—Original—

The validity of anesthetic protocols for the surgical procedure of castration in rats

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Abstract: To achieve surgical anesthesia in animal experimentation, it is necessary to select the appropriate anesthetic protocol by considering its pharmacological properties and the surgical procedure to be performed. However, few studies have investigated the validity of anesthetic protocols under surgical conditions in small rodents. The present study aimed to clarify the pharmacological properties of 4 anesthetic protocols during the surgical procedure of castration in rats. Eight-week-old male Wistar rats were anesthetized with anesthetics, including the combination of ketamine and xylazine (K/X), the combination of medetomidine, midazolam, and butorphanol (M/M/B), isoflurane, and sevoflurane. Castration was performed under each anesthesia, and anesthetic depth and times were assessed, as were vital signs. The injectable anesthetics were investigated at standard and high doses. The concentration of inhalant anesthetics was adjusted to 1.5 minimum alveolar concentration (MAC). K/X at both doses demonstrated sufficient anesthetic depth with rapid induction and recovery. However, bradycardia and hypothermia were prominent in high-dose K/X, indicating that the standard-dose is more appropriate for surgical anesthesia in castration procedures. M/M/B demonstrated high anesthetic sensitivity variation in individual animals. In contrast to injectable anesthetics, inhalant anesthetics provided stable anesthetic depth with less cardiorespiratory influence. Sevoflurane did not lead to a significant decrease in rectal temperature during the anesthetic period. Results of the present study revealed the optimal dose and pharmacological features of several anesthetic protocols for castration, and may contribute to the standardization of surgical anesthesia in rats.

Key words: anesthesia, refinement, rodent, surgery

Introduction

Anesthesia, which is a specific method of alleviating pain and distress in animals, is important in complying with the principle of the "3Rs", and is further indispensable for ensuring the reproducibility of experimental data [7]. Notably, appropriate anesthetic management is essential for achieving success in surgical experiments. Due to the recent growing interest in laboratory animal welfare, further optimization of laboratory animal anesthesia is now required. However, many aspects of surgical anesthesia in small rodents remain unclear and,

therefore, the pursuit of additional evidence is warranted.

When selecting appropriate anesthesia in individual animal experiments, it is important to evaluate the anesthetic risk to animals by considering the features of the anesthesia and the surgical procedure. General anesthesia for surgery is mainly divided into injectable and inhalation anesthetics. Typical injectable anesthetics used in rodents include the combination of ketamine and xylazine (K/X) [6, 15], and the combination of medetomidine, midazolam, and butorphanol (M/M/B) [10, 11]. Representative inhalation anesthetics include isoflurane and sevoflurane [5]. Each anesthesia exhibits individual pharmacological characteristics, and various factors, such as animal species, age, and strain can affect anesthetic sensitivity [7, 17, 19, 20, 22]. In addition, adequate anesthetic depth varies depending on the surgical procedure. Although recent studies have demonstrated the characteristics of various anesthetic protocols in small rodents [6, 10, 11, 20–22], they were based on non-operative conditions, and features of these anesthesia during surgical procedures remain unclear. In the present study, we evaluated the characteristics of various anesthetics under operative conditions in rats.

Materials and Methods

Animals

This study was approved by Institutional Animal Care and Use Committee of Azabu University, Kanagawa, Japan. Thirty-six 8-week-old male Wistar rats (Slc:Wistar, Japan SLC Inc., Shizuoka, Japan) were used in this study. The animals were housed in polycarbonate cages with wood shaving bedding (Soft chip, Japan SLC Inc.), which were changed twice per week. Rats were fed a pellet rodent diet (Labdiet, Japan SLC Inc.) and tap water. The facility was maintained at temperature of $22 \pm 1°C$ and humidity of $55 \pm 5\%$, with a 12-h light cycle. Before the experiment, the animals were acclimated at the facility for 1 week. After the whole experiment, the animals were euthanized using intraperitoneal administration of pentobarbital (Somnopentyl, Kyoritsu Seiyaku Co., Ltd., Tokyo, Japan) at a dose of 200 mg/kg.

