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Intravenous propofol, ketamine (ketofol) and rocuronium after sevoflurane induction provides long lasting anesthesia in ventilated rats

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Abstract: Rats are commonly used animals for laboratory experiments and many experiments require general anesthesia. However, the lack of published and reproducible intravenous anesthesia protocols for rats results in unnecessary animal use to establish new anesthesia techniques across institutions. We therefore developed an anesthesia protocol with propofol, ketamine, and rocuronium for mechanically ventilated rats, and evaluated vital parameters and plasma concentrations. 15 male Sprague-Dawley rats underwent inhalation induction with sevoflurane and tracheal, venous and arterial cannulation. After established venous access, sevoflurane was substituted by propofol and ketamine (ketofol). Rocuronium was added under mechanical ventilation for 7 h. Drug dosages were stepwise reduced to prevent accumulation. All animals survived the observation period and showed adequate depth of anesthesia. Mean arterial pressure and heart rate remained within normal ranges. Median propofol plasma concentrations remained stable: 1, 4, 7 h: 2.0 (interquartile range (IQR): 1.8–2.2), 2.1 (1.8–2.2), 1.8 (1.6–2.1) μg/ml, whereas median ketamine concentrations slightly differed after 7 h compared to 1 h: 1, 4, 7 h: 3.7 (IQR: 3.5–4.5), 3.8 (3.3–4.1), 3.8 (3.0–4.1) μg/ml. Median rocuronium plasma concentrations were lower after 4 and 7 h compared to 1 h: 1, 4, 7 h: 3.9 (IQR: 3.5–4.9), 3.2 (2.7–3.3), 3.0 (2.4–3.4) μg/ml. Our anesthesia protocol provides stable and reliable anesthesia in mechanically ventilated rats for several hours.
Key words: anesthetics plasma concentration, rat anesthesia techniques, rat inhalation anesthesia, rat long time anesthesia, rat ventilation

Introduction

Rats are a commonly used animals for laboratory experiments and provide suitable models for physiology, toxicology, behavior, or nutrition [1]. Many rats are used in experimental surgery, therefore it is imperative that they all receive adequate anesthesia and analgesia to follow the ‘refinement’ principle of the 3R [2, 3]. Anesthesia should provide low side effects for reproducibility, validity, and stable experimental conditions [4]. Several procedures have been used in rodents [5–9]. But drugs are often applied by intraperitoneal injection with certain disadvantages like increased stress for the animal, uncertain needle placement, and inconstant plasma concentrations [10, 11].

Inhalation induction provides safe and painless delivery of anesthesia. Sevoflurane is suitable for inhalation induction, as it has a rapid onset of anesthesia and causes less airway irritation than other volatile anesthetics [6, 12, 13]. Nevertheless, isoflurane is commonly used due

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to low costs and broad experiences [6, 14]. With the increasing availability of volatile anesthesia in rodents and apparent benefits compared to injectable anesthetics [15, 16], there is an immediate need to describe methods providing safe and reliable use. However, conventional small animal ventilators can lead to high consumptions of volatile anesthetics and room air pollution when appropriate scavenger systems are missing [17, 18]. Therefore, maintenance of anesthesia over several hours using volatile anesthetics may be inconvenient and results in unnecessary prolonged exposure of staff. A combination of inhalation induction with intravenous maintenance reduces these drawbacks of current anesthesia in rodents and may improve analgesia and anesthesia.

Ketamine is a potent analgesic drug with an increasing anesthetic effect at higher doses and is frequently used in veterinary medicine [19]. Due to its psychotropic and cataleptic side effects, it should be combined with sedatives. Previous observations indicate that combinations of propofol with analgesics enhance the stability and safety of anesthesia in rats [20]. As propofol attenuates and ketamine improves cardiovascular function, we assumed them to be a reasonable combination for providing sufficient spontaneous breathing as well as sedation and anesthesia [9, 21, 22].

