Combined effects of dexmedetomidine and remifentanil on anesthetic requirement and cardiovascular and renal functions in dogs anesthetized with sevoflurane

犬のセボフルラン麻酔下における麻酔薬要求量と循環および腎機能に対するデクスメデトミジンとレミフェンタニルの併用効果

The United Graduate School of Veterinary Science

Yamaguchi University

Natsuki AKASHI

March 2021

Contents

General Introduction

Proper use of anesthetics, sedatives, and analgesics can alleviate pain, create amnesia, and produce muscle relaxation essential for safe and humane patient care. The term “balanced anesthesia” means the simultaneous use of multiple drugs that are targeted to provide specifically individual components of the surgical general anesthesia, that is, unconsciousness, amnesia, antinociception, muscle relaxation [29, 72]. This technique is advantageous to minimized doses of individual drugs and less adverse effects such as cardiovascular and respiratory depression, which may increase morbidity and mortality particularly in critically ill patients [29, 72].

From the viewpoint of balanced anesthesia, opioids and α_2-adrenoceptor agonists are useful adjunctive drugs for general anesthesia mainly due to their potent analgesic properties. Both opioids and α_2-adrenoceptor agonists can provide antinociceptive effects through acting on neurons intrinsic to the spinal cord, as well as on axon terminals arising from dorsal root ganglia or from descending modulatory pathways [4, 18, 36, 79]. Moreover, their combination is known to be potentially beneficial in enhancing analgesic utility [11]. In rodents, it has been revealed that opioids synergistically interact with α_2-adrenoceptor agonists for analgesia mainly at the spinal cord mediated by either intercellular or intracellular mechanisms [11, 51, 62, 66, 80]. However, translational research alongside clinical aspects on the interactions between opioids and α_2-adrenoceptor agonists are limited in small animal practice.

Dexmedetomidine is a highly selective α_2-adrenoceptor agonist with useful sedative and analgesic properties as an anesthetic adjunct [6, 34, 47]. Conventionally, the use of dexmedetomidine has been limited to healthy animals in sedation for minor procedures or

premedication before general anesthesia in veterinary medicine due to its cardiovascular side effects including vasoconstriction immediately after administration resulting in subsequent hypertension and decreases in cardiac index (CI) [6, 31, 34, 47, 56]. In recent years, constant rate infusion (CRI) method with dexmedetomidine, derived from human medicine, has been applied in veterinary medicine enabling to minimize side effects [37, 53] and to consistently provide its efficacy through the anesthetic period [54, 73, 74].

Remifentanil is a newer, synthetic μ-opioid analgesic that is used intraoperatively in veterinary medicine for its analgesic and anesthetic sparing effects [2, 48]. Remifentanil has very-short mean elimination half-lives that were reported to range from 3 to 6 min in dogs and is metabolized by nonspecific esterases in blood and tissues independent of hepatic and renal clearances [12, 27]. Therefore, its pharmacokinetics are characterized by a rapid onset and elimination from circulation, with no accumulation even with prolonged administration [7, 12, 27, 40]. The application of remifentanil brings certain advantages in facilitating rapid adjustments of its analgesic effect and in making recovery times brief, compared with other opioids [2, 48].

Despite their lots of potential benefits, no study was found in the literature that investigated the combined effects of dexmedetomidine and remifentanil in dogs. To verify the clinical efficacy and adaptability of a concurrent CRI of dexmedetomidine and remifentanil, this study aimed to reveal both anesthetic potency and simultaneous influences on cardiovascular side effects with a combination of these two drugs in anesthetized dogs with sevoflurane.

In chapter 1, to reveal efficacy as anesthetic adjuncts according to combined dose rates, we evaluate the effects of CRI of dexmedetomidine and remifentanil, alone and combined, on the

sevoflurane requirement for blunting response to noxious stimuli in healthy dogs, and determine interactions between these two drugs at different dose rates.

In chapter 2, to determine the acceptability in cardiovascular side effects as well as organ perfusion and function when dexmedetomidine and remifentanil are combined under general anesthesia, we evaluate cardiovascular and renal functions with the CRI of these two drugs alone and in combination in dogs anesthetized with sevoflurane.

Chapter I

Effects of constant rate infusions of dexmedetomidine, remifentanil and their combination on minimum alveolar concentration of sevoflurane in dogs

Introduction

Dexmedetomidine is a highly selective α_2-adrenoceptor agonist with useful sedative and analgesic properties as an anesthetic adjunct [6, 34, 47]. Dexmedetomidine may be administered as a constant rate infusion (CRI) in the perioperative period to provide an anesthetic sparing effect, hemodynamic stability and postoperative analgesia [54, 73, 74]. The cardiovascular side effects of dexmedetomidine, such as bradycardia with decreased cardiac output (CO), may be substantial in dogs at CRIs > 0.5 μg/kg/hr but are less pronounced at lower infusion rates [37, 53].

Remifentanil is a synthetic μ-opioid receptor agonist [7] that is used intraoperatively in veterinary medicine for its analgesic and anesthetic sparing effects [2, 48]. As remifentanil is metabolized by nonspecific esterases in blood and tissues, its pharmacokinetics are characterized by a rapid onset and elimination from circulation, with no accumulation even with prolonged administration [11, 27, 40].

Minimum alveolar concentration (MAC) is a standard measure of inhaled anesthetic potency, and it is defined as the partial pressure of inhaled anesthetics that preventing purposeful movement in 50% of subjects exposed to a noxious stimulus [14]. The MAC-sparing effect, which is a property of analgesic or sedative agents to reduce the requirement of inhaled anesthetics [57], has been experimentally demonstrated with a CRI of dexmedetomidine alone at 0.5 to 4.5 μg/kg/h with 18 to 69% of MAC reduction [24, 54, 78]. Several previous reports have shown additional MAC reduction with dexmedetomidine CRI in combination with lidocaine [43] or morphine–lidocaine–ketamine [13] in dogs. The MAC-sparing effect of remifentanil,

achieving a maximum MAC reduction of approximately 70%, has also been evaluated with infusion rates of 0.055 to 5.5 μg/kg/min using enflurane [40], 0.15 to 0.9 μg/kg/min using isoflurane [41] and 0.15 to 2.4 μg/kg/min using sevoflurane [46] in dogs.

A previous study demonstrated a positive interaction of dexmedetomidine and fentanyl in dogs resulting in an enflurane MAC reduction [65]. We assume that the combination of dexmedetomidine and remifentanil also provides effective MAC reduction. Additionally, the pharmacokinetics of remifentanil brings certain advantages in facilitating rapid adjustments of its analgesic and anesthetic-sparing effects and in making recovery times brief, compared with other opioids [2, 48]. However, no study was found in the literature that investigated the combined effects of dexmedetomidine and remifentanil on MAC of any inhaled anesthetic in dogs.

This study aimed to 1) evaluate the effects of infusions of dexmedetomidine and remifentanil, alone and combined, on the sevoflurane MAC in healthy dogs, and 2) document heart rate (HR) and blood pressure changes at different dose rates. We hypothesized that CRIs of dexmedetomidine and remifentanil alone would result in dose-dependent sevoflurane MAC reduction, and that their combination would produce a greater MAC reduction through a positive (additive or synergistic) interaction.

Materials and methods

Animals

A group of six adult neutered Beagle dogs (three males and three females) mean ± standard deviation, aged 3.1 ± 0.8 years and weighing 12.6 ± 1.4 kg were used in this study. The dogs were considered healthy according to physical examination, complete blood count and serum

biochemical analysis performed before the study. They were housed in cages and food, but not water, was withheld for 12 hr prior to anesthesia. This study was a randomized, crossover trial including five treatments according to dexmedetomidine infusion rate. The dogs were anesthetized on five occasions separated by ≥ 2 weeks and were administered one each of five treatments, according to random numbers generated from Microsoft Office Excel Version 14.5.7 (Microsoft Corporation, WA, USA). This study was approved by the Animal Research Committee of Tottori University (no. 16-T-19).

Anesthesia and instrumentation

Anesthesia was induced with sevoflurane (Sevoflo; DS Pharma Animal Health Co. Ltd, Japan) delivered through a facemask via a pediatric, semiclosed circle circuit (vaporizer setting 8%, oxygen 5 *l*/min) until endotracheal intubation could be performed. After intubation, the dogs were positioned in left lateral recumbency and anesthesia was maintained with an end-tidal sevoflurane concentration (ETSEV) of 2.5%, oxygen 2 *l*/min. Synchronized intermittent mandatory ventilation maintained the end-tidal carbon dioxide partial pressure ($ETCO_2$) at 35–45 mmHg (Aestiva 7900; GE Healthcare Japan Corporation, Japan). Tidal volume was 10 to 20 m*l*/kg with a minimum respiratory rate of 10 breaths/min. The esophageal temperature (T) was maintained at 37.5 to 38.5 °C with forced warm air (3M Bair Hugger Model 775 Patient Warming Unit; 3M Japan Limited, Japan). A lead II electrocardiogram (ECG), HR (obtained from ECG), peripheral hemoglobin oxygen saturation (SpO_2, measured by pulse oximetry), ETSEV, $ETCO_2$ and T were measured continuously (BSM-5192; Nihon Kohden Corporation, Japan). An 8-French catheter (Atom Multipurpose Tube; Atom Medical Corporation, Japan) was shortened and inserted through the L-shaped adaptor connecting the endotracheal tube to the

breathing circuit until the tip was at the distal end of the endotracheal tube for sampling of airway gases at 200 m*l*/min (BSM-5192; Nihon Kohden Corporation). The gas analyzer was calibrated using a commercial calibration mixed gas (3% isoflurane, 5% CO2, 40% N2O, 52% O2; Sensitivity Calibration Gas A-5; Nippon Megacare Co. Ltd, Japan) prior to each anesthesia. A jugular vein was catheterized with an 18-gauge, 4.5 cm catheter (Introcan Safety 3; B Braun Aesculap Japan Co. Ltd, Japan) for administration of lactated Ringer's solution (3 m*l*/kg/hr; Solulact; Terumo Corporation, Japan). The left and right cephalic veins were catheterized with 22-gauge, 2.5 cm catheters (Surflo; Terumo Corporation) for dexmedetomidine and remifentanil administration, respectively.

A dorsal pedal artery was percutaneously catheterized with a 24-gauge, 1.9 cm catheter (Surflo; Terumo Corporation) for blood collection and continuous measurement of systolic (SAP), mean (MAP), and diastolic (DAP) arterial pressures (BSM-5192; Nihon Kohden Corporation). The catheter was connected to a pressure transducer (DTXPlus; Argon Medical Devices Japan K.K., Japan) via 120 cm extension tubing (TOP blood pressure monitoring tube; TOP Corporation, Japan). The transducer was placed and zeroed to atmospheric pressure at the level of the midsternum and was calibrated utilizing a digital manometer (HT-1500NH; HODAKA Co. Ltd, Japan) prior to each anesthesia. The whole arterial measurement system was continuously flushed with heparinized (1 U/m*l*) saline.

