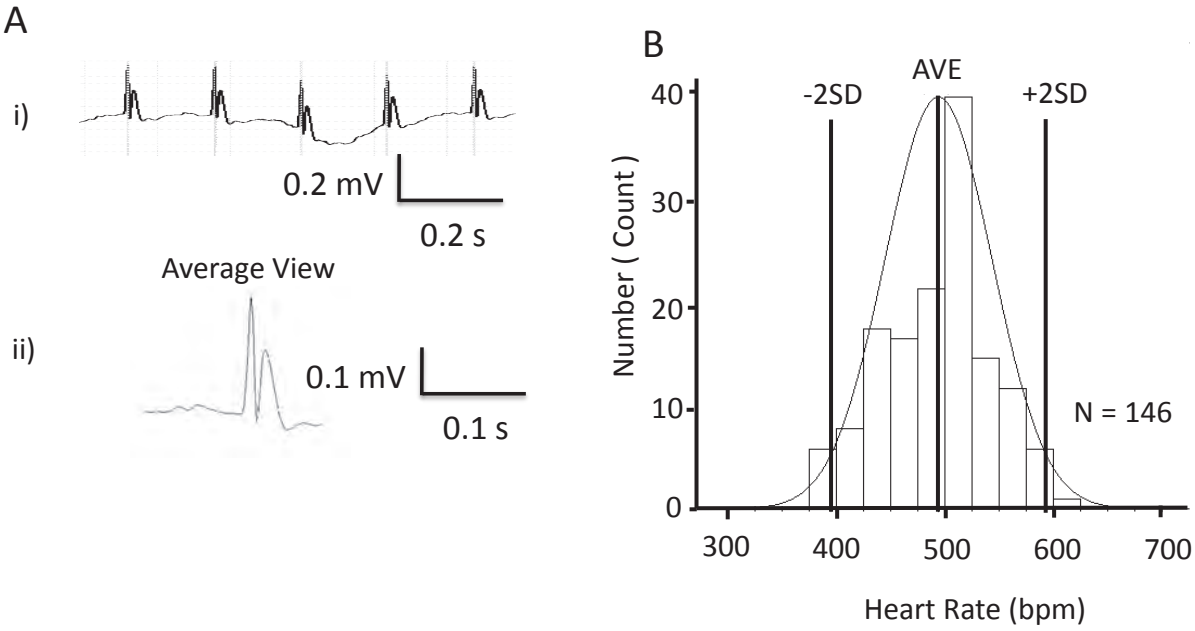

Figure 1 A
i) Representative ECG of mice with 2% isoflurane anesthesia.
ii) Averaging view of ECG with 2% isoflurane.
B) Histogram and frequency distribution curve of heart rate from 146 recordings with 2% isoflurane.

Table 1 ECG parameters under 2% isoflurane anesthesia

ECG parameters	PR Interval (ms)	P Duration (ms)	QRS Interval (ms)	QT Interval (ms)	QTc (ms)
Mean ± SEM	35.7 ± 0.9	18.5 ± 1.0	11.1 ± 0.1	23.6 ± 0.9	67.8 ± 0.3

ms), QRS interval (11.1 ± 0.1 ms), QT interval (23.6 ± 0.9 ms), and rate-corrected QT (QTc) interval (67.8 ± 0.3).

Figure 1B shows a histogram and a frequency distribution curve for heart rate from 146 recordings with 2% isoflurane anesthesia. Statistical analysis showed an appropriate heart rate with 2% isoflurane (494 ± 3.7 bpm, n = 146). Interestingly, calculated standard deviation of heart rate was large (57.3 bpm), which indicates that C57BL6 mice have various heart rates (494 ± 114.6 bpm, n = 146, mean ± 2SD, representing 95 % of total population), while their genetic background is homogeneous.

We next analyzed dose-dependent heart rate changes in response to various concentrations of isoflurane anesthesia (Fig. 2). Isoflurane anesthesia affected the heart rate in a dose-dependent fashion (Fig. 2A). It also affected QT time at high concentrations (Fig. 2B open circle), while calculated QT time was conserved (Fig. 2B closed circle), suggesting an appropriate level of isoflurane anesthesia for physiological analysis. With high-dosage of isoflurane (4%), some mice showed sick sinus syndrome (Fig. 2C) and these experiments were terminated.