In general, neuromuscular blocking is not necessary for mechanical ventilation [9]. However, anesthetic drug doses needed for a total depression of spontaneous breathing may cause severe hemodynamic instability [21, 23]. We therefore decided to use neuromuscular blocking to avoid spontaneous breathing efforts under mechanical ventilation, thereby accepting the trade-off that neuromuscular blocking increases the risk of undetected awareness because monitoring of anesthesia depth becomes more challenging [9, 24]. We used rocuronium, a non-depolarizing agent with a rapid onset, reversibility and low effects on hemodynamics which recommends it not only in veterinary but also in human medicine [25, 26].

Hardly any data is available for a safe, reliable, and stable procedure of intravenous anesthesia in ventilated rats. We therefore established and described an anesthesia procedure using sevoflurane, propofol, ketamine, and rocuronium for mechanically ventilated rats, and evaluated the stability of physiological variables and drug plasma concentrations.

Materials and Methods

The anesthesia procedure was validated in animals being part of a study that investigated ventilator-induced lung injury (Approval No. 45/2016 Landesamt für Soziales, Saarbrücken, Germany). The study protocol was approved and in accordance with the German Animal Welfare Act. To exclude potential influence of hormone status, only male animals were used.

Animals and housing

Fifteen male Sprague-Dawley rats (*Rattus norvegicus*) were randomly selected from the investigation groups of 8 and 12 ml/kg tidal volume using a random number generator (Excel 2010, Microsoft, Redmond, WA, USA) and drug plasma concentrations were measured in blood samples that were part of the primary investigation [27]. The animals were obtained from Charles River Laboratories (Sprague-Dawley IGS Rat (Crl:CD (SD)) Strain 001, Charles River Laboratories, Research Models and Services, Germany GmbH, Sulzfeld, Germany) weighing 328 ± 24 g (mean ± SD) with an age of 8 to 10 weeks at investigation time. The barrier of origin was free of the pathogens listed in the FELASA recommendations for the health monitoring of rats [28]. They were housed in polycarbonate group cages with 4 animals each (Type Eurostandard IV, Tecniplast, Buguggiate, Italy) in our institutional animal facility under controlled conditions with temperature 20 ± 2°C and 50 ± 5% relative humidity at a regular 12:12 h light-dark cycle. Tapvei aspen bedding and aspen wool (Tapvei Estonia OÜ, Harjumaa, Estonia) were used as bedding and nesting material. With absence of poor welfare signs (e.g., wounds, secretion of harderian gland, signs of dehydration, diarrhea, isolation from others, itching) which were monitored several times a day, the animals were ensured to be fit for use.

Rats had free access to water and standard pellet food (No. 1328, Altromin, Lage, Germany) ad libitum. Food was withheld 12 h before the experiments. The investigations were performed in the laboratory unit of the Department of Anesthesiology, Intensive Care and Pain Therapy in Homburg, Germany, and started between 8 and 9 AM. The rats were housed close to the location of the experiments for at least one week after delivery from Charles River for acclimation. They were transported to the experimental location no later than 24 h prior to the procedure.

Anesthesia, drug administration and preparation

Step 1. Inhalative Induction and central venous catheter

Anesthesia was induced with 5 vol% sevoflurane in medical oxygen (Sevorane, AbbVie, Wiesbaden, Germany). Oxygen was delivered with 1 l/min by a flow controller (Mediselect II, GCE Group AB, Malmö, Sweden) passing a sevoflurane vaporizer (DRÄGER Vapor 19.3 Sevoflurane, Lübeck, Germany) into a 9-liter box.

After 10 min of equilibration, animals were moved into the box with an onset of anesthesia after 30–60 s. The anesthetized but spontaneously breathing animal was placed supine on a warmed plate (details below). Sevoflurane was administered with 3 vol% in oxygen with 1 l/min flow over a mask (Fluovac Anesthetizing System, Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany), which included an anesthetic gas scavenging system (MiniVacTyp 812 & Veterinary Fluosorber, Hugo Sachs Elektronik-Harvard Apparatus). Sevoflurane was titrated within a range of 2.5–3.5 vol% to maintain adequate depth of anesthesia (see below, monitoring). Body temperature was monitored by a rectal probe and kept at 38 ± 1°C; therefore, a heat mat (Terra Plus Comfort Heat Mat, Terra Exotica, Alfeld, Germany) was placed under a stainless-steel plate. The mat was coupled with a thermostat (UT300 Universal Thermostat, Renkforce, Hirschau, Germany) that regulated it to 40 ± 0.5°C. After surgical preparation and catheterization of the right jugular vein with a polythene catheter (Fine-Bore Polythene Tubing, ID 0.58 mm OD 0.96 mm, Smith Medical ASD, Keene, NH, USA), we connected a 0.7 mm / 22 G injection needle (Microlance™, BD, Heidelberg, Germany) to the catheter and a standard 3-way tap.

