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ORIGINAL ARTICLE

EFFECTS OF ENFLURANE ON CARDIAC AUTONOMIC NERVOUS ACTIVITY IN MICE

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Abstract We examined the effects of enflurane anesthesia on the mouse cardiac autonomic nervous system using electrocardiogram (ECG) analysis. Enflurane had a lower effect on heart rate (HR) compared to isoflurane, which is more widely used in small animal studies. Under anesthesia with 3 or 4% of enflurane, administration of propranolol had a significant effect on HR. Enflurane increased R-R interval length in a dose-dependent fashion, and the R-R interval became unstable at high concentrations. Although HR decreased with high doses of enflurane, we observed normal sinus rhythm and no arrhythmia. These results suggest that the effect of enflurane anesthesia is acceptable even though the drug can lead to cardiac instability. No remarkable changes were observed in HR frequency. These results suggest that enflurane anesthesia may be suitable for cardiac autonomic nervous system analysis.

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Key words: Autonomic nervous system; enflurane; halothane; inhalation anesthesia; mice.

Introduction

The establishment of appropriate general anesthesia is important for accuracy in small animal studies. The number of genetically modified mouse species has been increasing every year. For the molecular investigation of cardiac function, the confounding influences of anesthesia in mice must be overcome[1, 2)]. Accurate assessment of basal cardiac function is particularly important in mice that have been modified to have cardiac diseases[3)]. Thus, establishing an appropriate general anesthesia that maintains cardiac autonomic nervous activity is important.

Enflurane (CHF2OCF2CHFCl), a traditional inhalational anesthetic agent, has a halogenated ester structure and has been widely used since 1963[4)]. However, some side effects have been observed in humans, including depression of myocardial contractility, reflex tachycardia, malignant hyperthermia, and acute renal failure[5)]. Therefore, enflurane was replaced by other anesthetics, such as isoflurane or sevoflurane. However, the effect of these agents on the cardiac nervous systems of small animals is unknown.

In this study, cardiac function was electrophysiologically investigated in mice under general anesthesia to clarify the effects of enflurane on cardiac autonomic nervous activity.

Materials and Methods

This study was performed in accordance with the institutional guidelines of Hirosaki University (Hirosaki, Japan) and was approved by the Animal Care and Use Committee.

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Ten week-old C57BL/6 mice, one of the most commonly used mouse lines in Japan, were purchased from Japan SLC Inc. (Hamamatsu, Japan) and housed under standard laboratory conditions. The animals were maintained for one week at a temperature of 22 ± 2° C and relative humidity of 57% on a 12-h light /dark cycle (lights on 08:00–20:00), with free access to food and water.

General anesthesia

Ten male C57BL/6 mice (12–16 weeks-old; weight, 32 ± 1 g) were used for these experiments. Anesthesia was induced by placing the mouse in an anesthesia induction chamber (15 × 15 × 7 cm) containing 2–4% enflurane and room air. In some experiments, 2% isoflurane was used instead of enflurane. Anesthesia was maintained for 45 min (anesthetic maintenance state). All experiments were conducted from 10:00 a.m. to 4:00 p.m[6].

ECG Evaluation

An electrocardiogram (ECG) was recorded (lead I) and HR and R-R intervals were simultaneously measured (ML846 Power Lab System, AD Instruments, Dunedin, New Zealand). Each ECG was analyzed using the manufacturer's software. Standard deviation of the R-R interval (SDNN) was measured as HR variability, which was considered an indicator of cardiac vagal control[7]. For pharmacological analysis, mice were administered propranolol (0.1 to 0.8 mg/kg) for sympathetic blockade.

Statistical analysis

The results are expressed as the mean ± standard error (S.E.). At first, Shapiro-Wilk test was examined. Then, results were analyzed with *post hoc* mean comparisons using the Newman-Keuls multiple-range test. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test; p-values < 0.05 were considered to be significant.

