Exp. Anim. 65(4), 393–402, 2016

—Original—

Different sensitivity to the suppressive effects of isoflurane anesthesia on cardiorespiratory function in SHR/Izm, WKY/Izm, and Crl:CD (SD) rats

Kouki KATO[1)], Jun WAKAI[2)], Kazunori OZAWA[3)], Miho SEKIGUCHI[3,4)], and Kiyoaki KATAHIRA[3)]

[1)]*Center for Laboratory Animal Science, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan*
[2)]*Department of Laboratory Animal Medicine, Institute for Biomedical Sciences, Iwate Medical University, 2-1-1 Nishitokuta, Yahaba-cho, Shiwa-gun, Iwate 028-3694, Japan*
[3)]*Medical-Industrial Translational Research Center, Fukushima Global Science Center, Fukushima Medical University, 1-Hikarigaoka, Fukushima, Fukushima 960-1295, Japan*
[4)]*Laboratory Animal Research Center, Fukushima Medical University School of Medicine, 1-Hikarigaoka, Fukushima, Fukushima 960-1295, Japan*

Abstract: Isoflurane is a widely used anesthetic, but its effects with increase in inspired concentration on cardiovascular function have not yet been clarified in rodents. Additionally, there are only a few studies comparing isoflurane-induced cardiorespiratory effects between rat strains. Thus, we investigated the differences in cardiorespiratory responsiveness to increasing concentration of inspired isoflurane in SHR/Izm, WKY/Izm and Crl:CD (SD) rats, by increasing the setting values of vaporizer's dial indicator. The rats were anesthetized with 1.5% isoflurane, and electrocardiograms, blood pressure, and respiratory rate were recorded simultaneously. Thereafter, the inspired concentration was increased stepwise to 2%, 3%, 4%, and 5%, and cardiorespiratory parameters were obtained at each concentration. Under anesthesia at more than 4%, although prolongation of the RR and PR intervals was observed in all strains, shortening of the QT_C interval was found only in SHR/Izm rats. From frequency domain analysis of heart rate variability, an increase in LF/HF ratio and a decrease of HF components were observed in SHR/Izm and WKY/Izm rats, respectively, with 5% isoflurane anesthesia. Blood pressure and heart rate were remarkably reduced in SHR/Izm rats at higher concentrations, whereas the reduction was smallest in WKY/Izm rats among the three strains examined. Respiratory rate was inspired concentration-dependently decreased in all strains. These results suggested that SHR/Izm rats are more sensitive to suppressive effects of isoflurane anesthesia on cardiovascular function among these rat strains.

Key words: cardiorespiratory function, Crl:CD (SD), isoflurane anesthesia, SHR/Izm, WKY/Izm

Introduction

Refinement, one of the "3Rs" principles proposed by Russel and Burch in 1959 [29], to reduce to an absolute minimum the amount of distress imposed on the animals that are used for scientific procedures applies to all aspects of procedures performed on them. Refinement is currently defined as any approach that avoids or minimizes the actual or potential pain, distress, and other adverse effects experienced at any time during the life

(Received 28 January 2016 / Accepted 13 May 2016 / Published online in J-STAGE 10 June 2016)
Address corresponding: J. Wakai, Department of Laboratory Animal Medicine, Institute for Biomedical Sciences, Iwate Medical University, 2-1-1 Nishitokuta, Yahaba-cho, Shiwa-gun, Iwate 028-3694, Japan

of the animals involved, and which enhances their well-being [6]. To comply with this principle, appropriate use of anesthetics is recommended to minimize the pain and distress caused to animals during the surgical treatments.

Volatile anesthetics such as halothane, isoflurane, sevoflurane, and desflurane are frequently used for the general anesthesia of rodents, rabbits, and dogs [3, 14, 16]. Among these, isoflurane is widely used for anesthesia of rodents [27, 37] due to its safety and inexpensive delivery [12, 17]. Inhalation anesthesia is known to affect cardiovascular function. As well as other halogenated inhalation anesthetics, isoflurane is known to reduce arterial blood pressure in a dose-related manner in humans [38] and also in laboratory animals, including dogs [31], rabbits [26], mice [37], and rats [33]. In these experiments, isoflurane anesthesia was used at a constant concentration (1–2.5%) for at least 20 min [26, 31, 33, 37]. However, the effects of increasing isoflurane concentration on cardiovascular function have not yet been fully examined. In mice, left ventricular function was found to reduce in response to increasing inspired isoflurane concentration from 0.5% to 5%, in which transthoracic echocardiographic data were obtained during 2 min of isoflurane inhalation at each concentration [15]. Therefore, it is expected that cardiovascular suppression by isoflurane inspiration is caused with increase in the inspired concentrations within mins.

In research on cardiovascular systems and the pathogenesis of cardiovascular diseases such as hypertension, stroke, and cerebral infarction, spontaneously hypertensive rats (SHR) is often used as a disease model. Although there were only a few studies on the effects of isoflurane anesthesia on cardiorespiratory function in SHR rats, it was reported that inspired concentration of 1.2% for 1 h decreased mean blood pressure (MBP) and heart rate (HR) in the strain [33]. In the control strain Wistar Kyoto rats (WKY) [33] and normotensive Sprague-Dawley (SD) rats [11, 32], it was reported that inspiration of isoflurane concentration at 1–2.5% for more than 30 min caused decrease in MBP and HR. On the other hand, the mechanisms for blood pressure lowering was suggested to be different between SHR and other two strains, and it was reported that a reduction in blood pressure was caused by decreased cardiac output in SHR rats [33], whereas it was caused by reduced systemic vascular resistance in WKY rats and SD rats [32, 33]. Therefore, it is suggested that cardiovascular function is suppressed by isoflurane, but the detail of suppressive effects on cardiovascular function differs between hypertensive and normotensive rats. In these previous studies using SHR, WKY, and SD rats, inspired concentration of isoflurane was at a constant level, and it is unknown that to what extent the cardiorespiratory function is suppressed in these rat strains by rapid increase in inspired isoflurane concentration. Considering the differences in hemodynamic response to isoflurane among SHR and normotensive rats [33], we hypothesized that increased inspired isoflurane concentration acutely suppresses cardiorespiratory function in SHR, WKY and SD rats but there are some differences in cardiorespiratory responsiveness to isoflurane anesthesia based on the differences in the state of blood pressure and/or rat strain.