Step 2. Intravenous anesthesia

Syringes 1+2 (Table 1) were connected to the central venous catheter. Syringe pump 2 was started with an admission rate of 30 mg/kg/h for propofol and ketamine (Propofol 2%, Fresenius, Bad Homburg, Germany; Ketamine 100 mg/2 ml, Rotexmedica, Luitré, France). Another syringe pump with syringe 1 containing balanced electrolyte solution was started and adjusted to reach a total fluid intake of 10 ml/kg/h.

Sevoflurane was reduced to 2.5 vol% with infusion the start of intravenous anesthetics. When anesthetics reached the vein, it was reduced to 1.5 vol%. Two minutes thereafter, sevoflurane was stopped.

Step 3. Tracheotomy and arterial access

Under intravenous anesthesia with ketofol, a tracheotomy was performed via midline neck incision and introduction of a steel cannula (tracheal cannula, Luer Lock, OD 2.0 mm, length 13 mm, Hugo Sachs Elektronik Harvard Apparatus). The left internal carotid artery was also catheterized after surgical preparation with a polythene catheter (Fine-Bore Polythene Tubing, ID 0.58 mm OD 0.96 mm, Smith Medical ASD) and connected to a pressure transducer (PMSET 1DT-XX 1 ROSE, Beckton Dickinson, Franklin Lakes, NJ, USA).

Step 4. Neuromuscular blockade and ventilation start

Neuromuscular blockade was induced by intravenous injection of 10 mg/kg rocuronium prior to mechanical ventilation (Esmeron, N.V. Organon, Oss, Netherlands). Then, syringe 2 containing propofol and ketamine was replaced by syringe 3 containing propofol, ketamine, and rocuronium according to the anesthesia protocol in

Table 1. Anesthesia protocol: content of the syringes and drug dosing during the experiment

Syringe contents			
	Syringe 1:	Syringe 2:	Syringe 3:
	Balanced electrolyte solution	13 ml balanced electrolyte solution 5 ml Propofol 20 mg/ml 2 ml Ketamine 50 mg/ml	8 ml balanced electrolyte solution 5 ml Propofol 20 mg/ml 2 ml Ketamine 50 mg/ml 5 ml Rocuronium 10 mg/ml
Resulting concentration of the drugs		Propofol: 5 mg/ml Ketamine: 5 mg/ml	Propofol: 5 mg/ml Ketamine: 5 mg/ml Rocuronium: 2.5 mg/ml
	Total = 20 ml	Total = 20 ml	Total = 20 ml
Infusion protocol			
During preparation	Mixed in Syringe 2 Propofol 30 mg/kg/h	 Ketamine 30 mg/kg/h	
During ventilation	Mixed in Syringe 3 Propofol 25 mg/kg/h	 Ketamine 25 mg/kg/h	 Rocuronium 12.5 mg/kg/h
Adaptation of infusion rate	Reduction of 0.1 ml/h once every hour for Syringe 2 or 3 Raise of 0.1 ml/h once every hour for Syringe 1 (no weight adaptation)		
Minimal drug dosage	Propofol 15 mg/kg/h	Ketamine 15 mg/kg/h	Rocuronium 7.5 mg/kg/h

Table 1. The initial dosage during mechanical ventilation was 25 mg/kg/h of both propofol and ketamine and 12.5 mg/kg/h of rocuronium. The mechanical ventilation was started immediately after neuromuscular blockade.