Results

Heart rate (HR) and SDNN

The ECGs under 2, 3, or 4% enflurane revealed a regular pattern indicative of physiological pacemaking and excitation propagation (Fig. 1Ai). Corresponding averaged ECG signal changes under 2, 3, or 4% enflurane are aslo shown in Fig. 1Aii. Increased concentrations of enflurane induced elongation of the R-R interval (Fig. 1A). No arterial fibrillation, atrial paroxysmal contraction, ventricular paroxysmal contraction, or any other serious arrhythmia was detected in any of the mice. Each R-R interval was considered to be within a normal sinus rhythm range. P-Q and Q-T durations were equally elongated, and QRS was not affected (data not shown). Heart rate (HR) changes under enflurane (2–4%) or isoflurane (2%) anesthesia are shown in Fig. 1B; enflurane reduced HR in a concentration-dependent manner. Compared with enflurane, administration of 2% isoflurane significantly decreased HR. HRs with mice given 2 or 3% enflurane were significantly greater than those administered 2% isoflurane ($^*P < 0.05$). Considering the basal heart rate (ca. 530 bpm) without anesthesia in our former study, enflurane might be useful for physiological analysis[6]. In Fig. 1C, SDNN is shown for mice receiving 2% enflurane or isoflurane. There were no statistical differences between the two groups.

Sympathetic blockade with Propranolol

We further analyzed the impact of sympathetic blockade on HR during anesthesia using propranolol (Fig. 2). Enflurane anesthesia (2 to 4%) decreased HR in a concentration-dependent manner (Fig. 1B). Propranolol administered in addition to enflurane resulted in a further, significant reduction in HR. Propranolol (0.1 to 0.8 mg/kg) significantly

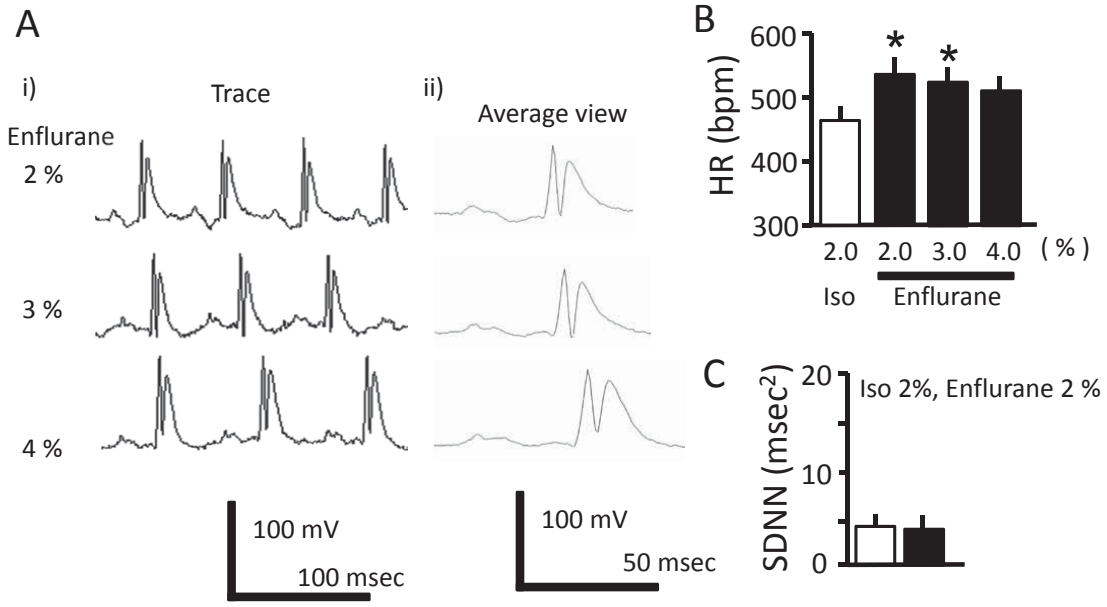

Figure 1 A) Representative traces of electrocardiogram with 2–4 % enflurane anesthesia (i). Representative averaging view of electrocardiogram with 2–4 % enflurane anesthesia (ii).
B) Statistical analysis of heart rate (HR) with 2% isoflurane anesthesia (open bar) or enflurane anesthesia (2–4 %, closed bar). $^*P < 0.05$, difference between 2% isoflurane anesthesia and enflurane anesthesia ($n = 8$ to 22).
C) Statistical analysis of standard deviation of the R-R interval (SDNN) during 2% isoflurane (open bar) or enflurane (closed bar) anesthesia.