Arterial blood (1 m*l*) was collected using 2 m*l* heparinized syringe (PICO 50; Radiometer K.K., Japan) after aspiration of 3 m*l* blood. Following sample collection, the blood was returned to the dog and the catheter was flushed with 3 m*l* of heparinized saline. pHa, arterial partial pressure of carbon dioxide ($PaCO_2$) and arterial partial pressure of oxygen (PaO_2) were measured after each MAC determination and corrected to body temperature (ABL-5; Radiometer K.K.).

The analyzer was calibrated automatically every 4 hr by two-point correction and every 30 min by one-point correction with dedicated known-concentration solutions and gas mixtures.

Drug administration and procedures

Approximately 30 min after induction of anesthesia with ETSEV at 2.5%, the dogs were administered saline (Otsuka Normal Saline; Otsuka Pharmaceutical Factory Inc., Japan) or dexmedetomidine (Precedex; Maruishi Pharmaceutical Co. Ltd, Japan) via the left cephalic vein (Fig. 1). The loading dose (LD, administered over 10 min) and CRI, respectively, in each treatment were: Saline treatment, saline 6 m*l*/kg and 1 m*l*/kg/hr; Dex0.1 treatment, dexmedetomidine 0.1 μg/kg and 0.1 μg/kg/hr; Dex0.5 treatment, dexmedetomidine 0.5 μg/kg and 0.5 μg/kg/hr; Dex1.0 treatment, dexmedetomidine 1.0 μg/kg and 1.0 μg/kg/hr; Dex5.0 treatment, dexmedetomidine 5.0 μg/kg and 5.0 μg/kg/hr. Sevoflurane MAC was determined (T1) after allowing 60 min for equilibration. The baseline MAC (sevoflurane alone) was determined only at T1 with saline treatment. Following the T1 determination of MAC with saline or dexmedetomidine, remifentanil (Ultiva; Janssen Pharmaceutical K.K., Japan) CRI was initiated at 0.15 μg/kg/min via the right cephalic vein. After 30 min for equilibration, MAC was determined (T2). Then, the remifentanil CRI was successively increased to 0.60 and 2.40 μg/kg /min and after 30 min for equilibration at each rate, MAC was determined (T3 and T4, respectively). Dexmedetomidine and remifentanil were prepared separately in saline solutions for CRI at a rate of 1 m*l*/kg/hr using two syringe pumps (TOP-551VC; TOP Corporation). These pumps were calibrated according to the manufacturer's specifications prior to the study. The LDs of saline or dexmedetomidine were administered by temporarily increasing the infusion rates to 6 m*l*/kg/hr for 10 min. After the MAC determination was completed at T4, all drug administrations

were discontinued, and the dog was allowed to recover from anesthesia. During recovery, meloxicam (0.2 mg/kg; Metacam; Boehringer Ingelheim Vetmedica Japan Co. Ltd, Japan) was administered subcutaneously.

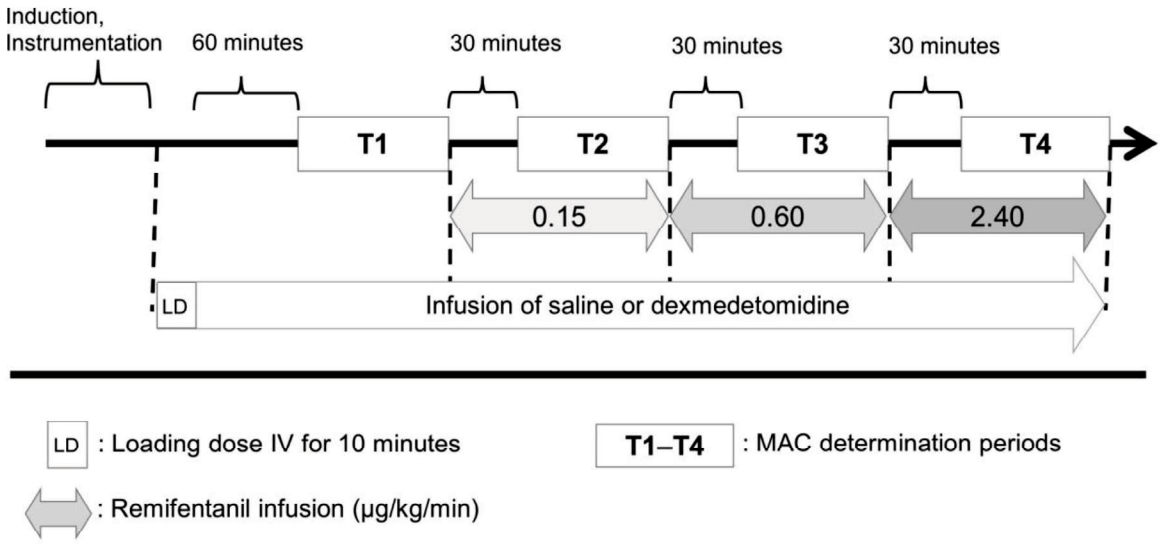

Figure 1. Study timeline. After induction of anesthesia, dogs were administered an infusion of saline or dexmedetomidine (0.1, 0.5, 1.0 or 5.0 μg/kg/hr) randomized for five anesthetic episodes separated by ≥ 2 weeks. The sevoflurane minimum alveolar concentration (MAC) was determined at T1. Then an infusion of remifentanil was started and continued at successively increasing dose rates (0.15, 0.60 and 2.40 μg/kg/min). MAC was determined at T2, T3 and T4.

MAC determination

HR, SAP, MAP, DAP, ETSEV, $ETCO_2$ and T were recorded 1 min prior to applying the electrical stimulation. An electrical current (50 V, 50 Hz, 10 ms) as a supramaximal noxious stimulus was generated by an electrical stimulator (SEN-3401; Nihon Kohden Corporation) connected via two 25-gauge needles (Terumo Corporation) placed subcutaneously, 5 cm apart, at

the cranial border of the proximal tibia. The stimulation protocol involved two single stimuli followed by two continuous stimuli of 3 sec duration with a 5 sec interval between all stimuli [75]. The stimulation was discontinued if purposeful movement was observed, defined as gross movements including head lifting and observable movement of limbs and/or trunk. Swallowing, back arching, chewing, spontaneous breathing efforts and movement in the pelvic limb that underwent electrical stimulation were not considered purposeful movements. If a purposeful movement was detected, or not detected, the ETSEV was increased by 10% or decreased by 20%, respectively [45]. The protocol was repeated following a 15 min equilibration period. The MAC was calculated as the mean of two consecutive ETSEV values with a positive response and negative response. The MAC was determined in duplicate and the final value was the mean of these two MAC determinations. Immediately after each MAC determination, pHa, $PaCO_2$ and PaO_2 were measured. MAC reduction values (%) were calculated as the percentage reduction from the baseline MAC at all time points in each treatment.

Pharmacodynamic analysis

The MAC reduction values were plotted against corresponding, logarithmically transformed infusion rates of remifentanil for each treatment. MAC reduction values in dexmedetomidine administered alone (values at T1 of Dex0.1, Dex0.5, Dex1.0 and Dex5.0 treatments) were also plotted against corresponding log-doses of dexmedetomidine. The dose-response curves were determined by least squares linear regression analysis between the infusion rate and the MAC reduction value. The infusion rates producing 50% of the maximal effect (ED_{50}) were obtained for dexmedetomidine alone, remifentanil alone and remifentanil combined with

dexmedetomidine. The data were analyzed using GraphPad Prism Version 7.00 (GraphPad Software, CA, USA).

Isobolographic analysis

The interactions between dexmedetomidine and remifentanil for MAC reduction were graphically assessed using the isobologram according to previous studies [17, 65]. The ED_{50} value for remifentanil combined with dexmedetomidine in each treatment was plotted against its corresponding infusion rate of dexmedetomidine with 95% confidence intervals (95% CI) on a quadratic plane graph (the y-axis was remifentanil and the x-axis was dexmedetomidine). A straight line (additivity isobole) was drawn between the ED_{50} value for remifentanil alone on the y-axis and that for dexmedetomidine alone on the x-axis with its 95% CI, which represents theoretical additivity of effects. If the ED_{50} value for remifentanil in combination with dexmedetomidine was plotted under or over the additivity isobole, and its 95% CI did not overlap with the 95% CI of the isobole, the interaction was considered synergistic or antagonistic, respectively. If its 95% CI overlapped with 95% CI of the isobole, the combination effect was considered additive.

Statistical analysis

Continuous data were assessed for normality using visual inspection of a scatter plot and the Shapiro–Wilk test. The analyses for the dose effect of remifentanil for each time point with regard to MAC, cardiovascular measurements, arterial blood gas variables, $ETCO_2$ and T were conducted using repeated measures one-way analysis of variance (ANOVA) and the post hoc multiple comparisons were performed with Bonferroni's test to identify differences within

treatments. One-way ANOVA was also used to examine the dose effect of dexmedetomidine for each treatment with regards to the above-mentioned variables, and the post hoc multiple comparisons were performed with Tukey–Kramer's test to identify differences among treatments. Differences were statistically significant when $p < 0.05$. The data was analyzed using GraphPad Prism Version 7.00 (GraphPad Software).

Results

All data, except ED_{50} values, are presented as mean ± standard deviation. ED_{50} values are presented as mean with 95% CI. The sevoflurane MAC at T1 with saline was 2.14 ± 0.29%, and administration of dexmedetomidine (Dex0.5, Dex1.0 and Dex5.0) significantly reduced the MAC values at T1 compared with saline values ($p = 0.010$ and 0.002, and $p < 0.0001$, respectively; Table 1). The MAC reduction values increased with infusion rates of dexmedetomidine and remifentanil (Table 2). The ED_{50} value was 0.54 (0.31–0.89) μg/kg/min for remifentanil alone and 2.99 (2.05–5.02) μg/kg/hr for dexmedetomidine alone. The values for remifentanil during Dex0.1, Dex0.5 and Dex1.0 were 0.35 (0.26–0.45), 0.12 (0.066–0.18) and 0.076 (0.033–0.13) μg/kg/min, respectively. The ED_{50} value for remifentanil with Dex5.0 was not acquired because the MAC reduction value at T1 (dexmedetomidine alone) was already higher than 50%. The ED_{50} values in Dex0.5 and Dex1.0 treatments were under the 95% CI of additivity isobole, but the value overlapped with the isobole in Dex0.1 treatment (Fig. 2). Thus, the combination effects for MAC reduction between dexmedetomidine and remifentanil were synergistic for Dex0.5 and Dex1.0 treatments and additive for Dex0.1 treatment. The interaction

in Dex5.0 treatment was not evaluated because of the inability to acquire the ED_{50} value as described above.

The HR was significantly decreased with Dex1.0 and Dex5.0 treatments compared with Saline treatment at all time points (Fig. 3A). With the Dex0.5 treatment, the HR decrease was found significant only at T1 ($p = 0.016$), but not during remifentanil infusion (T2 to T4) compared with Saline treatment. HR was also significantly decreased with remifentanil infusion at T4 compared from T1 with Saline, Dex0.1, and Dex1.0 treatments ($p = 0.023$, 0.029, and 0.002), respectively. In the Saline treatment, MAP was significantly increased by remifentanil at T4 compared with T2 ($p = 0.027$; Fig. 3b). In Dex5.0, MAP was significantly higher at T1 and T2 than saline ($p < 0.001$ and $p = 0.003$, respectively). No significant changes were observed with Dex0.1, Dex0.5 and Dex1.0 treatments (Fig. 3B). Similarly, the changes of MAP, SAP and DAP were significantly increased only with higher doses of remifentanil (T3 and T4) and dexmedetomidine (Dex1.0 and Dex5.0) (Table 3). $ETCO_2$ and T were within the controlled ranges. pHa, $PaCO_2$, PaO_2, $ETCO_2$ and T were not significantly different among treatments or time points throughout the study (data not shown).