Step 5. Adaptation of drug administration over time

The application rate of the anesthetic drugs in syringe 3 was reduced hourly by 0.1 ml/h and fluid administration by syringe 1 was consecutively increased by 0.1 ml/h. This resulted in an hourly reduction of 1.67 mg/kg/h for both propofol and ketamine and 0.84 mg/kg/h for rocuronium in an animal weighing 300 g (Table 2). With reaching a rate of 15 mg/kg/h of propofol/ketamine and 7.5 mg/kg/h of rocuronium, reduction was stopped (Table 1 and 2). Rats were sacrificed by an anesthetic drug overdose of propofol 7 h after the start of ventilation.

Monitoring

Anesthesia depth was monitored by respiratory rate and the hind limb withdrawal reflex. Sudden increase of respiratory rate after stimulation or a positive hind limb withdrawal reflex were interpreted as inadequate anesthesia depth for surgery based on criteria of Wixson *et al.* and Flecknell *et al.* [9, 29]. With absence of withdrawal responses, the surgical procedures were started. Anesthesia depth under neuromuscular blockade was monitored by blood pressure and heart rate; rapid increases (10% of baseline) induced by tail squeezes or hind limb stimulation were defined as signs of light anesthesia and were checked every 15 min. Based on available data, we defined a normal mean arterial pressure (MAP) with 110 ± 20 mmHg and a normal heart rate with 350–400 bpm [9, 30–33].

Breathing efforts without clinical signs of awareness (identified by alterations in the pressure/flow curves and by additional chest movements) were defined as an insufficient neuromuscular blockade.

After an anesthetic drug overdose at the end of the experiment, death was confirmed by observation of heart rate and blood pressure to ensure cardiac arrest.

Mechanical ventilation

Rats were ventilated with a tidal volume of 8–12 ml/kg and 63–42 breaths per min, adjusted to maintain a minute volume of 500 ml/kg (KTR-5 small animal ventilator, Hugo Sachs Elektronik Harvard Apparatus) to maintain normocarbia and normoxia. Inspired oxygen fraction was 0.5, and positive end-expiratory pressure (PEEP) 2 mbar. Inspired gases were delivered by pressure controllers (Swagelok, Solon, OH, USA) mixing nitrogen (gas generator, Genius NM32LA, Peak Scientific Instruments, Inchinnan, UK) and medical oxygen to adjust the inspired oxygen fraction monitored by an oximeter (GMH3695-GE / GOG-SET-H-GE, GHM Messtechnik, Regenstauf, Germany).

Ventilator setting (minute volume, peak expiratory pressure, respiratory frequency) and physiologic parameters were monitored with a computer system via PowerLab 8/35 device (ADInstruments, Dunedin, New Zealand) and collected by the LabChart 8.1 (ADInstruments). The system included a spirometer (FE 141 & MLT 1L), a temperature pod (ML309, ADInstruments), and a signal amplifier (Bio-Ampli ALF 24404-3, Alfos Elektronik, Biel-Benken, Germany).

Blood samples

All blood samples were taken from the arterial catheter. After discarding of 150 μl, arterial blood samples of 500 μl were taken at 1, 4, and 7 h after ventilation started. All blood tests were conducted from this sample. The blood was immediately transferred with a syringe to EDTA containing Eppendorf tubes for plasma analytics and pre-heparinized capillary tubes (safeCLINITUBES, Radiometer GmbH, Krefeld, Germany) for blood gas analysis (BGA). Plasma concentrations of propofol, ketamine, and rocuronium were measured by high-performance liquid chromatography coupled with a single mass spectrometer (HPLC-MS) [34]. Details are given in the supplement.

Arterial BGA was performed to monitor ventilation (ABL90 Flex, Radiometer GmbH). There are hardly any definitions of normal BGA parameter ranges in Sprague-Dawley rats. Referring to available data we defined normal ranges for this study (Table 3) [9, 31, 35–38].