In the present study, we examined the effects of increasing concentration of inspired isoflurane from 1.5% up to 5% on electrocardiogram (ECG), HR, arterial blood pressure, and respiratory rate in SHR/Izm, WKY/Izm and Crl:CD (SD) rats, in order to investigate the differences in cardiorespiratory responsiveness to isoflurane anesthesia among these rat strains.

Materials and Methods

This study was approved by the Ethics Committee for Animal Research of Fukushima Medical University (approval number: 25115). Animal experiments were carried out in accordance with the Guidelines for the Animal Experiments of Fukushima Medical University and the Act on Welfare and Management of Animals of Japan.

Animals

Male SHR/Izm (n=8) and WKY/Izm (n=8) rats were purchased from Japan SLC Inc. (Shizuoka, Japan) and Crl:CD (SD) (n=7) rats from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). These animals were acclimatized to the animal facility for more than 1 week, and then used for the study at 14 weeks of age. The animal room was maintained at a room temperature of 22 ± 2°C and a relative humidity of 55 ± 5% with a 12 h light/dark cycle (light period, 7:00–19:00). Commercial diet (Oriental Yeast Co., Tokyo, Japan) and ultrafiltrated water were given *ad libitum*.

Experimental protocol

For inhalation anesthesia of the rats, an isoflurane vaporizer (KN-1071; Natsume Seisakusho Co., Ltd., Tokyo, Japan) was used. The rat was placed in an anes-

thesia induction chamber (KN-1010; Natsume Seisakusho Co., Ltd.) and, by means of setting a dial of the vaporizer at 1.5%, isoflurane (Intervet, Inc., Tokyo, Japan) anesthesia mixed with room air was induced for 3 min. The air flow rate to the chamber was 5 l/min (manufacturer information). Practically, the mean induction times were 3.3 min in Crl:CD (SD), 3.5 min in WKY/Izm, and 3.3 min in SHR/Izm rats. After confirmation of the loss of righting reflex, the rat was moved into an aluminum shield chamber (50 × 40 × 78 cm) and placed in an abdominal position with a nose mask. Anesthesia was maintained with the dial held at 1.5% for 3 min, and then the cardiorespiratory parameters were recorded for 2 min as described below.

Subsequently, the dial indicator of isoflurane vaporizer was changed to 2% from 1.5%. The animal was left undisturbed for 1 min, and the cardiorespiratory parameters were obtained during a 2-min recording period. Similarly, the setting value of vaporizer's dial indicator was increased in a stepwise manner and cardiorespiratory parameters were obtained under the setting value of 3%, 4%, and 5%. After the recording under the set at 5%, the rat was relieved from anesthesia by removing the nose mask and observed until awakening. Endotracheal intubation in rats requires a certain amount of skill and endoscope for the successful intubation [20] and there were many reports using nose mask for isoflurane anesthesia in rats even in recent years [1, 2, 7, 40]. For practical data using nose mask, we used nose mask instead of endotracheal intubation in maintenance of isoflurane anesthesia. During the experiment, the temperature and humidity of the aluminum chamber were 23.5–28.6°C and 26–64%, respectively.

Electrocardiogram recording and analysis

A bipolar limb lead II was used for ECG recording. Needle electrodes subcutaneously attached to the limbs were connected to a bioamplifer (FE136, ADInstruments, Nagoya, Japan). ECG data were acquired at rate of 1,000/sec with an analog-to-digital converter (PowerLab4/26, ADInstruments) and analyzed for the following standard ECG variables by using analysis software (LabChart7 Pro ver7.12, ADInstruments): RR interval, PR interval, QRS duration, QT interval, QT_C interval, amplitude of T wave and HR. The QT_C interval was derived from the QT interval using Bazzet's formula: QT_C=QT Interval / √ (RR interval). These ECG variables were obtained by analysis of the ECG waveform during a 10 s noise-free period.

Frequency domain analysis of heart rate variability

In order to assess sympathetic and parasympathetic modulation of HR during isoflurane anesthesia, frequency domain indexes of HR variability were calculated using fast Fourier transform using analysis software (LabChart7 Pro ver7.12) to obtain low frequency (LF; 0.27–0.75 Hz) and high frequency (HF; 0.75–3.3 Hz) components in absolute values (ms^2) and the low frequency/high frequency (LF/HF) ratio. The frequency ranges were chosen based on the report by Murasato *et al*. [23]. The power of the HF band was considered as an index of parasympathetic nervous activity and it contains respiration-linked oscillations in HR [5], whereas that of the LF band was non-specific and contained both sympathetic and parasympathetic influences [13]. The LF/HF ratio estimated the fractional distribution of power and was regarded as an index of sympathovagal balance. For power spectral analysis of HR variability, we analyzed the same ECG data used in ECG analysis.

Arterial blood pressure measurement

The tail-cuff method has some advantages for blood pressure measurement in experimental animals [21]. The method is noninvasive, can be used for repeated measurement of blood pressure in same animal, and can be used to obtain data from large numbers of animals. Therefore, the tail-cuff method using an indirect blood pressure meter (BP-98A; Softron, Tokyo, Japan) was used in the present study. This apparatus has been used successfully in other cardiovascular studies using rats including SHR rats [18, 24, 35, 41]. In this study, systolic blood pressure (SBP), MBP, diastolic blood pressure (DBP), and pulse pressure (PP) were measured in the tail artery of rats. These parameters were measured once during each isoflurane concentration.

Respiratory rate measurement

Respiratory rate was measured using a force-sensitive sensor composed of a carbon fiber, as described previously [19]. The rat was placed in a prone position with the thoracoabdominal region in contact with the sensor. Pressure force caused by thoracoabdominal movement associated with respiration was transformed into electrical signals, amplified, and recorded. The respiratory rate was analyzed over 10 s during the same period as the ECG analysis was performed.