Table 1. Minimum alveolar concentration (MAC, %) of sevoflurane in six Beagle dogs before (T1) and during (T2–T4) remifentanil infusions at 0.15 (T2), 0.60 (T3), and 2.40 (T4) μg/kg/min in each treatment that was administered with saline or dexmedetomidine infused at 0.1, 0.5, 1.0 or 5.0 μg/kg/hr. Data are shown as the mean ± standard deviation.

Treatment	T1	T2	T3	T4
		Remifentanil infusion (μg/kg/min)		
	Saline	0.15	0.60	2.40
Saline	2.14 ± 0.29	1.38 ± 0.25[a)]	1.00 ± 0.27[ab)]	0.72 ± 0.13[ab)]
Dex0.1	1.84 ± 0.14	1.35 ± 0.15[a)]	0.90 ± 0.20[ab)]	0.66 ± 0.17[ab)]
Dex0.5	1.58 ± 0.38[c)]	1.05 ± 0.21[a)]	0.70 ± 0.14[ab)]	0.48 ± 0.10[ab)]
Dex1.0	1.48 ± 0.31[c)]	0.98 ± 0.21[ac)]	0.63 ± 0.20[abc)]	0.41 ± 0.13[abcd)]
Dex5.0	0.88 ± 0.15[cd)]	0.52 ± 0.21[acd)]	0.33 ± 0.20[abcd)]	0.25 ± 0.20[abcd)]

[a)] Significantly differ from T1 values within the treatment ($p < 0.05$); [b)] Significantly differ from T2 values within the treatment ($p < 0.05$); [c)] Significantly differ from Saline treatment at this time point ($p < 0.05$); [d)] Significantly differ Dex0.1 treatment at this time point ($p < 0.05$).

Table 2. The decrease of sevoflurane minimum alveolar concentration (%) from baseline (T1 of treatment saline) in six Beagle dogs before (T1) and during (T2–T4) remifentanil infusions at 0.15 (T2), 0.60 (T3), and 2.40 (T4) μg/kg/min in each treatment that was administered with saline or dexmedetomidine infused at 0.1, 0.5, 1.0 or 5.0 μg/kg/hr. Data are shown as the mean ± standard deviation.

Treatment	**T1**	**T2**	**T3**	**T4**
		Remifentanil infusion (μg/kg/min)		
	Saline	0.15	0.60	2.40
Saline	N/A	35 ± 12	53 ± 13	66 ± 7
Dex0.1	13 ± 7	37 ± 5	58 ± 9	69 ± 9
Dex0.5	27 ± 10	51 ± 5	67 ± 5	77 ± 5
Dex1.0	34 ± 9	55 ± 5	71 ± 7	81 ± 5
Dex5.0	59 ± 5	76 ± 9	85 ± 9	88 ± 10

N/A, not applicable.

Table 3. Systolic (SAP) and diastolic (DAP) arterial pressure that were measured along determination of each minimum alveolar concentration in six Beagle dogs with saline (T1) or remifentanil infusions at 0.15 (T2), 0.60 (T3), and 2.40 (T4) μg/kg/min in each treatment (administered with saline or dexmedetomidine infused at 0.1, 0.5, 1.0 or 5.0 μg/kg/hr). Data are shown as the mean ± standard deviation.

		T1	**T2**	**T3**	**T4**
			Remifentanil infusion (μg/kg/min)		
Variable	**Treatment**	Saline	0.15	0.60	2.40
SAP (mmHg)	Saline	109 ± 5	115 ± 9	128 ± 15[a)]	138 ± 15[ab)]
	Dex0.1	118 ± 8	118 ± 6	129 ± 13	138 ± 14
	Dex0.5	122 ± 14	120 ± 14	133 ± 15	138 ± 19
	Dex1.0	124 ± 9	124 ± 8	136 ± 13	140 ± 12
	Dex5.0	138 ± 16[cd)]	137 ± 17[c)]	140 ± 18	142 ± 22
DAP (mmHg)	Saline	64 ± 4	63 ± 9	70 ± 10	72 ± 9[b)]
	Dex0.1	74 ± 9	71 ± 12	77 ± 13	80 ± 16
	Dex0.5	80 ± 14	76 ± 11	86 ± 11	87 ± 17
	Dex1.0	86 ± 5[c)]	85 ± 9[c)]	92 ± 13[c)]	92 ± 13
	Dex5.0	96 ± 13[cd)]	92 ± 16[cd)]	94 ± 15[c)]	94 ± 15

[a)] Significantly differ from T1 values within the treatment ($p < 0.05$); [b)] Significantly differ from T2 values within the treatment ($p < 0.05$); [c)] Significantly differ from Saline treatment at this time point ($p < 0.05$); [d)] Significantly differ Dex0.1 treatment at this time point ($p < 0.05$).

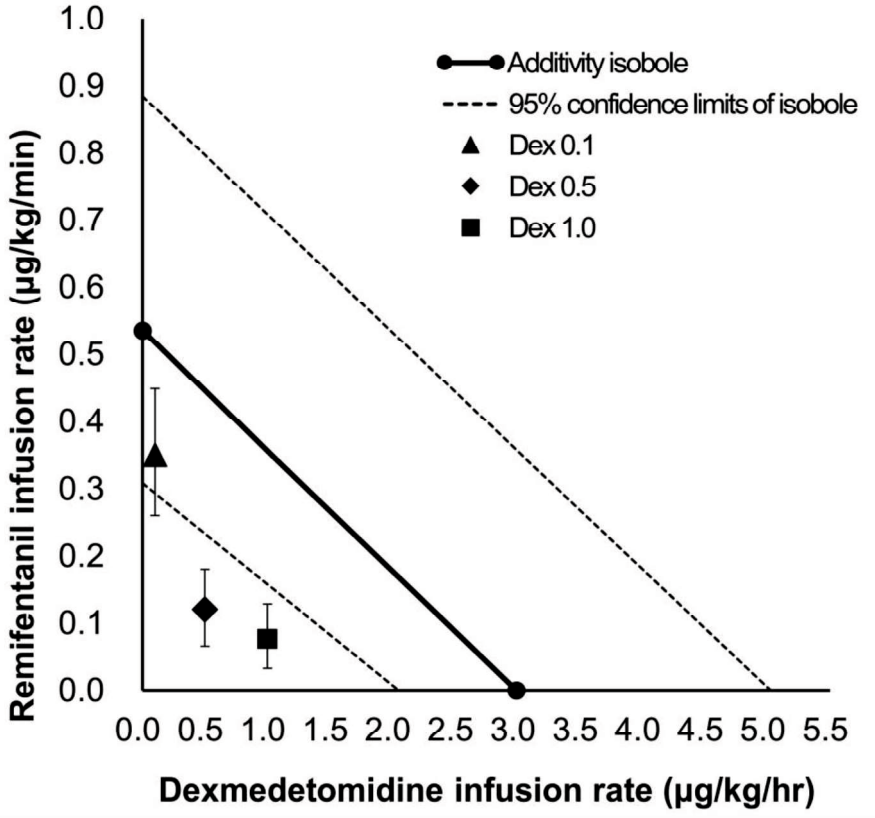

Figure 2. Isobolographic analysis of a 50% effect (ED_{50}) values for sevoflurane minimum alveolar concentration reduction by dexmedetomidine, remifentanil and their combination in six Beagle dogs. A straight line (additivity isobole) was drawn by connecting ED_{50} of dexmedetomidine alone (2.99 μg/kg/hr) on the x-axis and remifentanil alone (0.54 μg/kg/min) on the y-axis. The ED_{50} of remifentanil on Dex0.1 (0.1 μg/kg/hr), Dex0.5 (0.5 μg/kg/hr) and Dex1.0 (1.0 μg/kg/hr) treatments were plotted against the corresponding combined dexmedetomidine doses with 95% confidence intervals. The area surrounded by 95% confidence intervals (dotted lines) of the additivity isobole indicates theoretical plane of the additive combination effect. The ED_{50} and its 95% confidence intervals on Dex0.5 and Dex1.0 treatments were plotted in the area under 95% confidence intervals of the isobole, which indicates there were synergistic interactions.

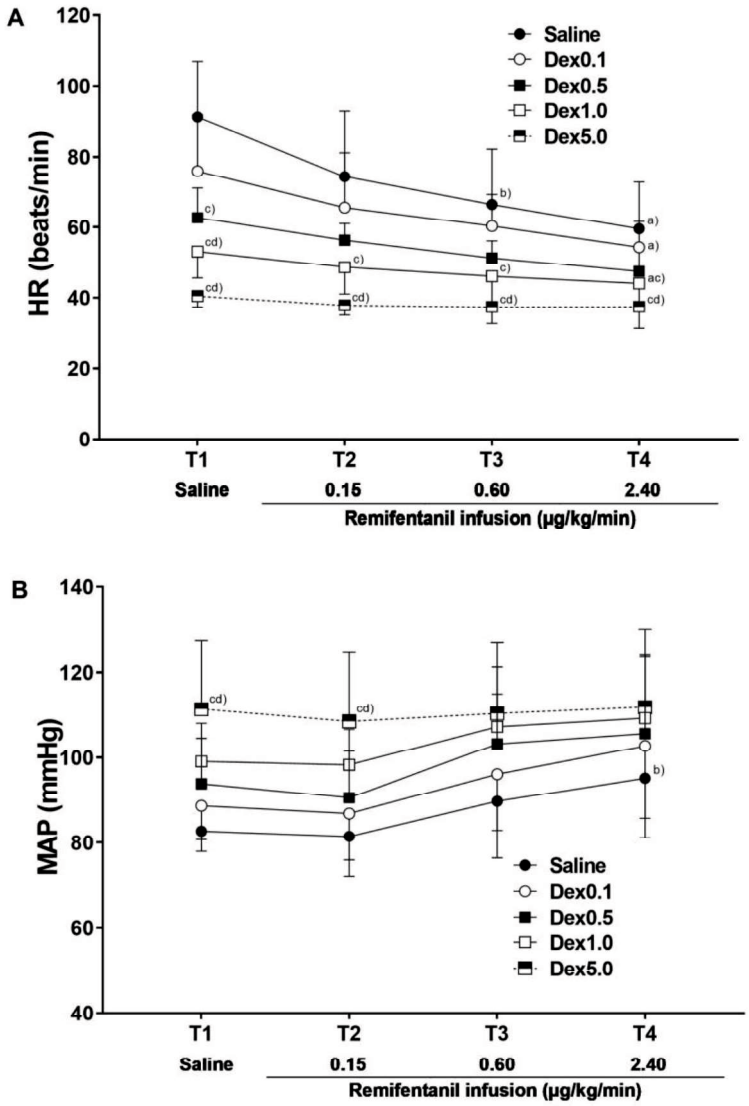

Figure 3. (A) Heart rate (HR) and (B) mean arterial pressure (MAP) measured at each minimum alveolar concentration determination in six Beagle dogs with saline (T1) or remifentanil infusions at 0.15 (T2), 0.60 (T3), and 2.40 (T4) μg/kg/min in each treatment [continuous infusions of saline or dexmedetomidine at 0.1 (Dex0.1), 0.5 (Dex0.5), 1.0 (Dex1.0) or 5.0 μg/kg/hr (Dex5.0)]. [a)] Significantly different from T1 values within the treatment ($p < 0.05$). [b)] Significantly different from T2 values within the treatment ($p < 0.05$). [c)] Significantly different from Saline treatment at the same time point ($p < 0.05$). [d)] Significantly different from treatment Dex0.1 at the same time point ($p < 0.05$). Data are shown as the mean ± standard deviation.