Table 2. Example infusion protocol for a rat weighing 300 g

	Balanced electrolyte solution (Syringe 1)	Propofol + Ketamine (Syringe 2)	Propofol + Ketamine + Rocuronium (Syringe 3)
During preparation	1.2 ml/h	1.8 ml/h	Stop
During ventilation	1.5 ml/h	Stop	1.5 ml/h
Hourly dose adaptation	Increase of 0.1 ml/h	Stop	Reduction of 0.1 ml/h (= 1.7 mg/kg/h propofol and ketamine and 0.8 mg/kg/h rocuronium)

Table 3. Blood gas analysis

Parameter	Hours after ventilation start			Defined Normal Range
	1	4	7	
pH	7.42 (7.40–7.44)	7.41 (7.36–7.44)	7.38 (7.32–7.44)	7.35–7.45
Base Excess [mmol/l]	1.1 ((−0.3)–2.3)	–2 ((−3.5)–(−0.2))	–3.1 ((−4.8)–(−1.6))	0 ± 2
Bicarbonate [mmol/l]	25.1 (24.2–26.5)	23 (21.9–24.5)	22.5 (20.8–23.2)	22–28
pO2 [mmHg]	245 (226–262)	251 (223–262)	253 (221–279)	200–300
pCO2 [mmHg]	40 (36–43)	36 (33–40)	37 (34–42)	35–45
Oxygen saturation [%]	99 (99–99)	99 (99–100)	99 (99–100)	98–100
Hemoglobin [g/dl]	14.4 (14–14.9)	13.5 (12.7–14.1)	12.3 (11.4–13.3)	11–16
Lactate [mmol/l]	0.6 (0.5–0.7)	0.6 (0.5–0.6)	0.5 (0.4–0.6)	0–1
Glucose [mg/dl]	91 (83–95)	85 (67–95)	77 (67–99)	60–200
Sodium [mmol/l]	142 (141–143)	144 (143–144)	144 (144–145)	141–150
Potassium [mmol/l]	4.5 (4.3–4.7)	4.3 (4.0–4.5)	4.3 (4.0–4.6)	4.0–6.2
Calcium [mmol/l]	1.17 (1.07–1.32)	1.11 (0.98–1.27)	1.19 (1.08–1.26)	1.05–1.35

Data are given as median and IQR.

Statistics

Data were analyzed using SigmaPlot 12.5 (Systat, San Jose, CA, USA). For propofol plasma concentration, we estimated a difference in means of 0.5 μg/ml being relevant. With an expected standard deviation of 0.5, a power of 0.8 and an alpha of 0.05, the paired *t*-test sample size calculation yielded into 10 animals. We planned 15 animals, because there were only inconsistent data available to calculate sample size with regard to plasma concentration. Data were tested for normality by Shapiro-Wilk testing. Because most data were not normally distributed, results are presented as medians and interquartile ranges. Longitudinal comparisons were performed by one-way repeated measures ANOVA on ranks with multiple comparisons by Dunn's method. First measurements of mean arterial pressure and heart rate were defined as baseline. $P<0.05$ was considered statistically significant.

Results

Anesthesia and physiologic parameters

All animals survived the observation time. A positive hind limb withdrawal reflex before surgery, as a clinical sign of light anesthesia, was successfully treated with an increase of sevoflurane to 3.5 vol% (2 out of 15 animals). When the reflex was absent, surgical stimuli did not induce any movement or increases of respiratory rate, as clinical signs of potential awareness. With the start of intravenous anesthesia, the hind limb withdrawal reflex was always absent. No animal became apneic during spontaneous breathing. Following rocuronium injection, there was a decline in blood pressure with a maximum duration of one minute of approximately 10% from baseline pressure. None of the paralyzed animals showed signs of light anesthesia (e.g., rapid increases in blood pressure or heart rate, noticeable lacrimation), or ineffective neuromuscular blockade (e.g., breathing efforts) during mechanical ventilation. Blood pressure and heart rate slightly decreased over time, but did not fall below normal ranges (Fig. 1). Normothermia (37.5–38.5°C) was successfully maintained throughout the experiments. The total preparation procedure from induction to ventilation was performed in less than 30 min.

Blood gas analysis

The pulmonary gas exchange was sufficient. There was a slight metabolic acidosis after 7 h, but blood pH stayed within normal ranges (Table 3).