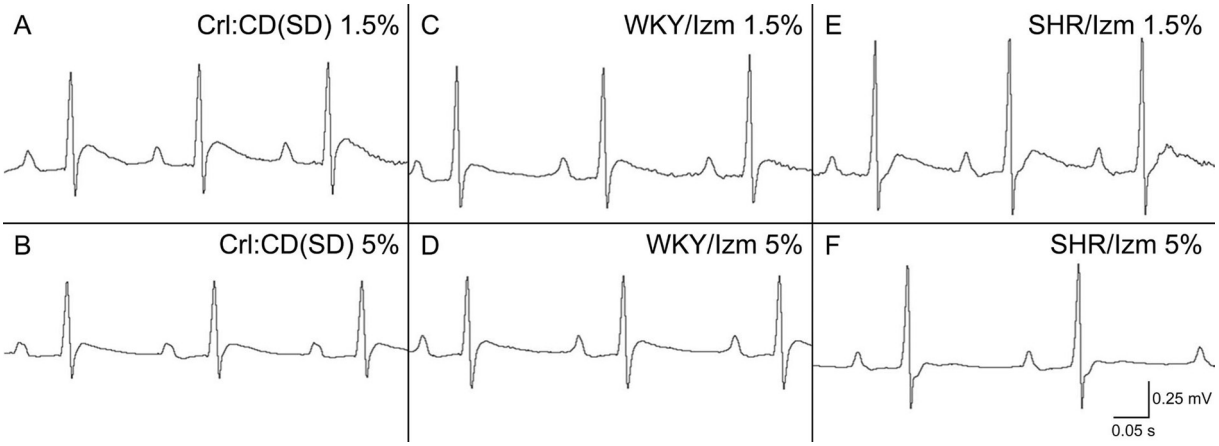

Fig. 1. Representative electrocardiogram (ECG) waveform in Crl:CD (SD), WKY/Izm, and SHR/Izm rats during 1.5% (A, C and E) and 5% (B, D and F) isoflurane anesthesia. Slight prolongation of RR interval was observed with 5% isoflurane compared with 1.5% isoflurane in all rats strains. In addition, flattening of the T-wave was observed in SHR/Izm rats during 5% isoflurane anesthesia.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). Statistical significance of differences in ECG parameters, HR variability, and respiratory rate between different concentrations of inspired isoflurane was determined by Friedman analysis, followed by Scheffe's method. Decreasing rates of MBP, HR, and respiratory rate in response to increasing inspired concentration from 1.5% to 5% was compared between different strains by Kruskal–Wallis test, followed by Scheffe's method. Differences were considered statistically significant at $P<0.05$.

Results

Electrocardiogram during isoflurane anesthesia

In both Crl:CD (SD) and WKY/Izm rats, no obvious abnormalities but only a slight prolongation of RR interval was observed after the concentration of inspired isoflurane was increased to 5%, compared with that under 1.5% (Figs. 1A–1D). In SHR/Izm rats, flattening of the T-wave was also observed at 5%, compared with that under 1.5% (Figs. 1E and 1F).

Electrocardiogram parameters during isoflurane anesthesia

In Crl:CD (SD) and SHR/Izm rats, RR interval was prolonged with increasing isoflurane concentration, and the interval during 4% and 5% isoflurane anesthesia were significantly different from with 1.5% isoflurane (Fig. 2A). In WKY/Izm rats, RR interval did not change from 1.5% to 4% isoflurane, but it increased significantly with 5% isoflurane in comparison with 2% isoflurane (Fig. 2A). As well as RR interval, PR interval was also prolonged with increasing isoflurane concentration in all strains (Fig. 2B). PR interval with 5% isoflurane anesthesia was significantly different from that with 1.5% or 2% isoflurane in all three strains (Fig. 2B). QRS duration did not change by increasing isoflurane concentration in any strain (Fig. 2C). Similarly, QT_C interval was not affected by increasing isoflurane concentration in Crl:CD (SD) and WKY/Izm (Fig. 2D) rats. However, in SHR/Izm rats, QT_C interval shortened in response to increasing isoflurane concentration and significant shortening was observed with 4% and 5% isoflurane as compared with 1.5% isoflurane (Fig. 2D). The amplitude of T wave in SHR/Izm became to be low with increasing concentration of inspired isoflurane, and the amplitude at 4% or 5% was significantly lower compared with that under 1.5%, 2% or 3% isoflurane (Fig. 2E). In Crl:CD (SD) and WKY/Izm, the amplitude of T wave with 5% isoflurane was significantly lower from that with 1.5% and 2% isoflurane (Fig. 2E).

Heart rate during isoflurane anesthesia

In Crl:CD (SD) and SHR/Izm rats, HR during 4% and 5% isoflurane anesthesia was decreased significantly compared with that during 1.5% or 2% (Table 1). In WKY/Izm rats, a significant decrease was observed only with 5% isoflurane in comparison with 2% isoflurane (Table 1).