Anesthetic protocols

The clinical agents investigated in the present study were as follows: ketamine hydrochloride (Ketalar, Sankyo Lifetech Co., Ltd., Tokyo, Japan); xylazine (Celac-

tar, Bayer Yakuhin Ltd., Tokyo, Japan); medetomidine hydrochloride (Domitol, Nippon Zenyaku Kogyo. Co., Ltd., Fukushima, Japan); midazolam (Dormicum, Astellas Pharma Inc., Tokyo, Japan); butorphanol (Vetorphale, Meiji Seika Pharma Co., Ltd., Tokyo, Japan); atipamezole (Antisedan, Nippon Zenyaku Kogyo Co., Ltd.); isoflurane (Isoflu, DS Pharma Animal Health Co., Ltd., Osaka, Japan); and sevoflurane (Sevoflo, DS Pharma Animal Health Co., Ltd.).

Castration was performed was performed under 4 anesthetic methods, including K/X, M/M/B, isoflurane, and sevoflurane. Two doses of M/M/B were evaluated: standard (M/M/B: 0.15/2.0/2.5 mg/kg) and 1.5 × standard-dose (M/M/B: 0.23/3.0/3.75 mg/kg) [11]. K/X was also evaluated in 2 doses: a standard (80/10 mg/kg) and high dose (100/10 mg/kg) [7]. The concentrations of isoflurane and sevoflurane during induction were adjusted to 5.0% and 8.0%, respectively. The concentration of inhalant anesthetics during maintenance were adjusted to minimum alveolar concentration (MAC) × 1.5 (isoflurane, 2.1%, sevoflurane, 4.1%) [4, 9]. Inhalant anesthesia was performed using a rodent inhalant anesthesia apparatus (SomnoSuite Small Animal Anesthesia System, Kent Scientific Corporation, Connecticut). According to a method described in a previous study by the authors [21], the flow rate of isoflurane and sevoflurane were determined using the following formula: Flow rate (ml/min)=0.65 × body weight (g). Induction was performed in an induction chamber and, after confirming the loss of righting reflex, rats were anesthetized via a nose-cone mask.

Surgery

Castration was performed in each rat under 1 of 4 types of anesthesia. After the administration of anesthesia, surgery was initiated when the anesthesia score reached≥3. Rats were placed in dorsal recumbency, and local hair was removed. After disinfection, a midline abdominal incision was performed. The testes were exposed, and the spermatic cord and blood vessel were then ligated using silk suture. After the removal of the testis on both sides, muscle layer and skin were sutured using nylon thread independently. In the M/M/B and K/X groups, the rats were administered atipamezole at a dose of 0.75 mg/kg, 30 min after administration of anesthetics. In isoflurane and sevoflurane groups, gas supply was stopped 30 min after the anesthetic induction.

When body movement or reflex was recognized during

Table 1. The description of anesthetic score

Score	Description
0	Animals shows all 4 reflexes
1	Animals lost 1 of 4 reflex
2	Animals lost 2 of 4 reflexes
3	Animals lost 3 of 4 reflexes
4	Animals lost all 4 reflexes

the operation, an additional injection of 25% of anesthetic was administered. A humane end point was assumed when the apparent body motion during movement (limb movements) was confirmed, and did not respond to additional administration or increase in maintenance anesthetic concentration. After undergoing the surgery, the rats were carefully observed for at least 1 day, after which they were euthanized.

Anesthetic depth and times

Anesthetic depth was evaluated according to the response to noxious stimuli [10, 19]. Using forceps, the withdraw reflex of the forelimb and hind limb, and reflexes of the eyelid, and tail pinch were observed. Anesthetic score (0 to 4) was then calculated in each rat according to the number of reflexes lost (Table 1). Surgical anesthetic depth was defined as a score≥3. During the surgical period, anesthetic depth was also evaluated according to voluntary movement or reflexes in response to incisions.

In each animal, induction time, anesthetic time, immobilization time, and recovery time were evaluated according to reflexes. Induction time was defined as the time elapsed for the anesthetic depth score to reach to 3 after administration. Anesthetic time was defined as the length of time the anesthetic score remained≥3. Immobilization time referred to the intervals between loss and recovery of the righting reflex. Recovery time was defined as the time to recovery of locomotor activity after atipamezole administration or discontinuation of gas supply in each animal.