Pharmacological responses with 2% and 3% isoflurane anesthesia were analyzed (Fig. 3). Representative recording of ECG at basal status with 2% (Fig. 3Ai) or 3% (Fig. 3Aii) isoflurane anesthesia (upper panels) and the response to propranolol (β-blocker, 0. 2 mg/kg, lower panels) are shown. Apparently, 2% isoflurane anesthesia resulted in significant elongation in the R-R interval in response to the β-blocker,

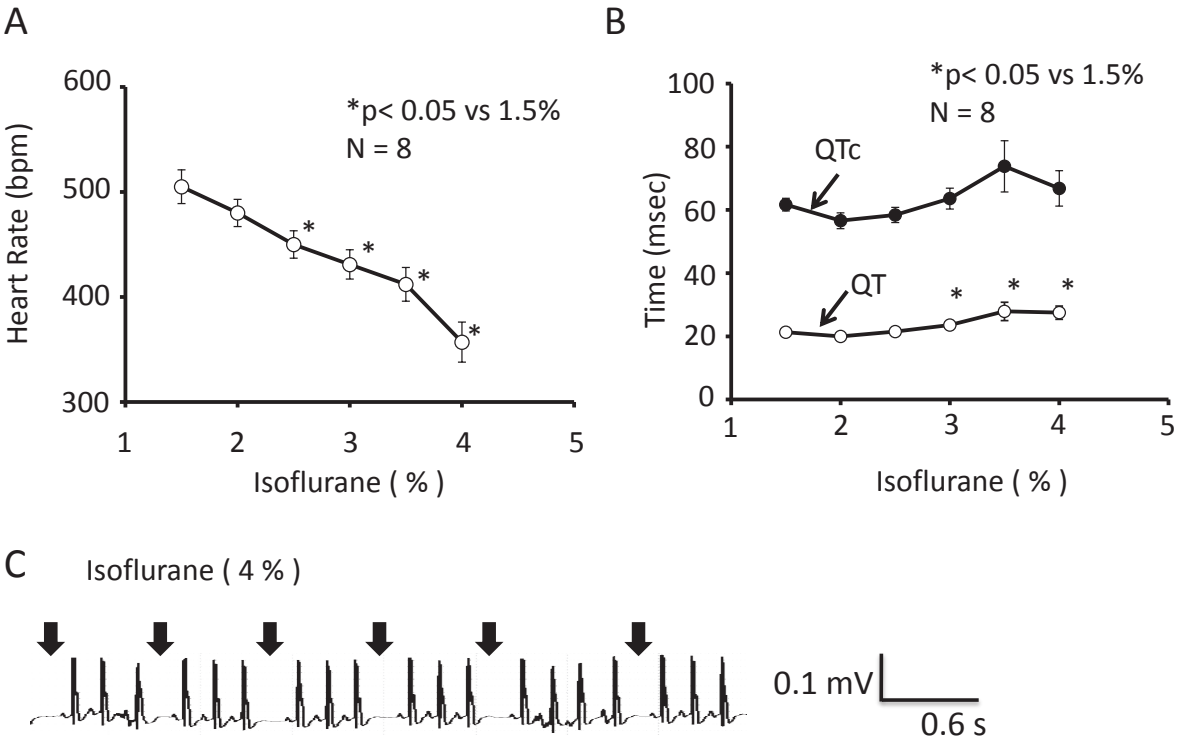

Figure 2 A) Dose-dependent changes in heart rate in response to various concentrations of isoflurane. $^{*}P < 0.05$, difference between 1.5% isoflurane anesthesia and other concentrations ($n = 8$).
B) Dose-dependent changes in QT time (open circle) and QTc time (closed circle) in response to various concentrations of isoflurane. $^{*}P < 0.05$, difference between 1.5% isoflurane anesthesia and other concentrations ($n = 8$).
C) Typical trace of sick sinus syndrome with 4% isoflurane anesthesia.

while 3% isoflurane anesthesia resulted in a marginal change in R-R interval.

Statistical analyses also showed a suitable response with 2% isoflurane anesthesia to propranolol (Fig. 3B basal and propranolol injection, open and dotted bar, respectively, $n = 8$). However, a marginal response was observed with 3% isoflurane anesthesia (Fig. 3B basal and propranolol injection, hatched and closed bar, respectively, $n = 8$).

