Inhalation Anesthesia Is Preferable for Recording Rat Cardiac Function Using an Electrocardiogram

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The effects of inhalation anesthesia (2% isoflurane, sevoflurane, or enflurane) and intraperitoneal anesthesia with pentobarbital (65 mg/kg) were compared in rats using an electrocardiogram (ECG) and determination of blood oxygen saturation (SPO2) levels. Following inhalation anesthesia, heart rate (HR) and SPO2 were acceptable while pentobarbital anesthesia decreased HR and SPO2 significantly. This indicates that inhalation anesthesia is more preferable than pentobarbital anesthesia when evaluating cardiovascular factors. Additionally, pentobarbital significantly increased HR variability (HRV), suggesting a regulatory effect of pentobarbital on the autonomic nervous system, and resulted in a decreased response of the baro-reflex system. Propranolol or atropine had limited effects on ECG recording following pentobarbital anesthesia. Taken together, these data suggest that inhalation anesthesia is suitable for conducting hemodynamic analyses in the rat.

Key words inhalation isoflurane anesthesia; pentobarbital; hemodynamic status; blood gas; rat

The selection of anesthetic regimens for surgery and physiological monitoring in small animals is important because many of these regimens affect cardio-respiratory function and other organ systems.1) Transgenic animals are sophisticated tools with which to probe protein function, serve as models of human disease, and act as hosts for the testing of gene replacement and other therapies. Transgenic mice represent the most widely used species of animal for these models but there is a relative paucity of functional in vivo studies in mice due, in part, to the technical difficulties associated with their small size and the lack of an extensive database on anesthetic procedures and normal physiological parameters. Thus, transgenic rats have been used as an alternative model to evaluate anesthetic procedures. In this animal model, the integration of foreign DNA into a fertilized oocyte by random chromosomal insertion is widely used.3) Recently, the transgenic rat has become a popular model with which to investigate human inherited cardiac disease. Electrophysiological studies in transgenic rats have characterized the electrical phenotype of the heart but little is known regarding the impact of experimental conditions or models on the outcome of such studies.

Several studies have examined the influence of commonly used anesthetics on cardiac function with non-invasive echocardiography.5,6) Although sodium pentobarbital and ketamine/xylazine are widely used to sedate small animals,7) inhalation anesthetic agents, such as isoflurane, possess significant advantages over injectable agents.8) Isoflurane anesthesia has relatively limited effect on hemodynamic status compared to injectable anesthetics such as pentobarbital and ketamine/xylazine.8,9) Additionally, since the classification of ketamine as a narcotic drug in Japan in 2006, its use has been reduced considerably. Thus, this study aimed to evaluate the hemodynamic parameters of rats following intraperitoneal pentobarbital anesthesia and inhalation anesthesia with isoflurane, sevo-

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Measurement of Blood Pressure

Male SD rats were anaesthetized using isoflurane (4% for induction and 2% for maintenance) or an intraperitoneal injection of pentobarbital (65 mg/kg). As enflurane and sevoflurane have higher minimum alveolar concentration (MAC), we used 2% for their inhalation anaesthesia for comparison. The carotid artery and vein were subsequently cannulated to record blood pressure and administer compounds, respectively. Blood pressure responses were recorded using a transducer (TP-400T; Nihon Kohden Ltd., Tokyo, Japan) connected to an amplifier (AP-601G; Nihon Koden Ltd.). The preparation was allowed to stabilize for at least 30 min before the start of the experiment. Data were A/D converted using PowerLab 4/20 (AD Instruments Ltd., Castle Hill, NSW, Australia) and recorded by a computer.

Statistical Analysis

Results are expressed as mean±standard error (S.E.). Statistical significance was determined by ANOVA followed by the Dunnett test; The p values less than 0.05 were considered to indicate significant differences.