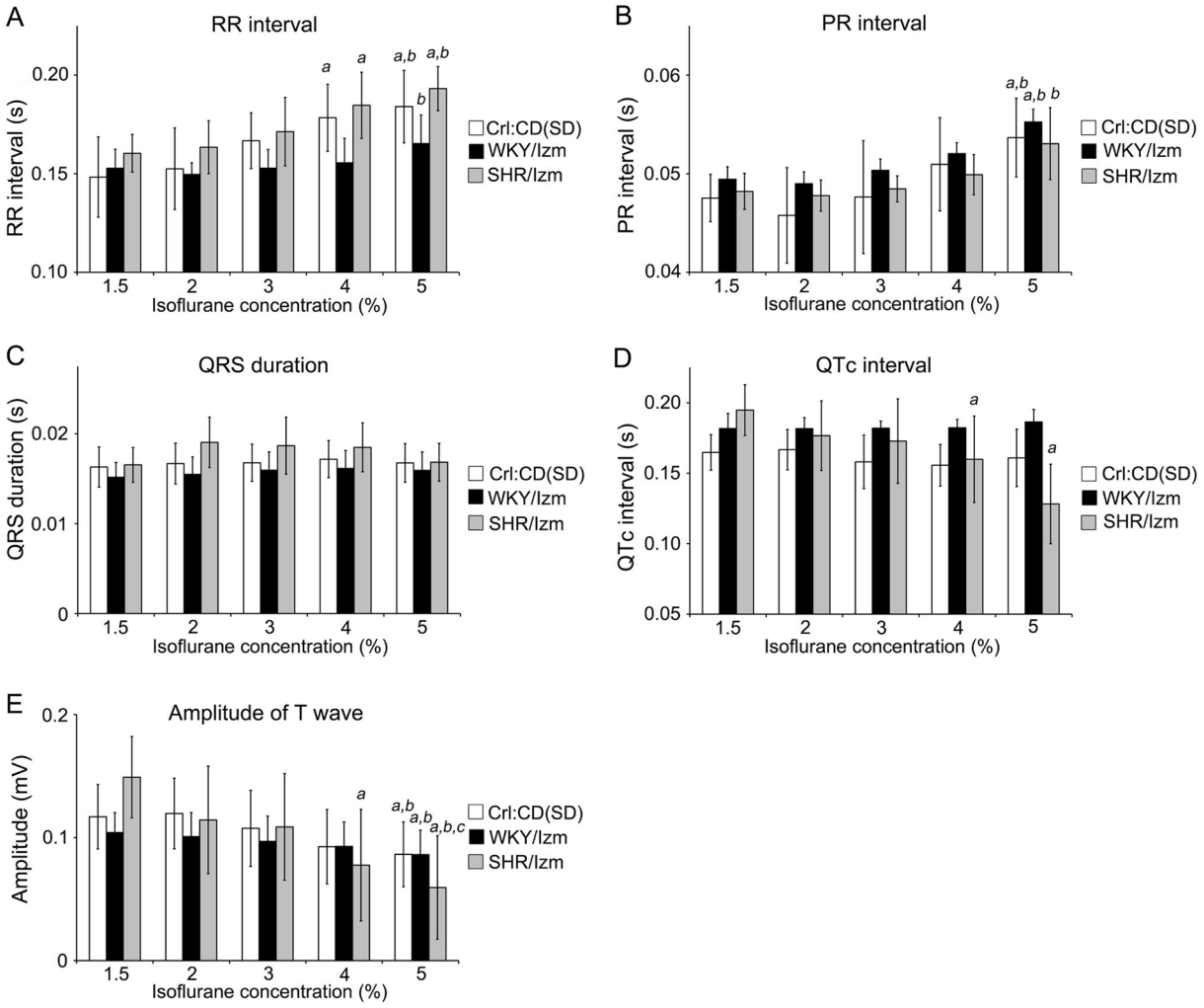

Fig. 2. Electrocardiogram (ECG)-measured RR interval (A), PR interval (B), QRS duration (C), QT_C interval (D), and amplitude of T wave (E) in Crl:CD (SD), WKY/Izm, and SHR/Izm rats during 1.5–5% isoflurane anesthesia. Data are expressed as mean ± SD. $^aP<0.05$ vs. 1.5% isoflurane, $^bP<0.05$ vs. 2% isoflurane, $^cP<0.05$ vs. 3% isoflurane.

Heart rate variability during isoflurane anesthesia

In Crl:CD (SD) rats, no significant changes in LF component, HF component, or LF/HF ratio were observed in response to increasing isoflurane concentration (Table 2). In WKY/Izm rats, the HF component during 5% isoflurane anesthesia was decreased significantly compared with 1.5% and 3% isoflurane (Table 2). In SHR/Izm rats, however, the LF component and LH/HF ratio with 5% isoflurane were significantly increased compared with 1.5% or 3% isoflurane (Table 2).

Arterial blood pressure during isoflurane anesthesia

A decrease in SBP was observed in all strains with increasing isoflurane concentration. SBP during 4% and 5% isoflurane anesthesia decreased significantly compared with 1.5% isoflurane (Fig. 3A). Furthermore, SBP in Crl:CD (SD) and SHR/Izm rats with 5% isoflurane was lower than that with 2% isoflurane. MBP also declined in Crl:CD (SD) and SHR/Izm rats, but not in WKY/Izm rats, with increasing isoflurane concentration (Fig. 3B). In both Crl:CD (SD) and SHR/Izm rats, MBP with 4% and 5% isoflurane was significantly different from that with 1.5% or 2% isoflurane (Fig. 3B). As well as MBP, DBP and PP decreased significantly during 5% isoflurane anesthesia compared with 1.5% or 2% isoflurane in Crl:CD (SD) and SHR/Izm rats, but these values were unchanged in WKY/Izm rats (Figs. 3C and 3D). As shown in Fig. 3, the suppressive effects of isoflurane anesthesia on blood pressure were more profound in SHR/Izm rats among the three rat strains examined. High

Table 1. Heart rate in SHR/Izm, WKY/Izm, and Crl:CD (SD) rats during isoflurane anesthesia (bpm)

Strain	Isoflurane concentration				
	1.5%	2%	3%	4%	5%
Crl:CD (SD)	412.8 ± 70.1	401.2 ± 66.5	361.9 ± 27.8	339.1 ± 31.8[a]	328.8 ± 31.3[a, b]
WKY/Izm	394.2 ± 25.4	401.6 ± 16.4	394.1 ± 24.2	387.7 ± 29.0	365.4 ± 30.0[b]
SHR/Izm	375.4 ± 22.3	369.3 ± 29.3	353.7 ± 38.5	327.3 ± 30.0[a]	311.5 ± 18.2[a, b]

Data are expressed as mean ± SD. [a]P<0.05 vs. 1.5% isoflurane, [b]P<0.05 vs. 2% isoflurane.

Table 2. Frequency domain analysis of heart rate variavilitty in SHR/Izm, WKY/Izm, and Crl:CD (SD) rats during isoflurane anesthesia

Strain	Index	Isoflurane concentration				
		1.5%	2%	3%	4%	5%
Crl:CD (SD)	LF (ms^2)	0.11 ± 0.15	0.07 ± 0.15	0.23 ± 0.41	0.21 ± 0.24	0.37 ± 0.49
	HF (ms^2)	2.87 ± 2.10	2.61 ± 2.60	2.60 ± 3.09	2.48 ± 3.11	2.14 ± 2.11
	LF/HF (ratio)	0.05 ± 0.07	0.04 ± 0.08	0.30 ± 0.62	0.30 ± 0.31	0.26 ± 0.20
WKY/Izm	LF (ms^2)	0.03 ± 0.04	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.10 ± 0.09
	HF (ms^2)	1.70 ± 1.00	1.70 ± 1.14	1.60 ± 1.19	1.52 ± 1.15	0.68 ± 0.80 [a, c]
	LF/HF (ratio)	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.46 ± 0.61
SHR/Izm	LF (ms^2)	0.07 ± 0.07	0.22 ± 0.41	0.10 ± 0.14	0.66 ± 0.53	1.23 ± 0.55 [a, c]
	HF (ms^2)	2.11 ± 2.92	2.92 ± 2.25	2.11 ± 1.53	2.91 ± 2.70	1.75 ± 1.10
	LF/HF (ratio)	0.04 ± 0.04	0.04 ± 0.05	0.07 ± 0.12	0.78 ± 0.90	0.92 ± 0.47 [c]

Data are expressed as mean ± SD. [a]P<0.05 vs. 1.5% isoflurane, [c]P<0.05 vs. 3% isoflurane.