Plasma concentrations of drugs used

The overall median plasma concentrations were 1.9 (1.7–2.2) μg/ml propofol, 3.8 (3.4–4.1) μg/ml ketamine and 3.3 (2.8–3.9) μg/ml rocuronium. Propofol plasma concentrations remained stable throughout the experiments. Ketamine plasma concentrations differed sig-

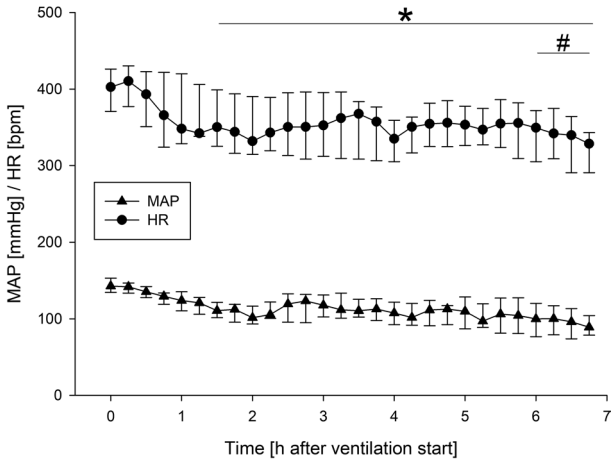

Fig. 1. Heart rate (HR) and mean arterial pressure (MAP) during ventilation time. Data are given as median and IQR. First measurements were defined as baseline. N=15. MAP: *$P<0.05$ vs. baseline; HR: #$P<0.05$ vs. baseline.

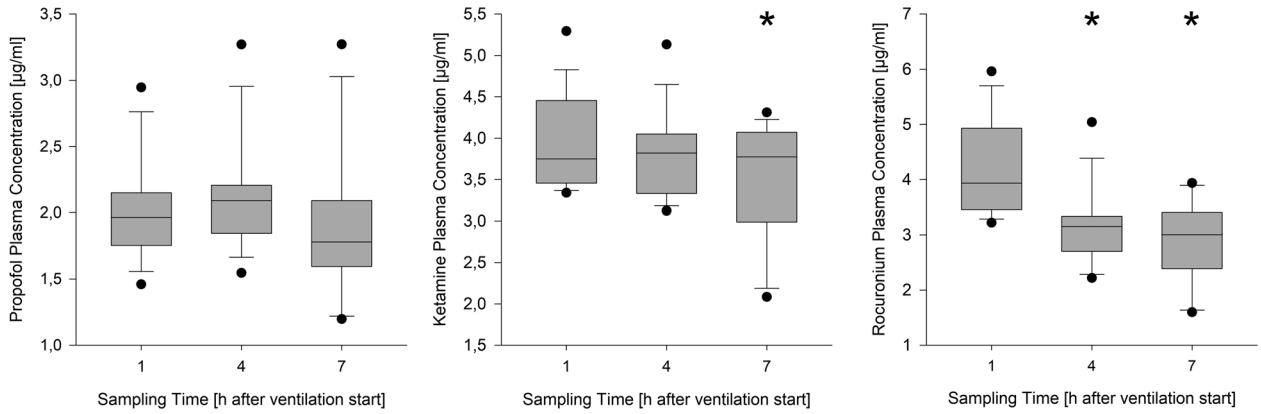

Fig. 2. Plasma concentrations of propofol, ketamine, and rocuronium. N=15, **P*<0.05 vs. 1 h.

nificantly between 1 and 7 h of mechanical ventilation. Rocuronium plasma concentrations were significantly lower after 4 and 7 h compared to 1 h (Fig. 2).

Discussion

Anesthesia management and physiologic parameters

The inhalative induction of anesthesia with sevoflurane avoided stressful events for the animals. Surgical preparation was performed under 2.5–3.5 vol% sevoflurane, which is about 0.9–1.3 times the minimum alveolar concentration (MAC) for rats [39]. This dosage provided good conditions for preparation without signs of awareness or stress (absence of the hind limb withdrawal reflex and lacrimation, stable respiratory rate). The subsequent transition from inhalative to intravenous anesthesia was simple to handle and accomplished in about five minutes.