Vital signs monitoring

Vital signs, including rectal temperature, pulse rate, respiratory rate, and oxygen saturation (SpO_2) were monitored before and after anesthesia. Rectal temperature was measured by inserting a thermal probe (Right Temp, Kent Scientific Corporation) into the colorectum. pulse rate and SpO_2 were measured using a rodent vital sign monitor (PhysioSuite, Kent Scientific Corporation)

by attaching pulse oximeter to hind limb of the animals. Respiratory rate was defined as the number of thoracic movements/min. During anesthesia, the rats were positioned on a nylon pad to provide consistent temperature underneath. Whole vital signs were measured before induction and every 5 min for 30 min.

Statistical analysis

Data are expressed as mean ± SD. One-way analysis of variance (ANOVA) was performed to compare each parameter in each anesthetic group. Following the ANOVA, multiple comparisons were performed using the Tukey-Kramer test. Following repeated measures ANOVA, the Dunnet *t* test was used to compare baseline values with those measured at other time points; $P<0.05$ was considered to be statistically significant. All analyses were performed using commercially available statistical software (Stat Mate IV; ATMS 160 Co., Ltd., Tokyo, Japan).

Results

In the present study, no animals experienced intraoperative death, and all rats survived for at least 24 h after surgery.

Anesthetic depth and times

The anesthetic depth score in all rats in every anesthetic group reached 3. As a result, in isoflurane, sevoflurane, and both of K/X groups, every rat showed enough anesthetic depth during entire surgical period. However, in the M/M/B group at standard-dose, 1 rat exhibited insufficient anesthetic depth during laparotomy; nevertheless, castration was completed after additional administration of anesthetic.

Induction, anesthetic, immobilization, and recovery times are summarized in Table 2. There was no significant difference in induction time among the groups. Mean anesthetic time in every anesthetic group was approximately 25 min, and castration was completed within the time. One rat that received M/M/B at high-dose exhibited delayed awakening from anesthesia (recovery time, 54 min). As a result, the mean immobilization and recovery times in the high-dose M/M/B group were longer than those in the other 5 anesthetic groups.

Vital signs

Differences in baseline values of vital signs of each

Table 2. Induction, anesthetic, immobilization time, and recovery times in each anesthesia

Anesthesia	Induction time	Anesthetic time	Immobilization time	Recovery time
K/X (80/10 mg/kg)	5.8 ± 3.0	25.5 ± 3.4	34.8 ± 3.6	4.2 ± 2.0
K/X (100/10 mg/kg)	3.8 ± 1.1	26.9 ± 3.8	31.6 ± 9.2	5.4 ± 2.4
M/M/B (0.15/2.0/2.5 mg/kg)	4.9 ± 2.0	28.3 ± 3.4	39.5 ± 9.0	11.1 ± 9.3
M/M/B (0.23/3.0/3.75 mg/kg)	4.6 ± 1.7	27.3 ± 2.5	68.5 ± 42.7*	28.4 ± 19.9*
Isoflurane (1.5 MAC)	6.5 ± 1.6	29.0 ± 1.1	29.0 ± 1.1	1.2 ± 0.6
Sevoflurane (1.5 MAC)	4.7 ± 1.5	28.4 ± 0.4	28.4 ± 0.4	1.7 ± 0.4