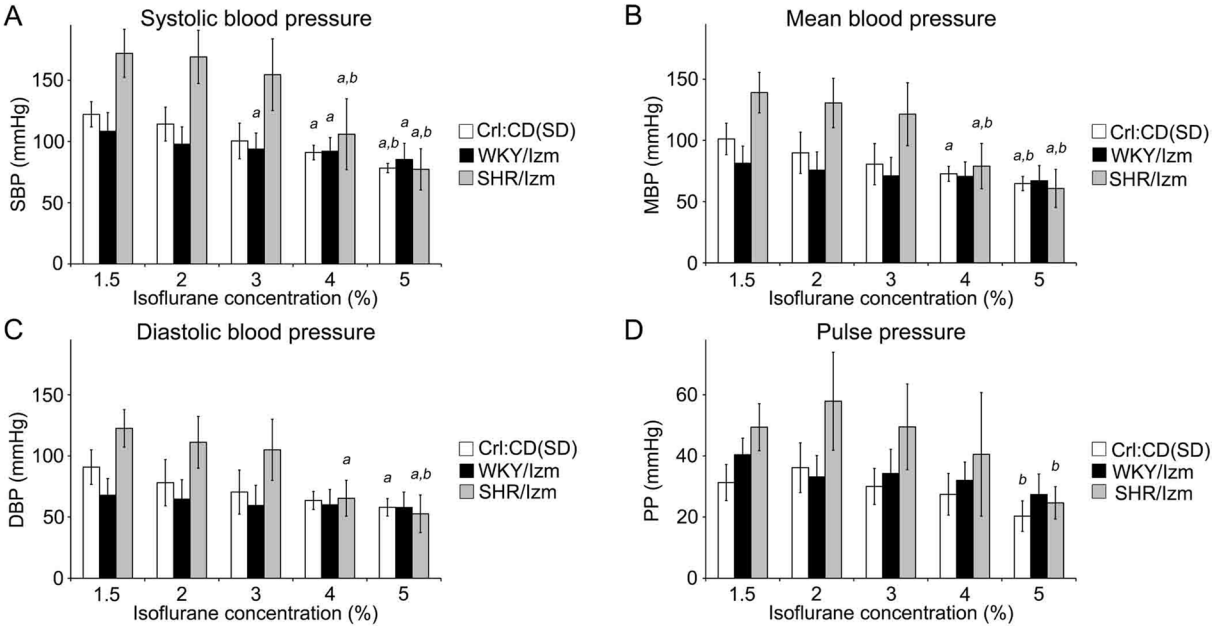

Fig. 3. Systolic (A), mean (B), and diastolic blood pressure (C), and pulse pressure (D) during 1.5–5% isoflurane anesthesia. Data are expressed as mean ± SD. [a]P<0.05 vs. 1.5% isoflurane, [b]P<0.05 vs. 2% isoflurane.

blood pressure level with 1.5% isoflurane in SHR/Izm rats declined to the same level as in Crl:CD (SD) and WKY/Izm rats when using 5% isoflurane.

Respiratory rate during isoflurane anesthesia

In all strains, respiratory suppression was observed with increasing isoflurane concentration (Table 3). Respiratory rate during 5% isoflurane anesthesia was sig-

Table 3. Respiratory rate in SHR/Izm, WKY/Izm, and Crl:CD (SD) rats during isoflurane anesthesia (breaths/min)

Strain	Isoflurane concentration				
	1.5%	2%	3%	4%	5%
Crl:CD(SD)	80.2 ± 19.4	82.0 ± 16.9	55.9 ± 13.7	43.1 ± 12.9	36.3 ± 13.3 [a,b]
WKY/Izm	89.9 ± 6.1	91.0 ± 8.5	88.3 ± 6.6	71.0 ± 12.8	48.8 ± 11.3 [a,b,c]
SHR/Izm	89.6 ± 19.6	85.5 ± 10.3	64.6 ± 12.1	46.6 ± 6.7[a]	39.0 ± 5.2 [a,b]

Data are expressed as mean ± SD. [a]$P<0.05$ vs. 1.5% isoflurane, [b]$P<0.05$ vs. 2% isoflurane, [c]$P<0.05$ vs. 3% isoflurane.

nificantly decreased compared with 1.5% and 2% isoflurane. Furthermore, in SHR/Izm rats, respiratory rate with 4% isoflurane was significantly lower compared with 1.5% isoflurane. However, in WKY/Izm rats, respiratory rate did not change up to 3% isoflurane, and respiratory rate with 5% isoflurane was significantly lower than with 1.5–3% isoflurane.

Decrease in rates of mean blood pressure, heart rate, and respiratory rate with increasing inspired isoflurane concentration from 1.5% to 5%

In order to compare the suppressive effect of isoflurane anesthesia on cardiorespiratory function among the three rat strains, the decrease in rate of MBP, HR, and respiratory rate with increasing inspired isoflurane concentration from 1.5% to 5% were calculated (Fig. 4). The decrease in rate of MBP in Crl:CD (SD), WKY/Izm, and SHR/Izm rats was 64.5 ± 6.2%, 82.9 ± 13.3%, and 44.2 ± 12.4%, respectively. This change was significantly greater in SHR/Izm rats compared with WKY/Izm rats. The decrease in rate of HR in Crl:CD (SD), WKY/Izm, and SHR/Izm rats was 80.8 ± 10.7%, 92.9 ± 8.3%, and 83.2 ± 5.7%, respectively, which was significantly greater in Crl:CD (SD) and SHR/Izm rats compared with WKY/Izm rats. Finally, the decrease in rate of respiratory rate in Crl:CD (SD), WKY/Izm, and SHR/Izm rats was 48.8 ± 22.9%, 53.9 ± 9.3%, and 45.2 ± 11.0%, respectively, and there were no significant differences between rat strains.

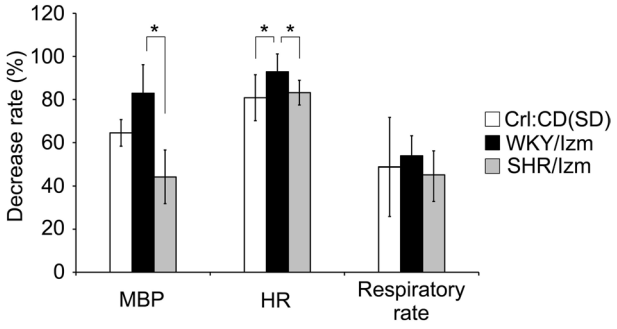

Fig. 4. The decrease rate of mean blood pressure (MBP), heart rate (HR), and respiratory rate during 5% inspired isoflurane anesthesia as compared with those under 1.5% inspired isoflurane anesthesia. *$P<0.05$ vs. WKY/Izm rats.