Rocuronium was injected using the same venous access as used for propofol and ketamine. Thus, a reservoir of anesthetic drugs within the 3-way tab and the catheter, which represents a volume of approximately 0.3 ml, was flushed into the animal. This corresponds to a bolus of about 1 mg of propofol and ketamine. We suspect that this drug bolus impaired cardiovascular function causing the observed short initial decrease in heart rate. Thereafter, heart rate and blood pressure decreased slightly but stayed within normal ranges. The animals were hypertensive during the first hours and declined to normotension. This might be an effect of the initially higher ketamine dose which has been described to increase blood pressure [9, 33, 40]. The decrease of blood pressure and heart rate over time may have resulted from anesthesia and mechanical ventilation which attenuates cardiac performance by intrathoracic pressure variation [9, 41].

We found a sufficient pulmonary gas exchange during mechanical ventilation [9, 29, 35]. Although the base excess decreased over time, animals stayed within normal pH-range which does not seem to be relevant during ventilation time [31, 35].

Animals were fasted for 12 h prior to the procedure due to the underlying breath analysis. We note though that if not explicitly needed for the research question, fasting should better be avoided in rodents in view of the risk of metabolic problems, stress and also the fact that these animals cannot vomit [42].

Rational for neuromuscular blocking

We decided to add a neuromuscular blocking agent, due to the main focus on breath analysis in our research department, which requires stable tidal volumes without spontaneous respiratory movements. Considering the impossibility of monitoring anesthesia depth by movements or withdrawal reflex, this may increase the risk of awareness [9, 24]. To minimize these concerns, all surgical procedures were performed before neuromuscular blocking. As soon as the rats were paralyzed, we monitored anesthesia depth by hemodynamic responses to stimulation to ensure adequate anesthesia depth. Finally, we propose the administration of ketofol and rocuronium with one syringe to eliminate the risk of continued relaxation in case of accidental discontinuation of analgesia and sedation.

Plasma concentrations and application

Median plasma concentrations of propofol remained almost constant, and the observed clinical effect was similar in all animals. The overall median ketamine plasma concentration was 3.8 (3.4–4.1) μg/ml with our anesthesia protocol. We found a significantly lower ketamine concentration at 7 h compared to 1 and 4 h. However, even the two animals with the lowest concentra-

tions (2.1 and 2.3 μg/ml) showed no signs of insufficient anesthesia.

Our resulting plasma concentrations are consistent with findings of intravenous dosing of Radford *et al.* and underline the importance of continuous application to achieve constant plasma concentrations [43]. A selection of available data on the injectable anesthetic drugs used in this study is given in Table 4. Brookes *et al.* similarly used a "step down infusion" scheme, either for ketamine or propofol/fentanyl, and found stable anesthesia under even lower plasma concentrations at the end of the experiment compared to our procedure [33].

Although there are reports on the combination and interaction of propofol and ketamine in Sprague-Dawley rats, it is known that both drugs accumulate over time [10, 44–47]. Our findings in plasma concentration underline that a stepwise reduction is necessary to avoid accumulation over several hours.

Of note, a mixture of propofol with other drugs or electrolyte solutions in a single syringe is usually not recommended, as this may lead to a destruction of the oil-water balance of the propofol emulsion. Nevertheless, our results show that our mixture was safe to use, but we recommend avoiding further dilution.

Rocuronium plasma concentrations were lower at 4 and 7 h compared to 1 h after infusion start with similar concentrations at 4 and 7 h. This indicates that steady-state was finally reached, but the loading dose of rocuronium led to initially increased plasma concentrations.

Available data on rocuronium dosage is inconsistent (Table 4) [49–53]. We decided to take 10 mg/kg as loading dose and a continuous infusion rate of 7.5–10 mg/kg/h to ensure sufficient neuromuscular blockade. This dosage is quite high compared to the ED90 (dose that leads to a 90% block) of 1.3 mg/kg in rats [54], but it is in line with findings from Osawa *et al.* to achieve a full block [50]. To our knowledge, there are no studies evaluating rocuronium plasma concentrations during continuous intravenous infusion in rats. However, our results suggest that rocuronium does not accumulate in rats. In summary, a reduction of the induction dose and a lower but constant maintenance rate of rocuronium may provide more stable plasma concentrations than in our setting.