Discussion

The present study measured sevoflurane MAC-sparing effects resulting from administration of dexmedetomidine and/or remifentanil in dogs. The sevoflurane MAC of 2.14% in this study is similar to previously reported values of 1.83% [43], 1.86% [24] and 2.24% [46]. MAC is affected by hypothermia, severe hypotension, hypoxemia and hypercapnia [57]. Based on the measurements of body temperature, blood pressures and arterial blood gases during this study, these abnormal conditions were not present and thus, did not influence the data.

The present study determined that remifentanil alone reduced sevoflurane MAC in a dose-dependent manner. The remifentanil infusion rate of 0.60 μg/kg/min was chosen from published clinical studies assessing remifentanil effects in dogs [2, 48]. Lower and higher infusion rates (0.15 and 2.4 μg/kg/min) were included to improve the reliability of the dose-response analysis [46]. Our results were comparable with the values reported in a previous study at remifentanil rates of 0.15, 0.60 and 2.40 μg/kg/min decreased sevoflurane MAC by 31, 50 and 67%, and ED_{50} was 0.53 μg/kg/min in dogs [46]. Other previous studies have also reported dose-dependent MAC reduction with remifentanil infusions in dogs. Michelsen et al. reported an ED_{50} as 0.72 μg/kg/min for enflurane MAC reduction using infusion rates of remifentanil from 0.055 to 5.5 μg/kg/min [40]. Monteiro et al. also investigated the isoflurane MAC-sparing effect of remifentanil CRI at 0.15 to 0.90 μg/kg/min and reported an ED_{50} of 0.20 μg/kg/min in dogs [41]. Variations in the reported ED_{50} values may be the result of different experimental conditions and inhalant anesthetics among studies.

The dose-dependent sevoflurane MAC reduction was also observed with administration of dexmedetomidine alone. The dexmedetomidine dose rates evaluated in this study were chosen from previous studies in dogs [24, 54, 73]. In the present study the sevoflurane MAC value with Dex0.1 was not significantly different from saline. This result is comparable with a previous report that dexmedetomidine (0.1 μg/kg/hr) did not significantly reduce the isoflurane MAC in dogs [54]. The sevoflurane MAC reduction of 27% by dexmedetomidine (0.5 μg/kg/hr) is similar to values obtained in previous studies of 18–30% isoflurane MAC reduction in dogs [13, 54]. Higher infusion rates of dexmedetomidine of 1.5, 2.0 and 4.5 μg/kg/hr decreased sevoflurane MAC by 43%, 44% and 69%, respectively [24, 43] and CRI of 3.0 μg/kg/hr decreased isoflurane MAC by 41% and 59% [54, 78]. Although it is difficult to make direct comparisons between the values because of differences in infusion rates among these studies, MAC reduction in a dose-dependent manner was consistently observed during dexmedetomidine CRI.

The present study showed synergistic interaction between dexmedetomidine and remifentanil for sevoflurane MAC reduction using isobolographic analysis. The synergistic effect with dexmedetomidine and μ-opioid agonist is consistent with the result of a previous study that the combination of successive doses of single IV administrations of dexmedetomidine (0.03–3.0 μg/kg) and a fentanyl infusion (0.05 μg/kg/min) clearly revealed synergistic interaction on enflurane MAC reduction in dogs [65]. Our study assessed interaction according to a fixed, consistent infusion rate of dexmedetomidine, referring to its current usage in the perioperative period [54, 73, 74], and suggested clinically preferable dose rates (0.5 to 1.0 μg/kg/hr) of dexmedetomidine CRI in terms of effective MAC reduction combined with opioids. It is important to assess interaction alongside infusion rates, because the interaction is not merely a property of the two drugs but also depends on the combined doses [69]. The combination of

dexmedetomidine (0.1 μg/kg/hr) with remifentanil was determined to be additive, suggesting that the dexmedetomidine dose rate was too low to achieve synergistic interaction.

The current study did not assess the interactions according to remifentanil infusion rates, and thus could not suggest any preferable dose rates with remifentanil. On the other hand, the results of this study showed that the required remifentanil dose rates for ED_{50} (0.12 and 0.076 μg/kg/min) during dexmedetomidine CRIs at 0.5 and 1.0 μg/kg/hr, respectively, were decreased compared to that for remifentanil alone (0.54 μg/kg/min). In a clinical setting, we suggest that lower remifentanil infusion rates will be adequate under the co-administration of dexmedetomidine CRI than those used in current practice.

The detailed mechanism of the synergism was not addressed in this study. However, it has been demonstrated that opioids synergistically interact with α_2-adrenoceptor agonists for analgesia mainly at the spinal cord [11, 51, 62, 66, 80]. Both opioids and α_2-adrenoceptor agonists can provide antinociceptive effects through acting on neurons intrinsic to the spinal cord, as well as on axon terminals arising from dorsal root ganglia or from descending modulatory pathways [4, 18, 36, 79]. Coincident inhibition of these series of neurons in anatomically analogous sites would produce synergistic analgesia. Further, it has been proposed that inhaled anesthetics produce immobility mainly through spinal mechanisms; a direct suppression of the motor neuron and an analgesic effect on the dorsal horn [3, 39]. It is possible that the synergism between dexmedetomidine and remifentanil at the spinal cord may have contributed to the synergistic MAC reduction observed in the present study.

Cardiovascular changes reported in the present study are comparable to results from previous studies in dogs [42, 46, 54]. Remifentanil induces bradycardia associated with increasing vagal tone via stimulation of μ-opioid receptors [67], whereas dexmedetomidine

stimulates the baroreceptor reflex to the peripheral vasoconstriction mediated via α_{2b}-adrenoceptors [47]. An increase in blood pressure would be also induced by the modulation of peripheral vasodilation owing to a reduction in the sevoflurane dose [49].

The cardiovascular effects provoked by the individual doses of remifentanil and dexmedetomidine were observed even when these drugs were combined. Salmenperä et al. reported that the synergistic interaction between fentanyl and dexmedetomidine for enflurane MAC-sparing effect might not be a clinical advantage in terms of avoiding bradycardia in dogs [65], and a similar situation was observed in the present study. A previous study indicated that dexmedetomidine infusion at 3.0 μg/kg/hr, with reduced isoflurane concentration as an equivalent of 1.3 MAC, decreased the CI to 56%, whereas the decrease in CI was 17% and might be clinically acceptable at 0.5 μg/kg/hr in dogs [53]. Remifentanil also decreased CI accompanied with decreased HR at 0.15–0.90 μg/kg/min [42], and the bradycardia effect could develop at higher infusion rates as shown presently and previously [40, 46] in dogs anesthetized with inhaled agents. There should be concern about decreases in HR when these drugs are combined at higher doses. HR changes may be acceptable when the drugs in combination are administered at low doses, but further studies are required to determine the degree of cardiovascular changes when dexmedetomidine and remifentanil are combined.

The current study contains some limitations. Measurement of plasma drug concentrations would have contributed to the assessment of the results by describing any changes in pharmacokinetics of the drug combination compared with the drugs administered alone. An equilibration time for dexmedetomidine was determined based on similar studies evaluating MAC-sparing effect with LD for 10 min followed by CRI for 60 min of dexmedetomidine [24, 78]. Remifentanil was also equilibrated with reference to previous studies determined MAC after

a 30 min CRI [40, 41, 46]. However, the equilibration times used in this study could have been supported or deemed inadequate by measurements of plasma drug concentrations. Second, the number of animals used in this study was relatively small. It is possible that some of the variables could have shown significant changes or synergy with Dex0.1 treatment if more dogs had been studied.

In conclusion, the present study evaluated sevoflurane MAC-sparing effects resulting from infusions of dexmedetomidine and remifentanil, alone and combined, in healthy dogs. A combination of dexmedetomidine and remifentanil enhanced their dose-dependent sevoflurane MAC-sparing effects through clear synergistic interactions when dexmedetomidine was administered at 0.5 and 1.0 μg/kg/hr. The combination of dexmedetomidine and remifentanil significantly reduced the requirement of sevoflurane for blunting response to pain and may contribute to improve the efficiency of antinociception during surgical general anesthesia in dogs.

Chapter II

Cardiovascular and renal effects of constant rate infusions of remifentanil, dexmedetomidine and their combination in dogs anesthetized with sevoflurane

Introduction

Dexmedetomidine, a dextro optical isomer of medetomidine, is an α_2-adrenoreceptor agonist that induces sedation and analgesia [47]. Dexmedetomidine also has cardiovascular side effects, including bradycardia, decreased CO, and increased systemic vascular resistance [44, 47, 53]. In addition, dexmedetomidine and the other α_2-adrenoreceptor agonists have been known to have a remarkable diuretic property; inhibition of arginine vasopressin (AVP) secretion has been proposed as one of the mechanisms for this diuretic property [9, 16, 58, 63, 76]. Dexmedetomidine CRI have been applied perioperatively to consistently maintain its anesthetic-sparing and hemodynamic-stabilizing effects or to provide adjunctive analgesia in dogs [52, 54, 73, 74]. In the clinical setting, dexmedetomidine and medetomidine CRI are frequently co-administered with other analgesics, especially μ opioid agonists, during the perioperative period in dogs [20, 52, 59, 71, 73, 74].

Remifentanil is a very short-acting μ-opioid receptor agonist that induces a potent analgesic effect and is rapidly metabolized by nonspecific esterases in blood and tissues, independent of hepatic and renal clearances [7, 12, 27]; this pharmacokinetic property of remifentanil may be beneficial for both predictivity and adjustability on the drug effects even in patients who have compromised in the liver and kidneys. Previous studies have demonstrated that remifentanil significantly decreases CI accompanied by bradycardia in dogs anesthetized with inhaled anesthetics [15, 42]. In contrast to α_2-adrenoreceptor agonists, remifentanil *per se* was related to an increase in the plasma AVP concentrations [42] and opioids tended to cause reduction in the urine flow [1, 60].

In the previous chapter, we mentioned that a combination of remifentanil and dexmedetomidine in CRI synergistically reduced the sevoflurane requirement in dogs; however, bradycardia with the concurrent CRI of these drugs is a concern. It would be clinically beneficial to identify whether the hemodynamic depression under the co-administration of remifentanil and dexmedetomidine is acceptable enough to maintain tissue oxygenation and the functional blood flow in vital organs such as kidneys. Furthermore, although both remifentanil and dexmedetomidine potentially affect urine production and AVP secretion in different ways as noted above, no study has documented the combined effects of μ-opioid and α_2-adrenoreceptor agonists on renal functions.