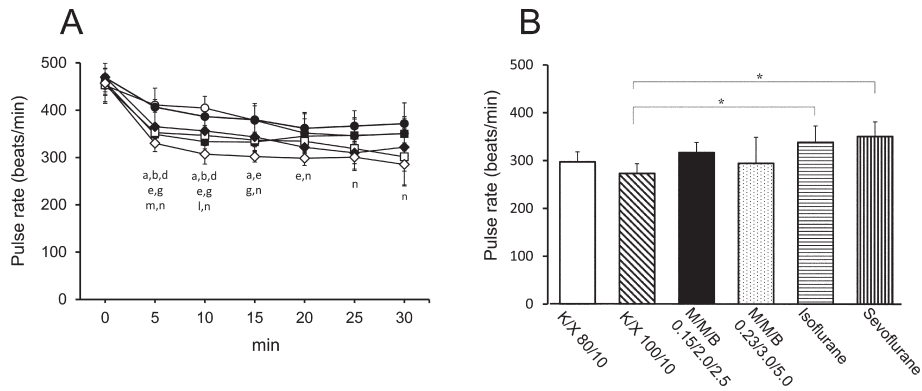*significant difference with other 5 groups.
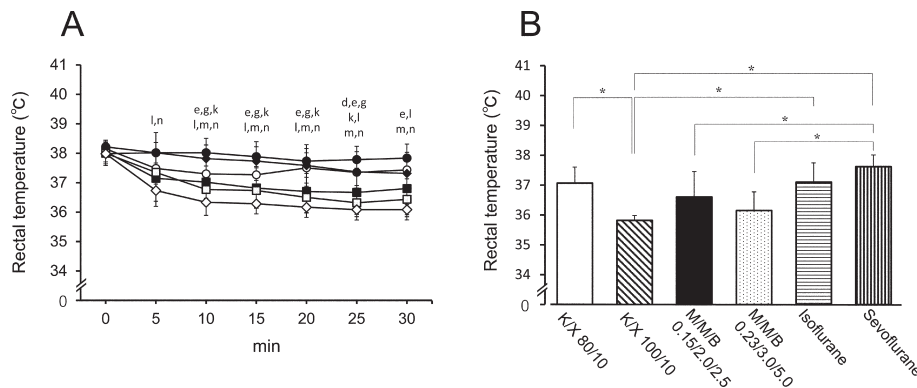
Fig. 1. Rectal temperature under each type of anesthesia. Black diamond: K/X at standard-dose (80/10 mg/kg). White diamond: K/X at high-dose (100/10 mg/kg). Black square: M/M/B at standard-dose (0.15/2.0/2.5 mg/g). White square: M/M/B at high-dose (0.23/3.0/3.75 mg/kg). White circle: Isoflurane at 1.5 MAC. Black circle: Sevoflurane at 1.5 MAC. A: time course of rectal temperature in each group. B: minimum value of rectal temperature in study period in each group. *$P<0.05$. Anesthetics were administered at 0 min. d: Isoflurane − M/M/B at high-dose. e: Isoflurane − K/X at high-dose. g: M/M/B at standard-dose − Sevoflurane. k: K/X at standard-dose − M/M/B at high-dose. l: K/X at standard-dose − K/X at high-dose. m: Sevoflurane − M/M/B at high-dose. n: Sevoflurane − K/X at high-dose.

group were not statistically significant. The time course and minimum value of each vital sign in the 6 anesthetic groups are shown in Figs. 1–4. In the M/M/B group at standard-dose and the K/X group at high-dose, significant decreases in rectal temperature were observed 5 min after anesthesia administration. In the high-dose M/M/B, standard-dose K/X, and isoflurane groups, significant decreases in rectal temperature were observed 10 min after anesthesia administration. In contrast, the sevoflurane group did not exhibit a decrease in rectal temperature during the entire anesthetic period. In the high-dose K/X group, minimum value of rectal temperature was lower than that in the isoflurane, sevoflurane, and standard-dose K/X groups (Fig. 1). Decreases in pulse rate were observed during the entire anesthetic period in every group (Fig. 2). Inhalant anesthesia with isoflurane and sevoflurane led to higher pulse rate values compared with high-dose K/X. In every anesthesia, a significant decrease in respiratory rate was observed in

all anesthesia groups from 5 min after administration (Fig. 3). Remarkably, the minimum values of respiratory rate in the inhalant anesthesias were higher than that in injectable anesthesias. Similar to respiratory rate, a significant decrease in SpO$_2$ was observed in every anesthetic group 5 min after administration, and minimum value of SpO$_2$ in the inhalant anesthesias were significantly higher than those for the injectable anesthesias (Fig. 4). Compared with M/M/B at standard-dose, M/M/B at high-dose resulted in significantly lower SpO$_2$ values at 25 min.

Discussion

When performing surgery, the required anesthetic depth varies and is dependent on the procedure. In the present study, the optimal dose and pharmacological features of 4 anesthetics were evaluated in castration, which is one of several standard operative procedures