RESULTS

Typical traces of HR and the calculated SDNN following the administration of different anesthetic regimens (2% inhalation of isoflurane, sevoflurane, or enflurane or an intraperitoneal injection of pentobarbital) are shown in Fig. 1A. The $\text{SPO}_2$ percentage was relatively high following isoflurane or sevoflurane anaesthesia (isoflurane: 94.7±0.8%, $n=7$; sevoflurane: 94.6±0.3%, $n=7$, respectively, mean±S.E.M.), but enflurane resulted in a decreased $\text{SPO}_2$ saturation (87.8±1.0%, $n=6$). Intraperitoneal injection of pentobarbital resulted in a significantly lower $\text{SPO}_2$ (66.8±1.5%, $n=6$; Fig. 1B). HR was relatively high and stable during inhalation anaesthesia (isoflurane: 320±6, $n=10$; sevoflurane: 313±11, $n=6$; and enflurane: 303±5, $n=6$; Fig. 1B), while pentobarbital anaesthesia resulted in a significant decrease in HR (272±11, $n=6$; Fig. 1B). Therefore, inhalation anaesthesia resulted in acceptable HRs while enflurane decreased HR moderately. Pentobarbital anaesthesia decreased HR significantly relative to all inhalation anaesthetic regimens. However, pentobarbital anaesthesia resulted in increased HRV. Interestingly, pentobarbital significantly increased the calculated SDNN (18.8±4.5 ms$^2$, $n=6$), while inhalation anaesthesia resulted in stable results (isoflurane: 4.7±0.6 ms$^2$, $n=10$; sevoflurane: 5.1±0.7 ms$^2$, $n=6$; and enflurane: 5.3±0.9 ms$^2$, $n=6$).

Matsuda et al. reported that neither hypoxia nor hypercap-
nia was observed during isoflurane anesthesia with a ventilator system. Therefore, the present results are consistent with previous reports. Because inhalation anesthesia was associated with better HR and SPO2 results and because isoflurane is probably the most widely used inhalation anesthesia for animal experiments in Japan, the physiological and pharmacological responses under isoflurane anesthesia or pentobarbital anesthesia were then evaluated.

**Detailed Heart Rate Variability Changes** We further analyzed heart rate variability during isoflurane anesthesia or pentobarbital anesthesia. Figure 2A shows typical results of beat-to-beat dynamics with Poincare plots (RRn vs. RRn+1). Obviously, pentobarbital anesthesia showed fluctuated changes in beat-to-beat dynamics. In the frequency domain analysis, LF (0.2–0.75 Hz) and HF (0.75–2.5 Hz) components were resolved in power spectral density (lower panels). Statistical analysis of LF components resulted in significant increase in pentobarbital anesthesia (B). HF components were not significantly different (C). As expected from LF component, pentobarbital showed higher LF/HF ratio than inhalation anesthesia (D).

**Pharmacological Responses** Typical changes in HR and the SDNN following the administration of isoflurane anesthesia are shown in Fig. 3A. Typical traces of HR and SDNN following pentobarbital anesthesia are also provided (Fig. 3B).

The baro-reflex was analyzed to examine autonomic responses. Under isoflurane anesthesia, rats exhibited a significant increase in HR (Fig. 3A, arrow) while a limited response was observed under pentobarbital anesthesia (Fig. 3B, arrow).

The administration of propranolol (1.0 mg/kg, intraperitoneally (i.p.)) resulted in a decreased HR in isoflurane-anesthetized rats (Fig. 3A) and decreased changes in HR during pentobarbital anesthesia (Fig. 3B). In the latter group, the effect was limited and slow which indicates a lowered response to propranolol. Furthermore, pentobarbital anesthesia was associated with a high SDNN indicating an unstable HR that was probably related to its effects on respiration and decreased autonomic nerve tone. The effects of parasympathetic blockade were also analyzed using intraperitoneal injections of atropine (1.0 mg/kg, i.p.), a typical muscarinic receptor antagonist. A typical recording in response to atropine is shown (Fig. 3, right panel). Atropine significantly increased the HR during isoflurane anesthesia (Fig. 3A), while pentobarbital anesthesia induced limited and slow changes in HR (Fig. 3A, right lower panel); this indicates a lowered response following parasympathetic blockade. Atropine injections also reduced SDNN in pentobarbital-anesthetized rats, possibly due to blockade of cardiac vagal nerve tone. We also evaluated powerspectrum changes in response to propranolol during pentobarbital anesthesia (supplementary Fig. 2). Propranolol (1.0 mg/kg) clearly decreased LF/HF ratio from 0.243 to 0.059, indicating that this kind of HRV analysis is also applicable for small rodents, like former study.