Discussion

In the present study, we examined the effects of increasing concentration of inspired isoflurane on cardiorespiratory parameters in SHR/Izm, WKY/Izm and Crl:CD (SD) rats by increasing the values of vaporizer's dial indicator from 1.5% to 5%.

This study has limitation. Because the endo-tidal concentrations of isoflurane in rats were not measured during experiments, the inspired isoflurane concentration-dependency of the cardiorespiratory alterations is not exactly clear. Therefore, further studies are needed to clarify the relationship between inspired isoflurane concentrations and cardiorespiratory changes in SHR/Izm, WKY/Izm and Crl:CD (SD) rats. However, at least, the results of the present study indicated that increasing concentrations of inspired isoflurane induced the cardiorespiratory suppression in rats, especially, in SHR/Izm rats. That is to say, this study would suggest that SHR/Izm rats are more sensitive to suppressive effects of isoflurane anesthesia on cardiorespiratory function among the three rat strains.

It was revealed for the first time that the changes in ECG in SHR/Izm, WKY/Izm and Crl:CD (SD) rats were induced as the setting values of isoflurane vaporizer's dial increased. On the ECG, prolongation of the RR and PR intervals was observed during isoflurane anesthesia at more than 4% in all three rat strains. Although there were no reports on ECG wave changes in these rats under increased inspired isoflurane concentration, it was previously reported that anesthesia with 2% of inspired isoflurane in dogs did not affect PR interval compared

with in a conscious state [28], and in humans, PR interval remained unchanged with 1–2 minimum alveolar concentration (MAC) isoflurane [30]. In the present study, we did not carry out ECG measurements in conscious rats, so we cannot compare ECG parameters between during isoflurane anesthesia and in the conscious state. Nevertheless, the results of the present study suggested that isoflurane anesthesia might prolong both the RR and PR intervals as the inspired concentration increased. These intervals are known to be associated with alterations in HR, so it was considered that RR and PR intervals were prolonged in association with the decrease in HR during 4% or 5% isoflurane anesthesia. On the other hand, in SHR/Izm rats, the QT_C interval was shortened by isoflurane at more than 4%, and obvious flattening of the T-wave on ECG was also observed under the same concentration range. With regard to the effects of isoflurane on QT interval, prolongation of QT interval was reported with 2% and 1–2 MAC isoflurane in normotensive dogs [28] and humans [30], respectively. From these findings, it might be suggested that higher inspired concentrations of isoflurane may induce shortening of the QT interval, especially in hypertensive subjects. QT interval corresponds to depolarization and repolarization phases of action potentials of cardiac ventricular myocytes. In isolated ventricular myocytes of guinea-pig hearts, action potential duration was found to be increased by isoflurane at a low concentration (0.6 mM), whereas conversely it decreased at high concentration (1.8 mM) [36], suggesting a biphasic effect of isoflurane on cardiac myocyte action potential. In the present study, the QRS duration of the rats was not affected by increasing setting values of isoflurane vaporizer, indicating that isoflurane did not affect the depolarization time of ventricular muscle in SHR/Izm rats. Thus, it was thought that the dial settings of isoflurane vaporizer to 4% and 5% reduced the repolarization phase of ventricular muscle, leading to shortening of QT interval in SHR/Izm rats. In humans, several drugs, including digitalis, are known to induce shortening of QT interval [34], and a tall and peaked T-wave is a common feature in short QT syndrome [22]. However, to the best of our knowledge, there are no reports on the isoflurane-induced shortening of QT interval. It is conceivable that QT shortening with flattened T-wave in SHR/Izm rats response to increase in the dial setting of vaporizer is caused by different mechanisms than in normotensive subjects or other species. Although the exact mechanisms leading to QT shortening by isoflurane anesthesia was unclear from the present study, the results might suggest that isoflurane anesthesia, as its inspired concentration rises, increases the risk of QT interval shortening in SHR/Izm. In WKY/Izm and Crl:CD (SD), the lowering T wave without shortening of QT interval was observed under the dial setting of vaporizer to 5%, and possibly, shortening of QT interval might be caused in this normotensive two strains when the inspiration time of 5% isoflurane is more prolonged.

During 5% isoflurane anesthesia, LF components and the LF/HF ratio increased in SHR/Izm rats, and HF components decreased in WKY/Izm rats, suggesting that sympathetic modulation of HR is predominant with increase in inspired isoflurane concentration. These results are compatible with other *in vivo* findings that isoflurane anesthesia for up to 4% of end-tidal concentration dose-dependently increased renal sympathetic nerve activity in rabbits [25]. It might be possible that isoflurane, as its inspired concentration increases, may bring about an imbalance in sympathetic and parasympathetic modulation of HR in SHR/Izm and WKY/Izm rats. Although not significant, a similar tendency for alteration in frequency domain indexes was also observed in Crl:CD (SD) rats. Sympathetic activation during isoflurane anesthesia in rats might occur via irritation of the airway, the baroreceptor reflex, and direct stimulation of the central nervous system as suggested by Okamoto *et al.* [25].

Previously, there were some studies on the effects of inspired isoflurane on blood pressure and HR in SHR, WKY, and SD rats both at less than 3% inspired concentration and for at least 30 min [10, 11, 32, 33]. In the present study, it was revealed that inspired isoflurane concentration-dependently reduced arterial blood pressure and HR in all rat strains examined. Cole *et al*. [10] reported that MBP after isoflurane (1.65% end-tidal) inspiration for 120 min was changed to approximately 148, 94, and 101 mmHg in SHR, WKY, and SD rats respectively. MBP values in the present study after the dial setting of vaporizer was changed to 5% were approximately 61, 67, and 65 mmHg in SHR/Izm, WKY/Izm, and Crl:CD (SD) rats, respectively, and lower than the values reported by Cole *et al*. [10]. This finding might indicate that hypotensive effects of isoflurane, as its inspired concentration increases, occur immediately and strongly in these rat strains. Based on Darcy's law, MBP is expressed as shorthand by the following equation [4]:

MBP=cardiac output × systemic vascular resistance
=(HR × stroke volume) × systemic vascular resistance

In the present study, MBP under the setting value of 5% was approximately 60 mmHg in three rat strains, indicating that isoflurane inspiration by increasing the setting value of vaporizer to 5% induces similar suppressive state in cardiovascular system among the strains. HR in SHR/Izm rats tended to be consistently lower than other two strains under the dial settings of vaporizer from 1.5% up to 5%. Therefore, it seems to be that SHR/Izm rats, during inspiration of relatively low inspired concentration, exhibited high blood pressure due to high systemic vascular resistance and/or high stroke volume. On the other hand, as the setting values of vaporizer increased, it is considered that SHR/Izm rats exhibited profound decrease in blood pressure due to decrease in both systemic vascular resistance and stroke volume, in addition to decreased HR. Previously, it was reported that the mechanisms for blood pressure lowering was different between SHR and other two strains, and it was reported that a reduction in blood pressure was caused by decreased cardiac output in SHR rats [33], whereas it was caused by reduced systemic vascular resistance in WKY rats and SD rats [32, 33]. However, those results were obtained under isoflurane inspiration at a constant level of less than 3%. Thus, although both systemic vascular resistance and stroke volume were not measured in this study, hypotensive mechanism by inspiration of increased isoflurane concentration would be different from the mechanisms by relatively low level.