Fig. 2. Pulse rate under each type of anesthesia. Black diamond: K/X at standard-dose (80/10 mg/kg). White diamond: Ketamine at high-dose (100/10 mg/kg). Black square: M/M/B at standard-dose (0.15/2.0/2.5 mg/g). White square: M/M/B at high-dose (0.23/3.0/3.75 mg/kg). White circle: Isoflurane at 1.5 MAC. Black circle: Sevoflurane at 1.5 MAC. A: time course of pulse rate in each group. B: minimum value of pulse rate in study period in each group. *$P<0.05$. Anesthetics were administered at 0 min. a-o: a significant differences at $P<0.05$. a: Isoflurane − M/M/B at standard-dose. b: Isoflurane − K/X at standard-dose. d: Isoflurane − M/M/B at high-dose. e: Isoflurane − K/X at high-dose. g: M/M/B at standard-dose − Sevoflurane. l: K/X at standard dose − K/X at high-dose. m: Sevoflurane − M/M/B at high-dose. n: Sevoflurane − K/X at high-dose.
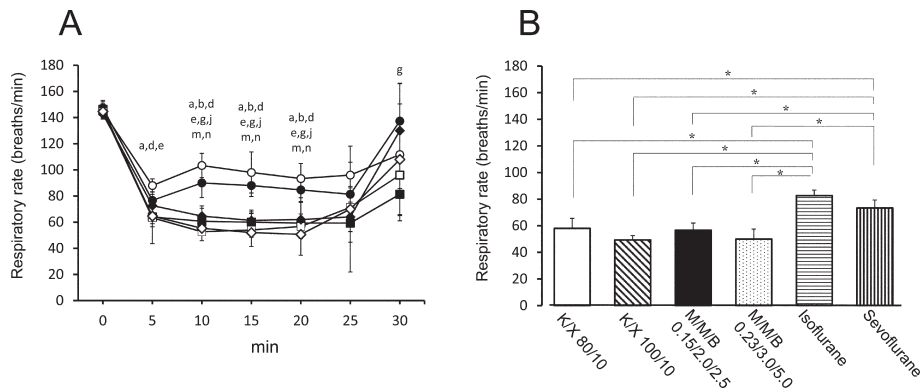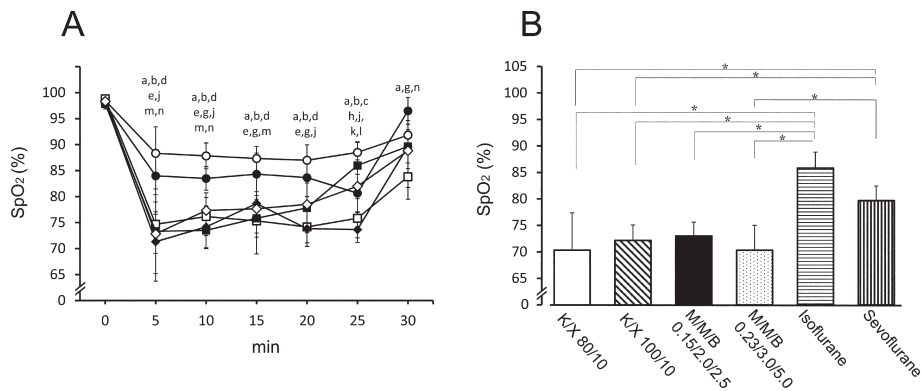

Fig. 3. Respiratory rate under each type of anesthesia. Black diamond: K/X at standard-dose (80/10 mg/kg). White diamond: Ketamine at high-dose (100/10 mg/kg). Black square: M/M/B at standard-dose (0.15/2.0/2.5 mg/g). White square: M/M/B at high-dose (0.23/3.0/3.75 mg/kg). White circle: Isoflurane at 1.5 MAC. Black circle: Sevoflurane at 1.5 MAC. A: time course of respiratory rate in each group. B: minimum value of respiratory rate in study period in each group. *$P<0.05$. Anesthetics were administered at 0 min. a-o: a significant differences at $P<0.05$. a: Isoflurane − M/M/B at standard-dose. b: Isoflurane − K/X at standard-dose. d: Isoflurane − M/M/B at high-dose. e: Isoflurane − K/X at high-dose. g: M/M/B − standard-dose − Sevoflurane. j: K/X at standard-dose − Sevoflurane. m: Sevoflurane − M/M/B at high-dose. n: Sevoflurane − K/X at high-dose.

performed in rats.