**Blood Pressure Changes** Typical blood pressure traces during inhalation anesthesia with isoflurane (Fig. 4A, upper...
panel) and following intraperitoneal injections of pentobarbital (65 mg/kg; Fig. 4A, lower panel) are provided. Isoflurane anesthesia and pentobarbital anesthesia resulted in a decrease in systolic blood pressure (isoflurane: 107.8±2.9, n=6; pentobarbital; 67.6±3.4, n=7, respectively), while diastolic blood pressure (isoflurane: 74.0±4.8, n=6; pentobarbital: 56.9±3.8, n=7, respectively) was preserved (Fig. 4B, closed bar).

DISCUSSION

This study assessed the hemodynamic effects of isoflurane inhalation anesthesia and pentobarbital anesthesia in rats. Pentobarbital is a commonly used injectable anesthesia in rodents, and isoflurane inhalation has been useful in many small animals.

In the present study, pentobarbital showed significantly decreased SPO$_2$ and heart rate (Fig. 1), which probably due to its...
suppressiv e effect on respiratory rhythm\textsuperscript{14} and hemodynamic effect on cardiovascular system.\textsuperscript{15} As we used relatively high-dosage of pentobarbital comparing with other study, its suppressive effect on the respiration might be the main reason for the significant changes.\textsuperscript{16} Therefore, we further evaluated dose-dependent (30, 50, 65 mg/kg) changes on SPO$_2$, heart rate, and SDNN (supplementary Fig. 3). As low (30 mg/kg) or middle (50 mg/kg) dose of pentobarbital showed no significant changes in heart rate comparing with isoflurane anesthesia, while significant decrease in SPO$_2$ was observed, suppressive effect of pentobarbital on the respiration apparently plays significant role. This suppressive effect might also be related to increased SDNN. Nevertheless, our present study indicates that low dose (30 mg/kg) of pentobarbital anesthesia has significantly increased SDNN, suggesting that pentobarbital might affect autonomic nerve regulation.

As this study was intended to find the most adequate anesthesia in cardiovascular analysis, it was quite important to know which kind of administration (inhalation or intraperitoneal injection, in this study) is more preferable for ECG recording. Induction of pentobarbital anesthesia is slower (ca. 5–10 min) than that of inhalation anesthesia (ca. 1–2 min with isoflurane), because of slow absorption of pentobarbital after intraperitoneal administration. Inhalation anesthesia is easy to control, although it needs special apparatus. Obviously, pharmacokinetics is a key factor for these differences. Under isoflurane anesthesia, HR and SPO$_2$ were higher and more stable than during pentobarbital anesthesia, suggesting that inhalation anesthesia is a more suitable method for evaluation of cardiovascular factors.

In addition, we evaluated blood pressure without anesthesia with a tail-cuff system (BP-98A; Softron, Tokyo, Japan) to compare with our blood pressure results. With a tail-cuff system, systolic blood pressure (129±2.4 mmHg, n=6) was higher than that of isoflurane anesthesia, whereas diastolic blood pressure (74±1.6 mmHg, n=6) showed no significant changes with isoflurane anesthesia. As pentobarbital anesthesia showed significantly decreased systolic and diastolic blood pressure, we think isoflurane anesthesia, which showed smaller effect on blood pressure, is probably better choice to evaluate cardiovascular analysis.