Figure 4 shows a histogram and frequency distribution curve of HRV parameters with 2% isoflurane anesthesia. Figure 4A shows a histogram of SDNN. The calculated SDNN with 2% isoflurane anesthesia was 1.84 ± 0.16 ms2 ($n = 53$). Figure 4B shows a histogram of LF (low frequency). The calculated LF with 2% isoflurane anesthesia was 0.27 ± 0.05 ms^2 ($n = 43$). Figure 4C shows a histogram of HF (high frequency). The calculated HF with 2% isoflurane anesthesia was 0.77 ± 0.06 ms^2 ($n = 35$). Figure 4D shows a histogram of the LF/HF ratio. The LF/HF ratio with 2% isoflurane anesthesia was 0.39 ± 0.02 ms^2 ($n = 31$).

Discussion

We studied the electrophysiological effects of isoflurane anesthesia in mice. In the present study, we examined the ECG parameters with 2% isoflurane inhalation anesthesia, and analyzed basal ECG parameters and standard HRV parameters. First, we examined various concentrations of isoflurane (1.5-4%) to decide on the proper isoflurane concentration. Isoflurane anesthesia decreased the heart rate in a dose-

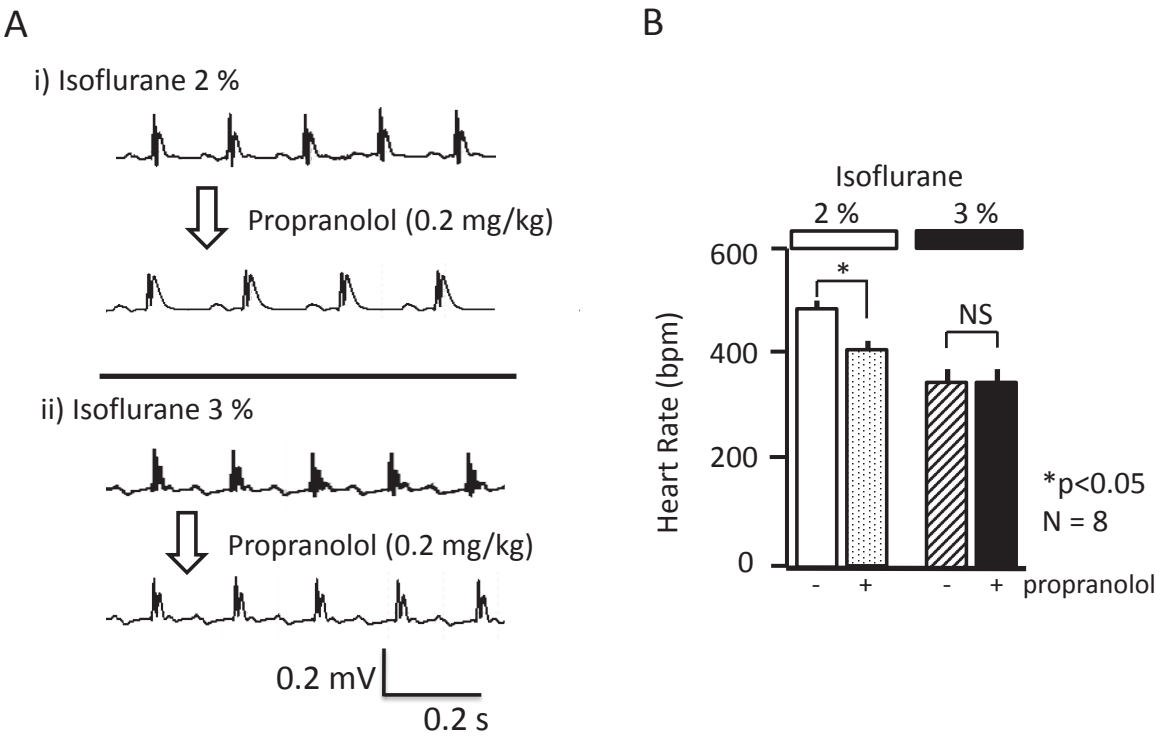

Figure 3 A) Representative ECG traces with 2% isoflurane anesthesia and response to propranolol.
i) Representative tracing of ECG during 2% isoflurane (upper panel) and ECG trace after propranolol (0.2 mg/kg, i.p.) injection.
ii) Representative tracing of ECG during 3% isoflurane (lower panel) and ECG trace after propranolol (0.2 mg/kg, i.p.) injection.
B) Statistical analysis of heart rate with 2% or 3% isoflurane anesthesia.
With 2% isoflurane anesthesia, propranolol (0.2 mg/kg i.p.) resulted in a significant decrease in heart rate (open bar; basal, dotted bar; propranolol injection). With a higher concentration of isoflurane (3%) anesthesia, the basal heart rate was lower. Propranolol injection resulted in marginal changes in the heart rate (hatched bar; basal, closed bar; propranolol injection). $^{*}P < 0.05$, difference between basal and propranolol injection ($n = 8$).