Ketamine is a representative anesthetic agent used in rodents. This drug is classified as a dissociative anesthetic agent, and does not provide deep anesthesia as a single agent [7]. Therefore, ketamine is usually administered concurrently with sedatives such as xylazine, medetomidine, and diazepam [3, 7]. In this study, the

most widely used combination of ketamine and xylazine was evaluated. The recommended dose of K/X in rats is 80–100 mg/kg and 10 mg/kg, respectively [7]. In the present study, we evaluated the effectiveness of K/X under surgical conditions at doses of 80/10 mg/kg and 100/10 mg/kg; sufficient surgical aesthetic depth was obtained using both doses. In addition, early awakening

Fig. 4. SpO$_2$ under each type of anesthesia. Black diamond: K/X at standard-dose (80/10 mg/kg). White diamond: Ketamine at high-dose (100/10 mg/kg). Black square: M/M/B at standard-dose (0.15/2.0/2.5 mg/g). White square: M/M/B at high-dose (0.23/3.0/3.75 mg/kg). White circle: Isoflurane at 1.5 MAC. Black circle: Sevoflurane at 1.5 MAC. A: time course of respiratory rate in each group. B: minimum value of respiratory rate in study period in each group. *P<0.05. Anesthetics were administered at 0 min. a-o: a significant differences at P<0.05. a: Isoflurane − M/M/B at standard-dose. b: Isoflurane − K/X at standard-dose. c: Isoflurane − Sevoflurane. d: Isoflurane − M/M/B at high-dose. e: Isoflurane − K/X at high-dose. g: M/M/B at standard-dose − Sevoflurane. h: M/M/B at standard-dose − M/M/B at high-dose. j: K/X at standard-dose − Sevoflurane. k: K/X at standard-dose − M/M/B at high-dose. l: K/X at standard-dose − K/X at high-dose. m: Sevoflurane − M/M/B at high-dose. n: Sevoflurane vs K/X at high-dose.

was achieved in rats, possibly by administration of the α$_2$ receptor antagonist atipamezole, which is antagonized by the α$_2$ receptor agonist, xylazine. However, K/X at high dose caused remarkable hypothermia and bradycardia during anesthesia. In a previous non-operative study, rats anesthetized with K/X (100/10 mg/kg), experienced cardiorespiratory depression [23]. Compared with previous non-operative studies, the bradycardia observed in the present investigation was more severe, suggesting that surgical invasion or bleeding affects pulse rate. From the current and previous findings, it is recommended that the dose of K/X is adjusted to 80/10 mg/kg for castration in rats, based on both anesthetic depth and safety.

Along with K/X, we investigated the anesthetic action of M/M/B, which is a major injectable anesthetic combination in Japan. The reported dose of medetomidine, midazolam, and butorphanol in rat anesthesia is 0.15, 2.0, and 2.5 mg/kg, respectively [11]. In the present study, we evaluated the standard-dose (0.15/2.0/2.5 mg/kg) and 1.5 times × high-dose (0.23/3/3.75 mg/kg) in mature rats. As a result, 1 rat administered at standard-dose exhibited insufficient anesthetic depth during castration, which was addressed by additional administration of the agent. In addition, another rat showed delay in awakening, despite atipamezole administration, sug-

gesting that the anesthetic sensitivity of M/M/B varies dramatically in individual animals. This difference appears to be related to the influence of drug metabolism. When M/M/B is intraperitoneally administered and absorbed into circulating blood via the portal vein, its anesthetic action is strongly influenced by a fast-pass effect because medetomidine and midazolam are primarily metabolized in the liver [1, 14]. The absorption rate via the portal vein varies depending on the site of intraperitoneal administration, and this difference may cause variation in anesthetic action. In the present study, M/M/B caused remarkable cardiorespiratory depression, which is similar to that in non-surgical conditions [11]. The level of cardiac and respiratory depression was comparable with that in K/X anesthesia. The decrease of rectal temperature in standard-dose group seems faster than that of high-dose group. This is due to the fact that one rat at standard-dose group showed remarkable hypothermia (35.8°C) at 5 min. Collectively, M/M/B is highly adaptable to castration procedures, although it appears necessary to devote attention to cardiorespiratory depression and delay in awakening. In the case of insufficient anesthetic depth, 25% additional administration of M/M/B will achieve sufficient surgical anesthetic depth for castration.