This study aimed to experimentally evaluate the changes in cardiovascular and renal functions as well as AVP secretion with the CRI of remifentanil and dexmedetomidine alone and in combination in dogs anesthetized with sevoflurane.

Materials and methods

Animals

Six healthy, adult neutered Beagle dogs (three males and three females) aged 3.0 ± 0.7 years (mean ± standard deviation [SD]) and weighing 10.2 ± 1.2 kg (mean ± SD), were included in this study. Health status was assessed before the study by physical examination, complete blood count, serum biochemical analysis, and urinalysis. The dogs were housed in cages; food, but not water, was withheld for 12 hr before anesthesia. This study was approved by the Animal Research Committee of Tottori University (no. 16-T-19).

Study design

Each dog was randomly anesthetized on four occasions with a ≥4-week washout period and received one of the following four treatments: CRI of saline at 2 ml/kg/min as control (C treatment); CRI of remifentanil (Ultiva; Janssen Pharmaceutical K.K.) at successive dose rates of 0.15, 0.60, and 2.40 μg/kg/min (R treatment); dexmedetomidine (Precedex; Maruishi Pharmaceutical. Co. Ltd), initially at a loading dose of 0.5 μg/kg intravenously (IV) over 10 min, followed by CRI at 0.5 μg/kg/hr (D treatment); and combined CRI of remifentanil and dexmedetomidine at the dose rates mentioned above (RD treatment).

Instrumentation

Anesthesia was induced by sevoflurane (vaporizer setting 8%; Sevoflo; DS Pharma Animal Health Co., Ltd.) in oxygen at 5 *l*/min, using a face mask connected to an anesthetic machine (Aestiva 7900; GE Healthcare Japan Corporation) through a pediatric, semi-closed circle circuit. After endotracheal intubation, the dogs were placed in the left lateral recumbent position, and the anesthesia was maintained with 2.5 to 3.0% sevoflurane in oxygen at 2 *l*/min. Pressure-controlled mechanical ventilation with a peak inspiratory pressure of 8 to 15 cm H_2O and a respiratory rate of 10 breaths/min (inspiration to expiration ratio: 1:2) were set to maintain normocapnia ($ETCO_2$ between 35 and 45 mm Hg). Airway gases were continuously sampled at 200 m*l*/min through an 8-French catheter (Atom Multipurpose Tube; Atom Medical Corporation) that was passed into the distal end of the endotracheal tube. The catheter was tightly inserted from the sampling port of the L-shaped adaptor, which connected the endotracheal tube to the breathing circuit. Prior to each experiment, the gas analyzer was calibrated using manufacturer-supplied calibration mixed gases. The esophageal temperature was controlled within 37.5 to 38.5°C using forced warm air (3M Bair Hugger Model 775 Patient Warming Unit; 3M Japan Limited). The left and right

cephalic veins were cannulated with 22-guage catheters (Surflo F&F; Terumo Corporation) for the administration of drugs and lactated Ringer's solution at a rate of 3 ml/kg/hr, respectively, throughout anesthesia. A 24-guage catheter (Surflo F&F; Terumo Corporation) was placed percutaneously into a dorsal pedal artery and connected to a transducer (DTXPlus; Merit Medical Devices Japan K.K.) by a 120-cm extension tubing (TOP blood pressure monitoring tube, TOP Corporation) for continuous measurements of systolic, mean, and diastolic arterial pressures (SAP, MAP, and DAP, respectively). After local anesthesia over the right jugular vein infiltrated with 1 mg of lidocaine (Xylocaine Injection 2%; Aspen Japan K.K., Japan), a 5-French, 75-cm thermodilution catheter (Swan-Ganz thermodilution catheter 132F5; Edwards Lifesciences Corporation, Japan) was inserted through the right jugular vein using a 5-French catheter introducer (XEMEX Introducer Set; Zeon Medical Inc., Japan). The thermodilution catheter was advanced into the pulmonary artery, as confirmed by the transitions of characteristic pressure waveform patterns under fluoroscopic guidance (SXT-1000A; Canon Medical Systems Corporation, Japan). The proximal and distal ports of this catheter were placed in the right atrium and the pulmonary artery, respectively, and were connected to pressure transducers (DTXPlus; Merit Medical Devices Japan K.K.) for continuous measurements of right atrial pressure (RAP) and mean pulmonary artery pressure (MPAP), respectively. Pulmonary artery temperature (PAT) was obtained from the thermistor at the distal port of the catheter. Pulmonary artery occlusion pressure (PAOP) was measured intermittently at the distal port by inflating the balloon at the tip of the thermodilution catheter with 0.7 m*l* of air. All transducers were placed and zeroed at the manubrium of the dogs and were calibrated with a digital manometer before each anesthesia. The entire pressure measurement system was filled with heparinized (1 U/m*l*) saline and was continuously flushed. The CO was measured using a handy bolus injection of 3-ml cold (1 to

5°C) 5% dextrose solution (5w/v% Glucose Injection; Terumo Corporation) through the proximal port of the thermodilution catheter at end expiration. Each CO determination included five measurements; the maximum and minimum values were discarded, and the average of the three remaining measurements that differed within 10% of each other was recorded as the CO value. For urine sample collection, an 8-French urinary catheter, which is 55 cm long for male (Silicon Foley Catheter; Kirikan Ltd, Japan) or 34 cm long for female (All Silicon Foley Catheter; Create Medic Co., Ltd, Japan) was inserted through the urethra and was held in place in the bladder by inflating the balloon. Pulse rate, peripheral oxygen saturation by pulse oximetry, $ETCO_2$, ETSEV, SAP, MAP, DAP, RAP, and esophageal temperature were monitored using a multiparametric device (BSM-5192; Nihon Kohden) throughout the anesthesia. Another monitor (BSM-5132; Nihon Kohden) was used to monitor lead II ECG, HR obtained by ECG, MPAP, PAT, and PAOP and for the measurement of CO.

Experimental protocol

A schematic representation of procedures and drug administrations is shown in a figure (Fig. 4). After the instrumentation period, ETSEV was adjusted to maintain 3.2%, which was 1.5 times that of the previously reported sevoflurane MAC value in chapter 1, and was equilibrated for 60 min, simultaneously with an administration of lactated Ringer's solution at a temporarily increased rate of 10 ml/kg/hr for hydration. Thereafter, 2 ml of the venous blood sample was collected. Then, inulin (Inulead Injection; Fuji Yakuhin Co. Ltd, Japan) and p-amino hippuric acid (PAH) (Sodium Para-Aminohippurate Injection 10%; Daiichi Sankyo Company, Limited, Japan) in saline solutions were given IV through the left cephalic vein at respective primary

loading doses of 15 and 6 mg/kg, followed by CRI at 0.2 and 0.3 mg/kg/min throughout the experiment, respectively, allowing 30 min for equilibration [38, 50].

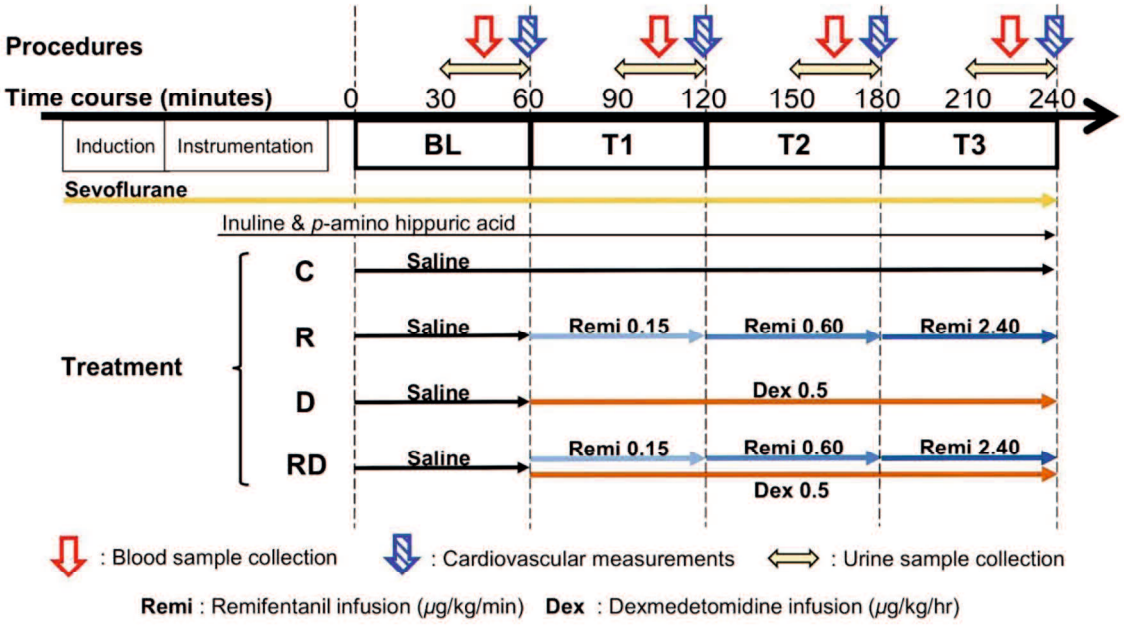

Figure 4. A schematic representation of procedures and drug administrations in each treatment (C, R, D or RD) in the present study. Measurements and sample collections were repeated every 60 min with saline as baseline (BL) and after drug administration assigned to each treatment until 240 min (T1, T2 and T3). Sevoflurane doses were adjusted to 1.5 times the minimum alveolar concentration (MAC) equivalent to sevoflurane alone during administration of remifentanil and/or dexmedetomidine infusions according to their MAC-sparing effects.

Following those equilibrations for anesthesia and infusions of inulin and PAH, the baseline measurement period (BL) was started at a set time of 0 min with the initiation of infusion of saline at 2 ml/kg/hr. The administration rate of lactated Ringer's solution was lowered to 3 ml/kg/hr, and it was continued throughout the experiment. After 30-min

equilibration for the infusion, the bladder was emptied, followed by continuous urine sample collection for 30 min (30−60 min). In the middle of the urine collecting period (45 min), 7 mL of the venous blood sample was collected from the proximal port of thermodilution catheter for the determination of plasma osmolality and electrolytes as well as plasma AVP, and serum inulin and PAH concentrations. An aliquot of 4 ml from the 7-ml blood sample was placed in a tube containing ethylene diamine tetra-acetic acid (EDTA) to obtain plasma. Another 2 ml was placed in a serum tube, and the remaining 1 ml was placed in a heparinized tube. At the end of the period (55−60 min), HR, ETCO2, ETSEV, SAP, MAP, DAP, RAP, MPAP, and PAT were recorded, and PAOP and CO were measured. Correspondingly, both arterial and mixed venous blood samples (0.5 ml, respectively) were anaerobically collected using 2-ml heparinized syringes (PICO 50; Radiometer K.K.) and were immediately placed in the analyzer (ABL-5; Radiometer K.K.) for the determination of pH, oxygen partial pressure, and carbon dioxide partial pressure, in both arterial (pHa, PaO2, and PaCO2 respectively) and mixed venous blood (pHmv, PmvO2, and PmvCO2, respectively). An additional 0.5 ml of the arterial blood sample was collected into an EDTA tube for measurements of hemoglobin (Hb) and hematocrit (Hct) (pocH-100iV Diff; Sysmex TMC Co. Ltd, Japan). The urine samples were collected (60 min) and stored on ice until centrifugation for separation of the supernatant after the measurement of urine volume using a plastic syringe and urine specific gravity (USG) using an analyzer (PAL-DOG&CAT; ATAGO Co. Ltd, Japan). All the samples were centrifuged at 2,000 g at 4°C for 15 min and stored until assay; plasma and serum samples were stored at −80°C and urine samples were stored at −30°C.