dependent fashion (Fig. 2A). Additionally, at a high concentration (4%), isoflurane anesthesia often resulted in arrhythmias. At 1% isoflurane anesthesia, we were unable to achieve appropriate anesthesia in terms of loss of the righting reflex. Thus, we decided to use 2% isoflurane as the standard concentration for proper inhalation anesthesia and further analyzed ECG parameters in detail.

Recently, transgenic mice have been used to evaluate the physiological importance of single genes. They have been used as models of human diseases. These studies are obviously useful for the development of new drugs. Anesthesia is often required for experimental interventions and phenotypic evaluations in transgenic mice. However, in such experiments, accidents due to anesthesia, such as death and unexpected hypotension, occur frequently. A wide range of anesthetic regimens has been used, depending on strain differences, previous experience, and institutional regulations[7]. Janssen *et al.* reported that isoflurane had lesser effects on systemic hemodynamic factors than pentobarbital anesthetics in mice[2]. Szczesny *et al.* also reported that isoflurane anesthesia was useful for experimental studies on mice, because of its simple administration, rapid induction, and easy control of the depth of anesthesia[8], while inhalation anesthesia needed a special apparatus.

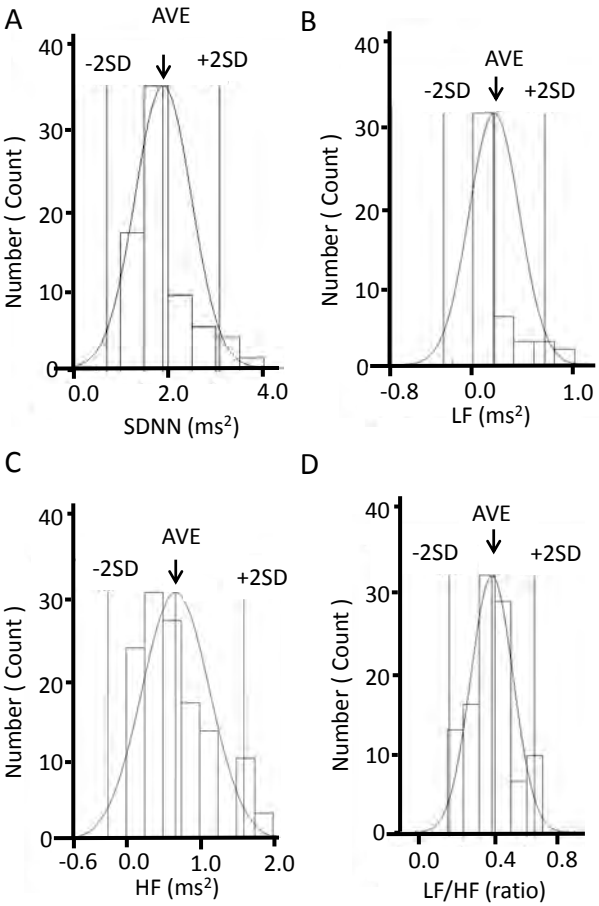

Figure 4 Histogram and frequency distribution curve of HRV parameters.
SDNN (A), LF (B), HF (C) and LF/HF ratio (D) with 2% isoflurane.
Average (AVE) and standard deviation (SD) values are indicated (AVE ± 2SD).

Nevertheless, inhalation anesthesia for mice has been performed in an only limited number of laboratories.

In isoflurane anesthesia, heart rate was preserved and stable, whereas the calculated standard deviation was large. In our previous study, pentobarbital anesthesia also showed conserved heart rate, while R-R intervals were not so stable. Apparently, the data obtained with 2% isoflurane anesthesia were sufficient for interpreting the results of pharmacological manipulation with an adrenergic β-blocker (propranolol).

In summary, we examined standard ECG parameters in a large number of C57BL6 mice with isoflurane anesthesia. Isoflurane anesthesia at 2% resulted in a desirable heart rate and a good response to propranolol. We also calculated standard HRV parameters during 2% isoflurane anesthesia. Our results indicate the importance of selecting an anesthesia methodology according to the purpose of an experiment in pharmacological studies.

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