As for the effects on respiration, isoflurane anesthesia was found to reduce respiratory rate in laboratory animals including rats [9], mice [8, 39], and rabbits [16]. In this study, we confirmed that isoflurane, as its inhalation concentration increased, decreased the respiratory rate in SHR/Izm, WKY/Izm, and Crl:CD (SD) rats. Because significant differences were not observed in decrease rate of respiratory rate, it is suggested that suppression level of respiratory function by increased inspired isoflurane concentration were similar between these strains examined.

In conclusion, we revealed the suppressive effects of isoflurane anesthesia on cardiorespiratory function in SHR/Izm, WKY/Izm and Crl:CD (SD) rats by changing the dial settings of isoflurane vaporizer from 1.5% up to 5%. The findings obtained from this study suggested that there are strain differences in sensitivity to the suppressive effects of isoflurane on cardiovascular function: SHR/Izm rats were relatively more sensitive, whereas WKY/Izm rats were relatively less sensitive.

References

1. Albrecht, M., Henke, J., Tacke, S., Markert, M., and Guth, B. 2014. Effects of isoflurane, ketamine-xylazine and a combination of medetomidine, midazolam and fentanyl on physiological variables continuously measured by telemetry in Wistar rats. *BMC Vet. Res.* 10: 198. [Medline] [CrossRef]
2. Albrecht, M., Henke, J., Tacke, S., Markert, M., and Guth, B. 2014. Influence of repeated anaesthesia on physiological parameters in male Wistar rats: a telemetric study about isoflurane, ketamine-xylazine and a combination of medetomidine, midazolam and fentanyl. *BMC Vet. Res.* 10: 310. [Medline] [CrossRef]
3. Almeida, D.E., Rezende, M.L., Nunes, N., and Laus, J.L. 2004. Evaluation of intraocular pressure in association with cardiovascular parameters in normocapnic dogs anesthetized with sevoflurane and desflurane. *Vet. Ophthalmol.* 7: 265–269. [Medline] [CrossRef]
4. Augusto, J.F., Teboul, J.L., Radermacher, P., and Asfar, P. 2011. Interpretation of blood pressure signal: physiological bases, clinical relevance, and objectives during shock states. *Intensive Care Med.* 37: 411–419. [Medline] [CrossRef]
5. Berntson, G.G., Bigger, J.T. Jr., Eckberg, D.L., Grossman, P., Kaufmann, P.G., Malik, M., Nagaraja, H.N., Porges, S.W., Saul, J.P., Stone, P.H., and van der Molen, M.W. 1997. Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34: 623–648. [Medline] [CrossRef]
6. Buchanan-Smith, H.M., Rennie, A.E., Vitale, A., Pollo, S., Prescott, M.J., and Morton, D.B. 2005. Harmonizing the definition of refinement. *Anim. Welf.* 14: 379–384.
7. Bunting, K.M., Nalloor, R.I., and Vazdarjanova, A. 2016. Influence of Isoflurane on Immediate-Early Gene Expression. *Front. Behav. Neurosci.* 9: 363. [Medline] [CrossRef]
8. Cesarovic, N., Nicholls, F., Rettich, A., Kronen, P., Hässig, M., Jirkof, P., and Arras, M. 2010. Isoflurane and sevoflurane provide equally effective anaesthesia in laboratory mice. *Lab. Anim.* 44: 329–336. [Medline] [CrossRef]
9. Chung, A., Fishman, M., Dasenbrook, E.C., Loparo, K.A., Dick, T.E., and Jacono, F.J. 2013. Isoflurane and ketamine anesthesia have different effects on ventilatory pattern variability in rats. *Respir. Physiol. Neurobiol.* 185: 659–664. [Medline] [CrossRef]
10. Cole, D.J., Marcantonio, S., and Drummond, J.C. 1990. Anesthetic requirement of isoflurane is reduced in spontaneously hypertensive and Wistar-Kyoto rats. *Lab. Anim. Sci.* 40: 506–509. [Medline]
11. Conzen, P.F., Vollmar, B., Habazettl, H., Frink, E.J., Peter, K., and Messmer, K. 1992. Systemic and regional hemodynamics of isoflurane and sevoflurane in rats. *Anesth. Analg.* 74: 79–88. [Medline] [CrossRef]
12. Dárdai, E. and Heavner, J.E. 1989. Comparison of respiratory and cardiovascular effects of halothane, isoflurane, and enflurane delivered via the Jackson-Rees breathing system in rats. New anaesthesia model for small animal surgery. *Z. Exp. Chir.*