After completion of the procedures at BL (60 min), CRI of saline, remifentanil alone, dexmedetomidine alone, or remifentanil combined with dexmedetomidine according to treatment

was initiated through the right cephalic vein. Considering the MAC-sparing effect, the ETSEV was reduced for each treatment to 1.5 times sevoflurane MAC with these CRI, as described in chapter 1, to maintain sevoflurane doses that were equipotent to the requirement as that when sevoflurane was administered alone. The procedures of sample collection and measurements noted above were repeated every 60 min until 240 min (T1: 60−120 min; T2: 120−180 min; T3: 180−240 min). For the C treatment, an administration of saline (2 ml/kg/hr) with 3.2% ETSEV was continued from T1 to T3. For the D treatment, an infusion of dexmedetomidine (initiated with LD of 0.5 μg/kg for 10 min followed by 0.5 μg/kg/hr CRI) with 2.4% ETSEV was administered from T1 to T3. For the R treatment, remifentanil was administered in increments at 0.15 (T1), 0.6 (T2), and 2.4 (T3) μg/kg/min with 2.1, 1.5, and 1.1% ETSEV, respectively. For the RD treatment, dexmedetomidine was consistently administered through T1 to T3 and was combined with remifentanil at the following infusion rates (μg/kg/min) with ETSEV: 0.15 with 1.6%, 0.60 with 1.1%, and 2.40 with 0.7% at T1, T2, and T3, respectively. Solutions for CRI of remifentanil and dexmedetomidine, as well as a mixture of inulin and PAH, were made separately in saline to infuse at a rate of 1 ml/kg/hr using syringe pumps (TOP-551VC; TOP Corporation) that were calibrated before the study, in accordance with the manufacturer's specifications. An additional infusion of saline (1 ml/kg/hr) was prepared for R and D treatments; thus, the volume for drug administration was equal to 2 ml/kg/hr between each treatment. After all the measurements were completed at T3, all drug administrations were discontinued, and all catheters were removed; the dog was then allowed to recover from anesthesia. Meloxicam (0.2 mg/kg; Metacam; Boehringer Ingelheim Vetmedica Japan Co. Ltd) was subcutaneously administered during the recovery period.

Sample collection and analysis

Urine (U_{Osm}) and plasma osmolality (P_{Osm}) were measured using a vapor pressure osmometer (Wescor Vapro 5520, Phoenix Science Inc., Tokyo, Japan). The concentrations of electrolytes (i.e., sodium, potassium, and chloride) in both the urine and heparin plasma samples were measured using a clinical blood biochemical autoanalyzer (Fuji DRI-CHEM 7000V; Fujifilm Corporation, Japan). In both the urine and serum samples, the inulin and PAH concentrations were determined using commercially available kits enzymatically (Diacolor Inulin Kit; Toyobo Co. Ltd, Japan) and calorimetrically (PAH Assay Kit; Sigma-Aldrich Japan Co., Japan). The analysis of plasma AVP concentrations using EDTA plasma samples was outsourced (BML Inc., Japan) and was conducted by radioimmunoassay.

Data analysis

The measured blood gas and hemodynamic values were used for the calculation of CI, stroke volume index (SVI), systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI), arterial (SaO_2) and mixed vinous ($SmvO_2$) hemoglobin saturation, arterial bicarbonate (HCO_3^-), base excess (BE), oxygen delivery index (DO_2I), oxygen consumption index (VO_2I), and oxygen extraction (O_2ER), with reference to previously published formulae [22, 56]. Urine output (UO) was calculated from the 30-min urine volume. Using urine volume and the measured values of osmolality and electrolytes, osmolar clearance (CL_{Osm}), free-water clearance (CL_{H2O}), fractional clearance of sodium (FE_{Na}), potassium (FE_K), and chloride (FE_{Cl}) were calculated, as previously reported [63, 76]. Glomerular filtration rate (GFR) and renal blood flow (RBF) were obtained according to the clearances of inulin and PAH, respectively [25, 38].

Statistical analysis

The visual inspection of scatter plot and the Shapiro–Wilk test were used to verify the normality of distribution of data. Data with normal distribution are presented as mean ± SD and were analyzed by repeated-measures one-way ANOVA with post hoc Bonferroni multiple comparisons test to evaluate the effects between all time points within each treatment and using one-way ANOVA with post hoc Tukey–Kramer's test to identify differences among the treatments at each time point. Data with non-normal distribution are presented as median with range (minimum, maximum) and were analyzed using Friedman tests for within-treatment analyses and Kruskal–Wallis test for between-treatment comparisons, with post hoc Dunn's multiple comparisons test, respectively. Significance was established at $p < 0.05$. All data were analyzed using commercially available software (Prism version 7.05; GraphPad Software).

Results

The cardiovascular and renal functional variables, and plasma AVP levels at BL did not differ among the treatments. For the C treatment, all the variables did not significantly different over time.

The HR was significantly decreased in the R, D, and RD treatments than in the respective BL values and C treatment (Table 4). At all the time points, the SVI significantly increased from BL only in the R treatment group. The CI significantly decreased from BL in the D and RD treatments. For comparisons among the treatment groups, the CI values were significantly lower in the RD treatment than C and R treatments. At some time points, a significant increase above the BL values was observed for SAP and MAP in R, D, and RD treatments and for DAP in D

and RD treatments. The SVRI was significantly increased from BL with D and RD treatments and was significantly higher at all time points with RD treatment than with R treatment. The PAOP significantly increased from BL in the D and RD treatments, and was significantly higher in the RD treatment group at T3 than in the other three treatment groups. The RAP significantly increased at T3 from BL in R and RD treatments. The MPAP significantly increased in the RD treatment from BL, but PVRI did not change through the experimental period.

In the RD treatment at T3, pHa significantly decreased and manifested as mild acidemia (7.32 ± 0.04) as compared to that with the C treatment (Table 5). The HCO_3^- and BE significantly decreased from BL in the R and RD treatments at T3. The Hct and Hb significantly increased from BL with D and RD treatments in certain time points. Furthermore, both $PmvO_2$ and $SmvO_2$ significantly decreased from BL in the D treatment at some time points and in the RD treatment at T3. The values of PaO_2, $PaCO_2$, pHmv, DO_2I, VO_2I, and O_2ER did not change significantly over time and among treatments. The values of SaO_2, $PmvCO_2$, and PAT remained unchanged throughout the study (data not shown).

No significant changes in GFR and RBF were detected among the time points or treatments (Table 6). For the R treatment, none of the renal function variables significantly differed from that at BL and with C treatment. The renal function variables changed mainly in the D treatment: UO, CL_{Osm}, and FE_{Na} significantly increased and U_{Osm} and USG decreased as compared to the BL. Individually, two dogs (dogs B and F) showed a marked increase in UO to >10 times the BL value with urine dilution (300–400 mOsm/kg for UOsm) (Fig. 5). In the other two dogs (dogs A and D), a moderate increase in UO (three to four times of the values at BL) was observed; the remaining two dogs did not show polyuria. Increases in the values of CL_{Osm} and FE_{Na} compared with BL were observed only in dogs B and F, both of which showed a

marked UO increase. A moderate increase in UO from BL was also observed in RD treatment in some dogs, although the changes were not statistically significant. No significant changes in CL_{H2O}, P_{Osm}, FE_K, and FE_{Cl} did not change though the experiment, except for the FE_{Cl} value in the RD treatment group at T1.

Plasma potassium concentration (mEq/l) decreased slightly but significantly in the D treatment at T3 (4.1 ± 0.4) and in the RD treatment at T2 (4.5 ± 0.4) and T3 (4.3 ± 0.3) from BL. Both sodium and chloride concentrations in plasma were within normal ranges and were not significantly changed through this experiment (data not shown).

The plasma AVP concentrations significantly increased from BL to T3 with R treatment (p = 0.042; Fig. 6B); however, the concentration significantly decreased at T3 with D treatment (p = 0.0002; Fig. 6C) and at T1 with RD treatment (p = 0.011; Fig. 6D) compared with BL, respectively. In the comparisons among the treatments at T3, plasma AVP concentration was significantly higher with RD treatment than with D treatment (p = 0.029).

Table 4. Cardiovascular variables (mean ± standard deviation) in six Beagle dogs before (baseline: BL) and during an infusion of saline as control (C), remifentanil at incremented doses of 0.15 (T1), 0.60 (T2) and 2.40 (T3) μg/kg/min (R), dexmedetomidine at 0.5 μg/kg/hr (D), or the combination of remifentanil and dexmedetomidine (RD) under sevoflurane anesthesia equipotent to 1.5 times of minimum alveolar concentration.