Limitations

Observation time was limited to 7 h of mechanical ventilation with an inspired oxygen fraction of 0.5. It remains unclear wether longer periods or other oxygen fractions would also be applicable with stable hemodynamics. Moreover, our experiments were lethal, so an experimental approach with recovery after anesthesia was not tested in this study. Additional monitoring, for

Table 4. Selection of literature reports on propofol, ketamine and rocuronium dosing in rats

Author (Year)	Dosing	Plasma concentration (if available)	Result
Ketamine			
Radford *et al.* (2017) [43]	5 mg/kg i.v. bolus, followed by 20 mg/kg/h for one hour	3.9 ± 1.4 μg/ml	Subanesthetic dose
Brookes *et al.* (2002) [33]	30–60 mg/kg i.v. bolus followed by 50–90 mg/kg/h "step down" infusion	0.8 ± 0.12 μg/ml at the end of experiment	Stable vital parameters under spontaneous breathing
Propofol			
Pal *et al.* (2017) [48]	48 mg/kg/h i.v.		Unconsciousness, changes in EEG
Tzabazis *et al.* (2004) [44]	44 mg/kg/h 5.6 µg/ml Target controlled infusion		Moderately deep anesthesia
Brookes *et al.* (2002) [33]	10–30 mg/kg i.v. bolus followed by 10–50 mg/kg/h Combined with fentanyl	6.7 ± 0.23 μg/ml	Stable vital parameters under spontaneous breathing
Brammer *et al.* (1993) [20]	12–23 mg/kg i.v. induction 40–60 mg/kg/h i.v. maintenance		Stable vital parameters
Rocuronium			
Liu *et al.* (2019) [49]	1.2 mg/kg i.v. Different doses (0.2–1.2 mg/kg i.v.)		Onset time 40.6 ± 3.09 s Half maximal inhibitory concentration 0.37 (0.35–0.38) mg/kg
Osawa *et al.* (2008) [50]	10 mg/kg i.v.		10 mg/kg i.v. represents ten times ED50 for diaphragm.
Braga *et al.* (2008) [51]	0.6 mg/kg i.v.		Blockade of tibialis anterior muscle of 66.7 ± 6.92%
Testelmans *et al.* (2006) [52]	6 mg/kg/h i.v.		Sufficient for 50% twitch reduction of plantar flection
Itoh *et al.* (2000) [53]	8.4 mg/kg/h i.v.		95% block of the first twitch response of train of four

example, electroencephalography or neuromuscular monitoring may also help to improve drug dosing and evaluation of anesthesia depth. We note though that common electroencephalographic measures are altered by ketamine, impeding their performance in estimating anesthetic depth [55, 56]. The smallest possible adaptation of infusion rate with our syringe pumps was 0.1 ml/h. Use of a more precise syringe pump had enabled more frequent adaptations to further improve stability of drug plasma concentrations.

Conclusion

We successfully established an anesthesia protocol with inhalation induction by sevoflurane, intravenous maintenance by propofol and ketamine, and neuromuscular blockade by rocuronium. Surgical preparation and mechanical ventilation for seven hours were enabled without anesthesia-related adverse events or deaths. Physiologic parameters remained within normal ranges and plasma concentrations of propofol and ketamine remained almost constant. If this anesthesia procedure is used in combination with rocuronium, we emphasize that this requires a tight control of physiologic parameters to ensure adequate depth of anesthesia. The presented rocuronium infusion scheme could be improved by a reduced induction dose and a lower but constant maintenance rate. Finally, this anesthesia protocol fulfills the general requirement of low side effects to provide reproducibility, validity, and stable experimental conditions. Our detailed description may help to refine anesthesia management for laboratory animals and reduce the number of animals needed to establish similar protocols in other centers.

Conflict of Interests

The authors declare that there is no conflict of interest.

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