It should be noted that inhalation anesthesia with isoflurane also showed a good response in baro-reflex analyses suggesting that this method is more preferable for physiological studies. Furthermore, the data obtained under isoflurane anesthesia was sufficient to interpret the results of pharmacological manipulation with an adrenergic β-blocker (propranolol) and a muscarinic antagonist (atropine). Thus, it is proposed here that isoflurane anesthesia is suitable for pharmacological experiments in which the hemodynamic state of small animals is evaluated.

Anesthesia is often required for experimental interventions and phenotypic evaluations in transgenic animals. Janssen et al. found that isoflurane has less of an effect on systemic hemodynamic factors relative to pentobarbital anesthesia.\textsuperscript{8} Similarly, Szczesny et al. reported that isoflurane anesthesia is useful for experimental studies because it is easy to administer and control the depth of anesthesia and it has a rapid induction of anesthesia, a low percentage of complications, and results in stable blood pressure and HR over a long period of time.\textsuperscript{17} In contrast, inhalation anesthesia requires special apparatus. Therefore, inhalation anesthesia in rats is performed in a limited number of laboratories; general anesthesia is typically induced using simple injection anesthetics, such as pentobarbital.

Janssen et al. reported a decreased HR during pentobarbital anesthesia, which is mainly related to the suppressive effect on respiration and partly due to its direct negative chronotropic action.\textsuperscript{8} This effect might be related to the decreased HR seen in the present study. Clearly, controlling the depth of anesthesia is considerably easier using inhaled compared to injected anesthetic regimen. Because heart rate regulation by autonomic nerve system is related to the balance between sympathetic and parasympathetic tonus, the decreased heart rate in the present study is probably mainly due to aforementioned suppressive effect on respiration and partly due to the effect of pentobarbital anesthesia on the sympathetic nerve system, although the well-known suppressive effect of pentobarbital on parasympathetic control may also partly play a role.\textsuperscript{18}

Both exercise and stimulation of the baro-reflex elicit physiological changes that are dependent on the integration of sympathetic and parasympathetic nervous system activity. In the present study, pentobarbital anesthesia resulted in a significant reduction of the baro-reflex while isoflurane anesthesia exhibited a more preferable response. Havel et al. found that halothane, another type of inhalation anesthesia, is less suppressive to the baro-reflex or to electrical activation of the parasympathetic input.\textsuperscript{19} The difference between these two anesthetic agents may be related to the suppressive effect of pentobarbital on the parasympathetic nervous system.

In the present study, the HR decrease induced by anesthesia was significantly less in inhalation-anesthetized rats than in pentobarbital-anesthetized rats (Fig. 1). Although they used mice, Zeller et al. measured HRV as the standard deviation of the beat interval.\textsuperscript{20} In their study, the intraperitoneal injection of pentobarbital increased the SDNN from 5.2±0.2 ms$^2$ in a conscious baseline state to 40.4±5.8 ms$^2$ under pentobarbital anesthesia. In this study, the SDNN under pentobarbital anesthesia was 18.8±4.5 ms$^2$. The discrepancy between these two studies might be due to the use of different animal species or perhaps other experimental conditions. Because the R–R interval in rats is ca. 200 ms and the R–R interval of mice is ca. 100 ms, the calculated SDNN in rats should be fourfold that of mice; however, the SDNN in the present study was only twofold greater. Nevertheless, isoflurane anesthesia resulted in acceptable SDNN (4.7±0.6 ms$^2$), suggesting that inhalation anesthesia is desirable when evaluating cardiovascular parameters.

CONCLUSION

In conclusion, pentobarbital anesthesia resulted in decreased HR and SPO$_2$, while inhalation anesthesia resulted in a desirable HR, SDNN, and SPO$_2$ in rats. These results underscore the importance of selecting an appropriate anesthetic regimen for pharmacological studies that is in line with the purpose of the experiment. Isoflurane inhalation anesthesia is a preferable candidate general anesthesia in small animals.

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