Variable	Treatment	BL	T1	T2	T3
HR (beats/min)					
	C	100.3 ± 18.0	101.5 ± 18.0[d)]	103.5 ± 16.2[cd)]	103.8 ± 16.1[cd)]
	R	102.7 ± 19.4	82.8 ± 12.0[a)]	76.8 ± 10.9[ab)]	71.5 ± 12.8[ab)]
	D	101.2 ± 14.3	79.3 ± 10.6[ab)]	79.5 ± 12.5[ab)]	80.0 ± 14.7[ab)]
	RD	104.5 ± 17.9	71.8 ± 7.8[ab)]	66.7 ± 8.4[ab)]	60.0 ± 11.2[ab)]
SAP (mm Hg)					
	C	100.3 ± 8.8	98.7 ± 11.2	97.5 ± 8.3[cd)]	100.8 ± 9.2[cd)]
	R	104.0 ± 10.6	109.0 ± 12.1	124.5 ± 13.5[ab)]	136.7 ± 14.7[ab)]
	D	100.0 ± 13.3	123.5 ± 14.1[a)]	121.5 ± 16.4[ab)]	124.0 ± 11.9[ab)]
	RD	97.8 ± 10.0	126.0 ± 25.5[b)]	130.8 ± 16.6[ab)]	145.3 ± 18.9[ab)]
MAP (mm Hg)					
	C	70.5 ± 6.2	69.5 ± 6.0	69.7 ± 4.5	71.0 ± 4.3[c)]
	R	72.7 ± 9.4	70.7 ± 9.3	81.3 ± 10.3	92.8 ± 12.2[ab)]
	D	70.0 ± 6.3	91.0 ± 13.6	88.5 ± 14.6[a)]	90.0 ± 11.5[a)]
	RD	67.7 ± 2.2	93.2 ± 23.3[b)]	97.3 ± 22.4[b)]	109.2 ± 19.8[ab)]
DAP (mm Hg)					
	C	58.7 ± 5.9	57.5 ± 5.4	58.0 ± 3.2	58.5 ± 3.4
	R	59.3 ± 8.7	56.0 ± 7.9	64.7 ± 8.4	75.5 ± 10.3
	D	58.0 ± 4.8	77.5 ± 11.7[a)]	76.2 ± 14.3[a)]	77.5 ± 11.9[a)]
	RD	56.8 ± 2.6	80.7 ± 23.3[bc)]	82.8 ± 22.9[b)]	93.3 ± 19.8[ab)]
RAP (mm Hg)					
	C	2.7 ± 0.5	2.7 ± 0.5	2.3 ± 0.5	2.7 ± 1.0[c)]
	R	2.5 ± 0.8	4.2 ± 1.8	4.2 ± 1.8	5.2 ± 1.7[ab)]
	D	2.7 ± 1.0	4.0 ± 1.1	3.7 ± 1.2	4.0 ± 1.7
	RD	2.5 ± 1.0	4.2 ± 2.0	4.8 ± 1.8[b)]	6.7 ± 1.4[abd)]
MPAP (mm Hg)					
	C	11.5 ± 1.5	11.5 ± 1.4	11.3 ± 1.0	11.5 ± 1.0
	R	11.2 ± 0.4	11.8 ± 2.7	12.5 ± 2.2	12.8 ± 1.7
	D	11.5 ± 1.0	12.3 ± 0.5	12.2 ± 1.2	12.0 ± 1.3
	RD	11.0 ± 1.1	12.3 ± 1.4[a)]	13.0 ± 0.9[a)]	14.0 ± 1.4[ab)]
PAOP (mm Hg)					
	C	5.2 ± 1.2	5.3 ± 1.2	5.0 ± 1.1	5.0 ± 1.7
	R	5.2 ± 1.2	5.7 ± 1.5	6.3 ± 1.6	6.8 ± 1.9
	D	4.8 ± 0.8	6.8 ± 1.5[a)]	6.5 ± 1.4[a)]	6.3 ± 0.8[a)]
	RD	5.0 ± 0.9	6.5 ± 2.1	8.0 ± 2.4[ab)]	9.7 ± 1.0[abcd)]
CI (l /min/m^2)					
	C	2.90 ± 0.46	2.89 ± 0.30	2.90 ± 0.38	3.03 ± 0.39
	R	3.05 ± 0.34	2.97 ± 0.48	3.09 ± 0.85	2.80 ± 0.88
	D	3.03 ± 0.37	2.28 ± 0.47[a)]	2.26 ± 0.43[a)]	2.36 ± 0.55[a)]
	RD	2.99 ± 0.50	2.14 ± 0.45[abc)]	2.12 ± 0.48[ac)]	1.92 ± 0.60[ab)]
SVI (ml /beat/m^2)					
	C	29.1 ± 5.9	29.0 ± 5.2	28.3 ± 4.2	29.5 ± 3.7[c)]
	R	30.2 ± 3.5	36.4 ± 6.9[a)]	39.8 ± 6.4[abd)]	38.5 ± 6.3[abd)]
	D	30.5 ± 6.5	29.1 ± 7.0	28.7 ± 6.0[c)]	29.5 ± 6.0[c)]
	RD	28.9 ± 4.4	29.7 ± 5.7	31.4 ± 4.1	31.4 ± 4.5

SVRI (dynes·sec/cm^5/m^2)					
	C	1929 ± 355	1861 ± 195[d)]	1879 ± 270[d)]	1826 ± 297
	R	1838 ± 135	1822 ± 359[d)]	2105 ± 542	2744 ± 1078
	D	1789 ± 202	3109 ± 529[abc)]	3067 ± 617[ab)]	3054 ± 787[a)]
	RD	1790 ± 314	3406 ± 882[abc)]	3623 ± 970[abc)]	4631 ± 1575[abc)]
PVRI (dynes·sec/cm^5/m^2)					
	C	177 ± 18	171 ± 29	175 ± 45	172 ± 48
	R	159 ± 32	171 ± 69	169 ± 54	182 ± 48
	D	180 ± 42	194 ± 29	201 ± 34	198 ± 35
	RD	166 ± 42	226 ± 56	200 ± 81	179 ± 30

HR, heart rate; SAP, systolic arterial pressure; MAP, mean arterial pressure; DAP, diastolic arterial pressure; RAP, right atrial pressure; MPAP, mean pulmonary artery pressure; PAOP, pulmonary artery occlusion pressure; CI, cardiac index; SVI, stroke volume index; SVRI, systemic vascular resistance index; PVRI, pulmonary vascular resistance index. [a)] significantly differ from respective baseline value ($p < 0.05$); [b)] significantly differ from C treatment at this time point ($p < 0.05$); [c)] significantly differ from R treatment at this time point ($p < 0.05$); [d)] significantly differ from D treatment at this time point ($p < 0.05$).

Table 5. Blood gas and oxygenation variables (mean ± standard deviation) in six Beagle dogs before (baseline: BL) and during an infusion of saline as control (C), remifentanil at incremented doses of 0.15 (T1), 0.60 (T2) and 2.40 (T3) μg/kg/min (R), dexmedetomidine at 0.5 μg/kg/hr (D), or the combination of remifentanil and dexmedetomidine (RD) under sevoflurane anesthesia equipotent to 1.5 times of minimum alveolar concentration.

Variable	Treatment	BL	T1	T2	T3
pHa					
	C	7.39 ± 0.02	7.37 ± 0.02	7.39 ± 0.01	7.38 ± 0.02
	R	7.37 ± 0.03	7.37 ± 0.03	7.38 ± 0.04	7.35 ± 0.04
	D	7.37 ± 0.03	7.38 ± 0.02	7.39 ± 0.05	7.37 ± 0.03
	RD	7.37 ± 0.02	7.35 ± 0.04	7.34 ± 0.04	7.32 ± 0.04[a)]
PaO_2 (mm Hg)					
	C	562 ± 39	561 ± 47	564 ± 48	570 ± 47
	R	560 ± 25	558 ± 30	559 ± 71	562 ± 35
	D	575 ± 40	578 ± 17	595 ± 23	573 ± 31
	RD	550 ± 45	571 ± 42	564 ± 40	559 ± 36
$PaCO_2$ (mm Hg)					
	C	39.2 ± 3.7	39.8 ± 2.0	38.2 ± 2.5	38.3 ± 3.1
	R	39.5 ± 3.3	40.0 ± 3.0	38.2 ± 5.9	39.8 ± 3.4
	D	39.3 ± 2.1	38.5 ± 1.2	37.0 ± 3.3	36.7 ± 2.2
	RD	40.7 ± 1.2	41.2 ± 3.3	41.2 ± 2.8	40.3 ± 2.3
HCO_3^- (mEq/l)					
	C	22.7 ± 1.6	22.3 ± 1.0	22.2 ± 1.0	22.1 ± 1.4
	R	22.2 ± 1.1	22.2 ± 1.1	21.4 ± 1.7	21.0 ± 1.2[a)]
	D	22.2 ± 1.3	21.9 ± 0.9	21.4 ± 0.8	20.7 ± 1.3
	RD	22.6 ± 1.3	22.0 ± 1.5	21.5 ± 1.4	20.1 ± 2.1[a)]
Base excess (mEq/l)					
	C	–2.3 ± 1.6	–2.9 ± 1.2	–2.8 ± 0.9	–3.0 ± 1.4
	R	–3.0 ± 1.1	–3.1 ± 1.3	–3.8 ± 1.4	–4.7 ± 1.5[a)]
	D	–3.1 ± 1.7	–3.2 ± 1.2	–3.6 ± 1.4	–5.0 ± 2.5
	RD	–2.8 ± 1.7	–3.6 ± 2.0	–4.2 ± 1.8	–6.1 ± 2.7[a)]
pHmv					
	C	7.33 ± 0.02	7.32 ± 0.02	7.34 ± 0.02	7.33 ± 0.02
	R	7.33 ± 0.02	7.32 ± 0.02	7.32 ± 0.04	7.32 ± 0.04
	D	7.33 ± 0.02	7.33 ± 0.03	7.33 ± 0.03	7.31 ± 0.04
	RD	7.32 ± 0.02	7.31 ± 0.06	7.30 ± 0.04	7.29 ± 0.05
$PmvO_2$ (mm Hg)					
	C	63.7 ± 3.2	64.3 ± 1.5	66.0 ± 5.1	68.2 ± 5.5[cd)]
	R	67.5 ± 5.6	62.3 ± 3.9	61.8 ± 9.1	58.0 ± 8.0[b)]
	D	65.5 ± 2.7	58.0 ± 4.5[a)]	56.7 ± 5.4[a)]	56.7 ± 6.1[ab)]
	RD	65.7 ± 2.7	56.2 ± 6.6[b)]	57.8 ± 4.6	53.3 ± 3.0[ab)]
$SmvO_2$ (%)					
	C	87.6 ± 1.5	88.0 ± 0.7	88.5 ± 2.0	89.4 ± 2.0
	R	89.1 ± 2.4	86.8 ± 2.3	85.9 ± 4.1	83.6 ± 5.5
	D	88.5 ± 1.2	84.2 ± 3.0[a)]	83.1 ± 4.3[a)]	82.9 ± 5.2
	RD	88.5 ± 1.3	82.6 ± 4.8[b)]	84.1 ± 3.0	80.9 ± 2.3[ab)]
Hct (%)					
	C	32.3 ± 3.4	32.5 ± 3.8	32.2 ± 3.5	31.8 ± 4.0
	R	32.8 ± 3.2	30.2 ± 0.4[d)]	31.2 ± 2.6[d)]	30.5 ± 3.3[d)]
	D	32.5 ± 3.4	36.2 ± 2.9[ac)]	37.2 ± 3.1[ac)]	38.2 ± 3.0[abc)]
	RD	32.5 ± 2.4	35.8 ± 3.3[ac)]	37.0 ± 3.7[c)]	37.8 ± 4.5[c)]

Hb (g/dl)					
	C	10.4 ± 0.8	10.8 ± 1.3	11.1 ± 1.3	11.2 ± 2.0
	R	10.5 ± 0.8	10.1 ± 0.3[d)]	10.3 ± 0.8[d)]	10.3 ± 1.3
	D	10.8 ± 1.2	12.1 ± 1.2[ac)]	12.5 ± 1.0[ac)]	12.7 ± 1.1[a)]
	RD	10.9 ± 0.6	12.0 ± 1.1[c)]	12.3 ± 1.3[c)]	12.5 ± 1.5
DO_2I (ml /min/m^2)					
	C	443 ± 60	461 ± 48	479 ± 86	505 ± 90
	R	481 ± 70	451 ± 79	484 ± 179	443 ± 192
	D	488 ± 41	404 ± 64	417 ± 76	437 ± 95
	RD	484 ± 75	378 ± 77	386 ± 104	357 ± 135
VO_2I (ml /min/m^2)					
	C	91 ± 11	93 ± 11	92 ± 14	93 ± 11
	R	92 ± 15	92 ± 10	103 ± 18	101 ± 20
	D	96 ± 9	92 ± 8	96 ± 10	98 ± 14
	RD	93 ± 13	90 ± 11	85 ± 11	85 ± 28
O_2ER (%)					
	C	21 ± 2	20 ± 1	19 ± 3	19 ± 3
	R	19 ± 2	21 ± 3	22 ± 4	25 ± 5
	D	20 ± 1	23 ± 2	24 ± 4	24 ± 5
	RD	19 ± 1	25 ± 5	23 ± 3	24 ± 5

pHa, arterial blood pH; PaO_2, arterial blood oxygen partial pressure; $PaCO_2$, arterial blood carbon dioxide partial pressure; HCO_3^-, arterial bicarbonate; pHmv, mixed venous blood pH; $PmvO_2$, mixed venous blood oxygen partial pressure; $SmvO_2$, mixed venous hemoglobin saturation; Hct, Hematocrit; Hb, Hemoglobin; DO_2I, oxygen delivery index; VO_2I, oxygen consumption index; O_2ER, oxygen extraction. [a)] significantly differ from respective baseline value ($p < 0.05$); [b)] significantly differ from C treatment at this time point ($p < 0.05$); [c)] significantly differ from R treatment at this time point ($p < 0.05$); [d)] significantly differ from D treatment at this time point ($p < 0.05$).