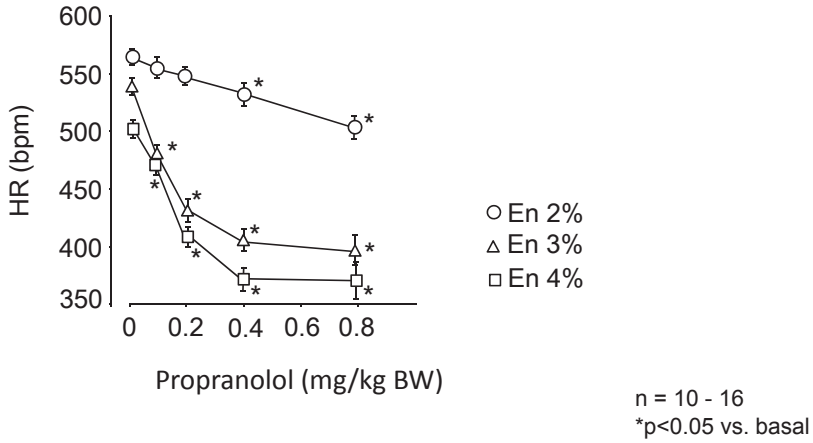

Figure 2 Dose-dependent changes in heart rate in response to various concentrations of propranolol. $^*P < 0.05$, difference between basal status of each anesthetic conditions ($n = 8$). (En: enflurane, 2%: ○, 3%: △, 4%: □)

decreased the HR in a dose-dependent manner, regardless of enflurane anesthesia concentration. In contrast, the degree of HR reduction differed between 2, 3, and 4% enflurane. The mice given 3 or 4% of enflurane showed a significant decrease in HR, even with 0.2 mg/kg of propranolol, while mice given 2% enflurane showed smaller HR changes in response to propranolol.

Frequency spectrum analysis

Figure 3A depicts the typical beat-to-beat dynamic results with Poincaré plots (R-R_n versus R-R_{n+1}) using 2–4% of enflurane.