The concentration of inhalant anesthetics is usually

determined by multiplying MAC by the coefficient corresponding to the surgical procedure [2]. In the present study, the concentration of isoflurane during maintenance was set to 2.1%, which corresponds to 1.5 MAC [4]. As a result, sufficient surgical anesthesia depth was achieved during surgery. Compared with the injectable anesthesias with M/M/B and K/X, inhalant anesthesia with isoflurane resulted in stable pulse rates, which is due to its lower influence on cardiac function [12]. In addition, respiratory rate and SpO$_2$ were higher than that for injectable anesthesia. However, in our previous study, respiratory rate decrease was prominent in isoflurane compared with K/X and M/M/B in mice [20]. The influence of isoflurane on respiratory function may be different among species. It is concluded that the adjustment of isoflurane concentration to 1.5 MAC provides sufficient anesthetic depth with less cardiorespiratory depression, and is recommend for surgical anesthesia for castration procedures in rats.

Sevoflurane is a representative inhalant anesthetic in human and veterinary medicine [16, 18]. It has a lower blood/gas partition coefficient compared with isoflurane, leading to rapid induction and recovery [13, 16]. In human medicine, sevoflurane is commonly used for pediatric anesthesia due to its low irritation [8]. Following these studies, we have recently demonstrated the utility of sevoflurane in 10-day neonatal rats under non-surgical conditions [19]. In the report, isoflurane anesthesia caused a delay in induction, and 25% of the animals died during anesthesia, while sevoflurane anesthesia yielded rapid induction and sufficient anesthetic depth without anesthetic death. In the present study, sufficient surgical anesthetic depth was achieved using sevoflurane at 1.5 MAC, with less cardiorespiratory depression in mature rats. The anesthetic action of sevoflurane was similar to that of isoflurane, and no significant difference in induction time was observed in both inhalant anesthesias. Compared with neonates, the advantage of sevoflurane over isoflurane appears to be diminished in mature rats. However, because sevoflurane has less influence on body temperature, it may be suitable for longer duration anesthesia, which demonstrates time-dependent hypothermia.

To perform surgical anesthesia in individual cases, it is necessary to adjust the dose and concentration properly by considering the invasiveness of the procedure. In this study, the action of 4 anesthetics during a castration procedure in rats was assessed, and all were adaptable to the surgical procedure. The recommended dose of K/X during castration is 80/10 mg/kg. Although M/M/B at both doses is adaptable to castration, its variation in anesthetic sensitivity should be considered. When performing castration, 1.5 MAC is an appropriate concentration for isoflurane and sevoflurane, which provides sufficient anesthetic depth with less cardiorespiratory depression. The extent of side effects varies depending on the anesthetic, but there are individual differences even with the same anesthesia method. Therefore, it is important to monitor vital signs during surgical anesthesia. In the present study, decrease of SpO$_2$ was observed in every anesthetic method, particularly in injectable anesthesias, indicating that it is necessary to pay attention to hypoxia during surgical anesthesia. The oxygen inhalation through mask will be effective for preventing the hypoxia induced by anesthesia and surgical invasion. Castration is a standard-surgical procedure in rodents, causing moderate surgical invasion to animals. Therefore, present result can be an indication for selecting anesthetsia in other standard surgical procedures. Strictly speaking, the required dose of anesthetic is dependent on the type of surgical procedure. Given the results of the present investigation, further studies assessing the effectiveness of anesthetic protocols in other surgical procedures are warranted.

Acknowledgment

References

1. Amrein, R. and Hetzel, W. 1990. Pharmacology of Dormicum (midazolam) and Anexate (flumazenil). *Acta Anaesthesiol. Scand. Suppl.* 92: 6–15, discussion 47. [Medline] [CrossRef]

2. Aranake, A., Mashour, G.A., and Avidan, M.S. 2013. Minimum alveolar concentration: ongoing relevance and clinical utility. *Anaesthesia* 68: 512–522. [Medline] [CrossRef]

3. Buitrago, S., Martin, T.E., Tetens-Woodring, J., Belicha-Villanueva, A., and Wilding, G.E. 2008. Safety and efficacy of various combinations of injectable anesthetics in BALB/c mice. *J. Am. Assoc. Lab. Anim. Sci.* 47: 11–17. [Medline]

4. de Wolff, M.H., Leather, H.A., and Wouters, P.F. 1999. Effects of tramadol on minimum alveolar concentration (MAC) of isoflurane in rats. *Br. J. Anaesth.* 83: 780–783. [Medline] [CrossRef]

5. Diven, K. 2003. Inhalation anesthetics in rodents. *Lab Anim. (NY)* 32: 44–47. [Medline] [CrossRef]

6. Dodelet-Devillers, A., Zullian, C., Vachon, P., and Beaudry,

F. 2016. Assessment of stability of ketamine-xylazine preparations with or without acepromazine using high performance liquid chromatography-mass spectrometry. *Can. J. Vet. Res.* 80: 86–89. [Medline]