Transplant. Kunstliche Organe 22: 50–54. [Medline]
13. Eckberg, D.L. 1997. Sympathovagal balance: a critical appraisal. *Circulation* 96: 3224–3232. [Medline] [CrossRef]
14. Gargiulo, S., Greco, A., Gramanzini, M., Esposito, S., Affuso, A., Brunetti, A., and Vesce, G. 2012. Mice anesthesia, analgesia, and care, Part I: anesthetic considerations in preclinical research. *ILAR J.* 53: E55–E69. [Medline] [CrossRef]
15. Gentry-Smetana, S., Redford, D., Moore, D., and Larson, D.F. 2008. Direct effects of volatile anesthetics on cardiac function. *Perfusion* 23: 43–47. [Medline] [CrossRef]
16. Hedenqvist, P., Roughan, J.V., Antunes, L., Orr, H., and Flecknell, P.A. 2001. Induction of anaesthesia with desflurane and isoflurane in the rabbit. *Lab. Anim.* 35: 172–179. [Medline] [CrossRef]
17. Henry, R.T. and Casto, R. 1989. Simple and inexpensive delivery of halogenated inhalation anesthetics to rodents. *Am. J. Physiol.* 257: R668–R671. [Medline]
18. Hussein, G., Goto, H., Oda, S., Sankawa, U., Matsumoto, K., and Watanabe, H. 2006. Antihypertensive potential and mechanism of action of astaxanthin: III. Antioxidant and histopathological effects in spontaneously hypertensive rats. *Biol. Pharm. Bull.* 29: 684–688. [Medline] [CrossRef]
19. Katahira, K. 1979. A new type of force-sensitive device using carbon fiber and its biomedical application. *Fukushima J. Med. Sci.* 26: 121–132. [Medline]
20. Konno, K., Shiotani, Y., Itano, N., Ogawa, T., Hatakeyama, M., Shioya, K., and Kasai, N. 2014. Visible, safe and certain endotracheal intubation using endoscope system and inhalation anesthesia for rats. *J. Vet. Med. Sci.* 76: 1375–1381. [Medline] [CrossRef]
21. Kurtz, T.W., Griffin, K.A., Bidani, A.K., Davisson, R.L., Hall, J.E., Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research 2005. Recommendations for blood pressure measurement in humans and experimental animals: part 2: blood pressure measurement in experimental animals: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Arterioscler. Thromb. Vasc. Biol.* 25: e22–e33. [Medline] [CrossRef]
22. Maluli, H.A. and Meshkov, A.B. 2013. A short story of the short QT syndrome. *Cleve. Clin. J. Med.* 80: 41–47. [Medline] [CrossRef]
23. Murasato, Y., Hirakawa, H., Harada, Y., Nakamura, T., and Hayashida, Y. 1998. Effects of systemic hypoxia on R-R interval and blood pressure variabilities in conscious rats. *Am. J. Physiol.* 275: H797–H804. [Medline]
24. Niwa, Y., Hiura, Y., Sawamura, H., and Iwai, N. 2008. Inhalation exposure to carbon black induces inflammatory response in rats. *Circ. J.* 72: 144–149. [Medline] [CrossRef]
25. Okamoto, H., Hoka, S., Kawasaki, T., Okuyama, T., and Takahashi, S. 1996. Dose-dependent increases in the renal sympathetic nerve activity during rapid increase in isoflurane concentration in intact, lower airway-deafferented, and baroreceptor-deafferented rabbits. *Anesthesiology* 84: 1196–1204. [Medline] [CrossRef]
26. Pac-Soo, C.K., Wang, C., Chakrabarti, M.K., and Whitwam, J.G. 2000. Comparison of the effects of inhalational anaesthetic agents on sympathetic activity in rabbits. *Eur. J. Anaesthesiol.* 17: 311–318. [Medline] [CrossRef]
27. Richardson, C.A. and Flecknell, P.A. 2005. Anaesthesia and post-operative analgesia following experimental surgery in laboratory rodents: are we making progress? *Altern. Lab. Anim.* 33: 119–127. [Medline]
28. Riley, D.C., Schmeling, W.T., al-Wathiqui, M.H., Kampine, J.P., and Warltier, D.C. 1988. Prolongation of the QT interval by volatile anesthetics in chronically instrumented dogs. *Anesth. Analg.* 67: 741–749. [Medline] [CrossRef]
29. Russell, W.M.S. and Burch, R.L. 1959. The principles of humane experimental technique. Methuen and Co. London. UK. 238pp.
30. Schmeling, W.T., Warltier, D.C., McDonald, D.J., Madsen, K.E., Atlee, J.L., and Kampine, J.P. 1991. Prolongation of the QT interval by enflurane, isoflurane, and halothane in humans. *Anesth. Analg.* 72: 137–144. [Medline] [CrossRef]
31. Seagard, J.L., Hopp, F.A., Bosnjak, Z.J., Osborn, J.L., and Kampine, J.P. 1984. Sympathetic efferent nerve activity in conscious and isoflurane-anesthetized dogs. *Anesthesiology* 61: 266–270. [Medline] [CrossRef]
32. Seyde, W.C. and Longnecker, D.E. 1984. Anesthetic influences on regional hemodynamics in normal and hemorrhaged rats. *Anesthesiology* 61: 686–698. [Medline] [CrossRef]
33. Seyde, W.C., Durieux, M.E., and Longnecker, D.E. 1987. The hemodynamic response to isoflurane is altered in genetically hypertensive (SHR), as compared with normotensive (WKY), rats. *Anesthesiology* 66: 798–804. [Medline] [CrossRef]
34. Shah, R.R. 2010. Drug-induced QT interval shortening: potential harbinger of proarrhythmia and regulatory perspectives. *Br. J. Pharmacol.* 159: 58–69. [Medline] [CrossRef]
35. Shindo, M., Kasai, T., Abe, A., and Kondo, Y. 2007. Effects of dietary administration of plant-derived anthocyanin-rich colors to spontaneously hypertensive rats. *J. Nutr. Sci. Vitaminol. (Tokyo)* 53: 90–93. [Medline] [CrossRef]
36. Suzuki, A., Aizawa, K., Gassmayr, S., Bosnjak, Z.J., and Kwok, W.M. 2002. Biphasic effects of isoflurane on the cardiac action potential: an ionic basis for anesthetic-induced changes in cardiac electrophysiology. *Anesthesiology* 97: 1209–1217. [Medline] [CrossRef]
37. Szczesny, G., Veihelmann, A., Massberg, S., Nolte, D., and Messmer, K. 2004. Long-term anaesthesia using inhalatory isoflurane in different strains of mice-the haemodynamic effects. *Lab. Anim.* 38: 64–69. [Medline] [CrossRef]
38. Torri, G. 2010. Inhalation anesthetics: a review. *Minerva Anestesiol.* 76: 215–228. [Medline]
39. Tsukamoto, A., Iimuro, M., Sato, R., Yamazaki, J., and Inomata, T. 2015. Effect of midazolam and butorphanol premedication on inhalant isoflurane anesthesia in mice. *Exp. Anim.* 64: 139–145. [Medline] [CrossRef]
40. Tsukamoto, A., Uchida, K., Maesato, S., Sato, R., Kanai, E., and Inomata, T. 2016. Combining isoflurane anesthesia with midazolam and butorphanol in rats. *Exp. Anim.* 65: 223–230. [Medline] [CrossRef]
41. Yoshida, Y., Shioi, T., and Izumi, T. 2007. Resveratrol ameliorates experimental autoimmune myocarditis. *Circ. J.* 71: 397–404. [Medline] [CrossRef]