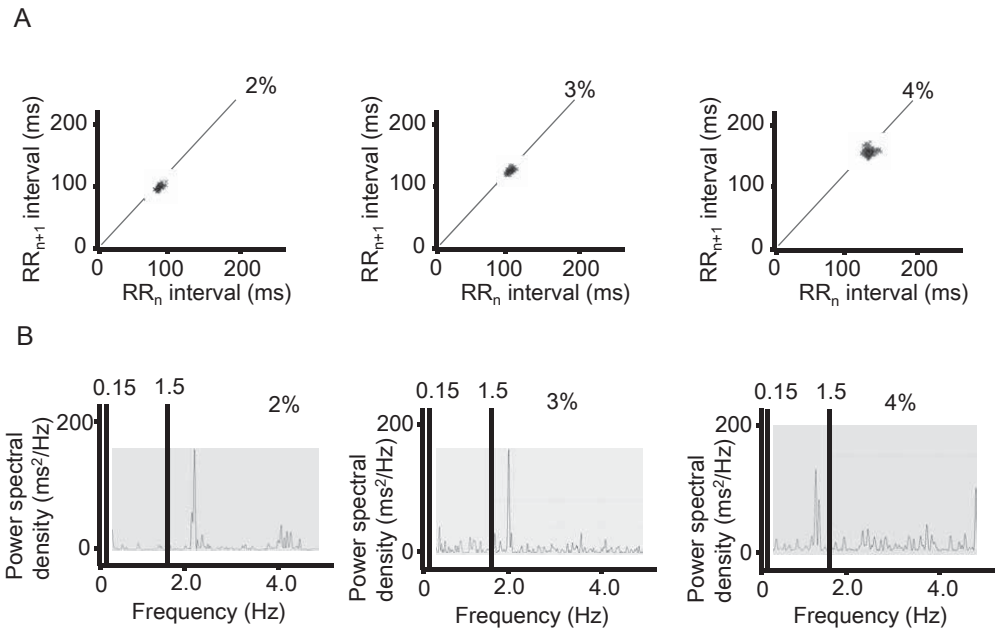

Figure 3 A) Representative HR variability analysis during 2% (left), 3% (middle), and 4% (right) enflurane anesthesia. Poincare plots (R-R_n vs. R-R_{n+1}) in which consecutive pairs of control period R-R intervals are graphed with the n^{th+1} R-R interval plotted against the n^{th} R-R period (upper panels).
B) Representative power spectral densities (lower panels) during 2% (left), 3% (middle), and 4% (right) enflurane anesthesia.

The concentration of enflurane was positively associated with an increase in R-R interval length.

Frequency domain analysis (Fig. 3B) was used to resolve low frequency (LF; 0.2–0.75 Hz) and high frequency (HF; 0.75–2.5 Hz) components into power spectral density values (Fig. 3B). The LF component did not change after 2, 3, or 4% enflurane anesthesia administration (18.4 ± 2.2, 12.7 ± 2.3, and 22.0 ± 2.6, for 2, 3, and 4% enflurane anesthesia, respectively). Statistical analysis of HF components also showed no significant changes (59.8 ± 5.2, 70.5 ± 4.0, and 66.6 ± 2.6, for 2, 3, and 4% enflurane anesthesia, respectively). Therefore, no significant changes in LF or HF components were observed after enflurane administration, suggesting that enflurane may be suitable anesthesia for physiological ECG analysis.

Discussion

In this study, enflurane anesthesia was shown to decrease HR in mice. However, enflurane anesthesia affected HR to a lesser degree than isoflurane anesthesia at the same concentration, suggesting that enflurane has less of an effect on autonomic nervous activity. Thus, for mouse experiments involving the autonomic nervous system, enflurane may be more appropriate than isoflurane, due to its reduced effects on HR. Enflurane also had no significant effect on LF or HF components. There were no differences in SDNN between isoflurane and enflurane anesthesia.

Propranolol administrated while under 3 or 4% enflurane anesthesia resulted in a further significant reduction in HR, suggesting that these concentrations are more suitable for physiological analysis. Small doses of propranolol (0.1 or 0.2 mg/kg) had no effect on HR when given in combination with 2% enflurane.

This may be because the minimum alveolar concentration (MAC) of enflurane is as high as 1.48; as such, 2% enflurane may not be sufficient to induce an appropriate level of anesthesia. Taken together, these data suggest that enflurane concentration should be higher than 3% for anesthesia.

R-R interval length increased with enflurane concentration. However, no arrhythmia was observed, even in mice given 4% enflurane. These results suggest that enflurane has acceptable effects on mouse cardiac function.

Maintaining cardiac function is imperative for genetically modified mice that may have deficient cardiac activity. These mice are often used to evaluate the physiological importance of a single gene in models of human disease. Anesthesia is often required for experimental interventions and phenotypic evaluations in gene-modified mice[8]. The development of a stable anesthetic method is important for accurate investigation in gene-modified mice. The results of this study show that enflurane may be a potentially beneficial anesthetic agent for maintaining cardiac autonomic function. However, medical use of halothane was discontinued in Japan in 2015.

In summary, enflurane is an appropriate general anesthetic agent with fewer side effects on cardiac function than isoflurane. A concentration of 3% enflurane for anesthesia was shown to be optimal in mice.

Acknowledgements

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