7. Fish, R.E. 2008. Anesthesia and Analgesia in Laboratory Animals. 2nd ed. *ACLAM*, Academic press, London.

8. Hobbhahn, J. and Funk, W. 1996. [Sevoflurane in pediatric anesthesia]. *Anaesthesist* 45:(Suppl 1): S22–S27. (in German) [Medline]

9. Kashimoto, S., Furuya, A., Nonaka, A., Oguchi, T., Koshimizu, M., and Kumazawa, T. 1997. The minimum alveolar concentration of sevoflurane in rats. *Eur. J. Anaesthesiol.* 14: 359–361. [Medline] [CrossRef]

10. Kawai, S., Takagi, Y., Kaneko, S., and Kurosawa, T. 2011. Effect of three types of mixed anesthetic agents alternate to ketamine in mice. *Exp. Anim.* 60: 481–487. [Medline] [CrossRef]

11. Kirihara, Y., Takechi, M., Kurosaki, K., Kobayashi, Y., Saito, Y., and Takeuchi, T. 2016. Effects of an anesthetic mixture of medetomidine, midazolam, and butorphanol in rats-strain difference and antagonism by atipamezole. *Exp. Anim.* 65: 27–36. [Medline] [CrossRef]

12. Murakami, M., Niwa, H., Kushikata, T., Watanabe, H., Hirota, K., Ono, K., and Ohba, T. 2014. Inhalation anesthesia is preferable for recording rat cardiac function using an electrocardiogram. *Biol. Pharm. Bull.* 37: 834–839. [Medline] [CrossRef]

13. Robinson, B.J., Uhrich, T.D., and Ebert, T.J. 1999. A review of recovery from sevoflurane anaesthesia: comparisons with isoflurane and propofol including meta-analysis. *Acta Anaesthesiol. Scand.* 43: 185–190. [Medline] [CrossRef]

14. Salonen, J.S. 1989. Pharmacokinetics of medetomidine. *Acta Vet. Scand. Suppl.* 85: 49–54. [Medline]

15. Shekarforoush, S., Fatahi, Z., and Safari, F. 2016. The effects of pentobarbital, ketamine-pentobarbital and ketamine-xylazine anesthesia in a rat myocardial ischemic reperfusion injury model. *Lab. Anim.* 50: 179–184. [Medline] [CrossRef]

16. Smith, I., Nathanson, M., and White, P.F. 1996. Sevoflurane-a long-awaited volatile anaesthetic. *Br. J. Anaesth.* 76: 435–445. [Medline] [CrossRef]

17. Sonner, J.M., Gong, D., and Eger, E.I. 2nd. 2000. Naturally occurring variability in anesthetic potency among inbred mouse strains. *Anesth. Analg.* 91: 720–726. [Medline] [CrossRef]

18. Steffey, E.P. 2002. Recent advances in inhalation anesthesia. *Vet. Clin. North Am. Equine Pract.* 18: 159–168. [Medline] [CrossRef]

19. Tsukamoto, A., Konishi, Y., Kawakami, T., Koibuchi, C., Sato, R., Kanai, E., and Inomata, T. 2017. Pharmacological properties of various anesthetic protocols in 10-day-old neonatal rats. *Exp. Anim.* 66: 397–404. [Medline] [CrossRef]

20. Tsukamoto, A., Serizawa, K., Sato, R., Yamazaki, J., and Inomata, T. 2015. Vital signs monitoring during injectable and inhalant anesthesia in mice. *Exp. Anim.* 64: 57–64. [Medline] [CrossRef]

21. 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]

22. Veilleux-Lemieux, D., Castel, A., Carrier, D., Beaudry, F., and Vachon, P. 2013. Pharmacokinetics of ketamine and xylazine in young and old Sprague-Dawley rats. *J. Am. Assoc. Lab. Anim. Sci.* 52: 567–570. [Medline]

23. Wellington, D., Mikaelian, I., and Singer, L. 2013. Comparison of ketamine-xylazine and ketamine-dexmedetomidine anesthesia and intraperitoneal tolerance in rats. *J. Am. Assoc. Lab. Anim. Sci.* 52: 481–487. [Medline]