Table 6. Renal function variables (mean ± standard deviation or median with range [minimum, maximum]) in six Beagle dogs before (baseline: BL) and during an infusion of saline as control (C), remifentanil at incremented doses of 0.15 (T1), 0.60 (T2) and 2.40 (T3) μg/kg/min (R), dexmedetomidine at 0.5 μg/kg/hr (D), or the combination of remifentanil and dexmedetomidine (RD) under sevoflurane anesthesia equipotent to 1.5 times of minimum alveolar concentration.

Variable	Treatment	BL	T1	T2	T3
UO (m*l* /min/kg)					
	C	0.015 (0.010, 0.022)	0.021 (0.011, 0.045)	0.017 (0.012, 0.032)	0.020 (0.013, 0.052)
	R	0.014 (0.011, 0.032)	0.015 (0.010, 0.019)	0.014 (0.010, 0.030)[d)]	0.017 (0.014, 0.040)
	D	0.015 (0.009, 0.020)	0.024 (0.017, 0.126)	0.035 (0.019, 0.228)[ac)]	0.054 (0.024, 0.264)[a)]
	RD	0.015 (0.012, 0.027)	0.024 (0.015, 0.060)	0.018 (0.012, 0.070)	0.017 (0.013, 0.029)[d)]
USG					
	C	1.041 ± 0.009	1.037 ± 0.014	1.041 ± 0.013	1.036 ± 0.014[d)]
	R	1.038 ± 0.012	1.046 ± 0.009[d)]	1.044 ± 0.010[d)]	1.037 ± 0.009[d)]
	D	1.039 ± 0.009	1.025 ± 0.012[c)]	1.019 ± 0.011[abc)]	1.017 ± 0.010[abc)]
	RD	1.038 ± 0.010	1.030 ± 0.010	1.034 ± 0.014	1.040 ± 0.010[d)]
U_{Osm} (mOsm/kg H_2O)					
	C	1051 ± 179	1013 ± 270	1068 ± 192	964 ± 272
	R	1018 ± 231	1093 ± 199[d)]	1120 ± 132[d)]	1021 ± 210[d)]
	D	996 ± 156	698 ± 240[c)]	574 ± 231[abc)]	510 ± 201[abc)]
	RD	996 ± 203	732 ± 206	854 ± 331	1054 ± 201[d)]
P_{Osm} (mOsm/kg H_2O)					
	C	295 ± 5	294 ± 3	295 ± 5	295 ± 4
	R	295 ± 5	292 ± 2	289 ± 3	289 ± 4
	D	294 ± 2	293 ± 2	291 ± 4	290 ± 3
	RD	293 ± 3	291 ± 2	292 ± 3	291 ± 4
CL_{Osm} (m*l* /min/kg)					
	C	0.056 (0.044, 0.066)	0.064 (0.051, 0.093)	0.060 (0.048, 0.090)	0.070 (0.054, 0.083)
	R	0.052 (0.048, 0.071)	0.055 (0.044, 0.066)	0.056 (0.042, 0.099)	0.063 (0.052, 0.106)
	D	0.051 (0.041, 0.059)	0.069 (0.044, 0.176)	0.075 (0.047, 0.245)[a)]	0.104 (0.057, 0.275)[a)]
	RD	0.050 (0.042, 0.075)	0.059 (0.045, 0.081)	0.055 (0.042, 0.073)	0.058 (0.048, 0.075)
CL_{H2O} (m*l* /min/kg)					
	C	–0.037 (–0.047, –0.034)	–0.046 (–0.058, –0.039)	–0.042 (–0.058, –0.036)	–0.044 (–0.054, –0.031)
	R	–0.037 (–0.047, –0.037)	–0.041 (–0.047, –0.027)	–0.042 (–0.070, –0.031)	–0.048 (–0.066, –0.036)
	D	–0.034 (–0.041, –0.025)	–0.042 (–0.055, –0.025)	–0.030 (–0.055, –0.017)	–0.029 (–0.074, –0.010)
	RD	–0.036 (–0.049, –0.026)	–0.035 (–0.044, –0.020)	–0.037 (–0.050, 0.017)	–0.044 (–0.050, –0.035)
GFR (m*l* /min/kg)					
	C	3.39 (2.82, 4.12)	2.84 (2.64, 4.27)	3.06 (2.78, 4.39)	3.19 (2.40, 5.52)
	R	3.56 (2.26, 4.42)	3.18 (2.60, 3.79)	2.90 (2.47, 4.50)	3.14 (2.10, 3.54)
	D	3.72 (2.63, 3.96)	3.18 (2.48, 4.57)	3.20 (2.65, 4.03)	3.37 (2.43, 4.72)
	RD	3.32 (2.73, 3.93)	3.45 (2.75, 4.20)	3.08 (2.63, 4.37)	3.39 (3.09, 4.30)
RBF (m*l* /min/kg)					
	C	12.4 (9.6, 19.8)	16.6 (10.6, 26.3)	19.6 (11.4, 26.3)	15.0 (11.0, 19.0)
	R	14.8 (7.4, 19.7)	12.6 (8.6, 15.1)	13.3 (10.3, 21.7)	17.0 (11.7, 25.5)
	D	11.7 (10.7, 19.8)	16.3 (10.2, 26.8)	18.0 (9.3, 19.7)	19.4 (14.5, 29.6)
	RD	12.0 (9.6, 15.9)	19.7 (9.3, 24.8)	19.5 (8.4, 22.6)	14.1 (10.0, 25.6)

FE_{Na} (%)					
	C	0.32 (0.15, 0.48)	0.77 (0.16, 0.98)	0.38 (0.11, 1.58)	0.67 (0.12, 1.43)
	R	0.27 (0.13, 0.41)	0.21 (0.16, 0.30)	0.25 (0.11, 0.42)	0.32 (0.17, 0.88)
	D	0.22 (0.16, 0.32)	0.34 (0.14, 3.73)	0.66 (0.24, 5.86)[a)]	0.93 (0.28, 10.18)[a)]
	RD	0.24 (0.15, 0.52)	0.27 (0.16, 0.36)	0.31 (0.10, 0.44)	0.29 (0.17, 0.61)
FE_K (%)					
	C	27.6 (22.3, 56.0)	37.6 (28.4, 51.5)	31.9 (15.4, 38.7)	32.5 (9.19, 47.5)
	R	37.3 (18.9, 55.5)	28.8 (16.5, 40.3)	36.0 (12.5, 42.0)	41.7 (17.9, 47.2)
	D	27.9 (19.5, 41.0)	38.1 (15.7, 74.4)	33.7 (21.1, 44.4)	43.4 (16.5, 69.6)
	RD	33.5 (18.3, 57.2)	27.3 (16.1, 32.5)	26.6 (17.2, 57.1)	28.6 (21.5, 44.8)
FE_{Cl} (%)					
	C	0.66 (0.27, 0.95)	0.92 (0.57, 1.30)	0.58 (0.33, 1.97)	0.89 (0.18, 1.92)
	R	0.54 (0.29, 1.40)	0.48 (0.40, 0.76)	0.54 (0.30, 1.50)	0.86 (0.39, 2.28)
	D	0.50 (0.25, 0.89)	0.75 (0.16, 3.39)	0.67 (0.51, 6.05)	1.26 (0.32, 10.17)
	RD	0.53 (0.28, 1.97)	0.34 (0.20, 0.74)[b)]	0.58 (0.088, 1.69)	0.92 (0.48, 1.71)

UO, urine output; USG, urine specific gravity; U_{Osm}, urine osmolality; P_{Osm}, plasma osmolality; CL_{Osm}, osmolar clearance; CL_{H2O}, free-water clearance; GFR, glomerular filtration rate; RBF, renal blood flow; FE_{Na}, fractional clearance of sodium; $FE_{K,}$ fractional clearance of potassium; FE_{Cl}, fractional clearance of chloride. [a)] significantly differ from respective baseline value ($p < 0.05$); [b)] significantly differ from C treatment at this time point ($p < 0.05$); [c)] significantly differ from R treatment at this time point ($p < 0.05$); [d)] significantly differ from D treatment at this time point ($p <0.05$).

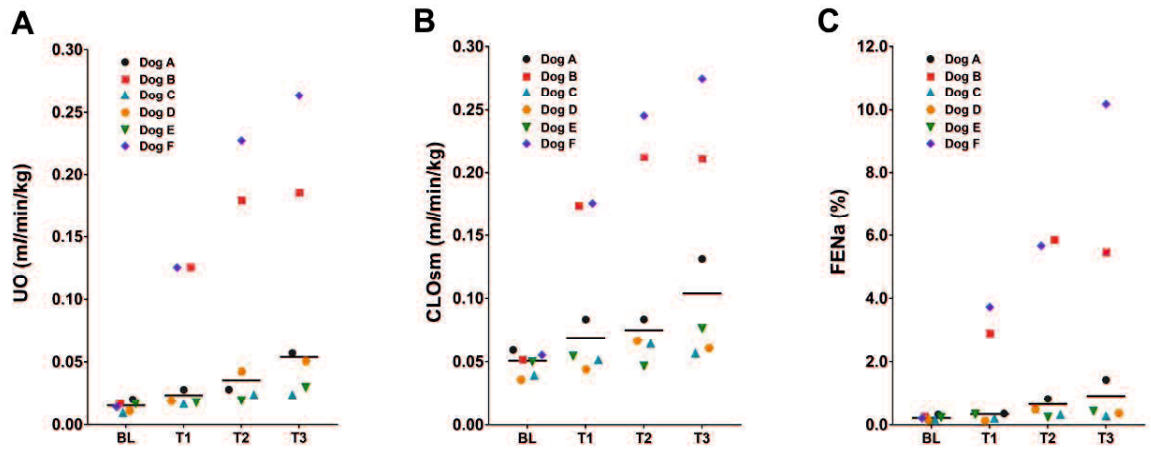

Figure 5. The median (bar) and individual values of (A) Urine output (UO), (B) osmolar clearance (CL_{Osm}), and (C) fractional clearance of sodium (FE_{Na}) in six Beagle dogs that before (baseline: BL) and after administration of dexmedetomidine infused at 0.5 μg/kg/hr (T1-T3).

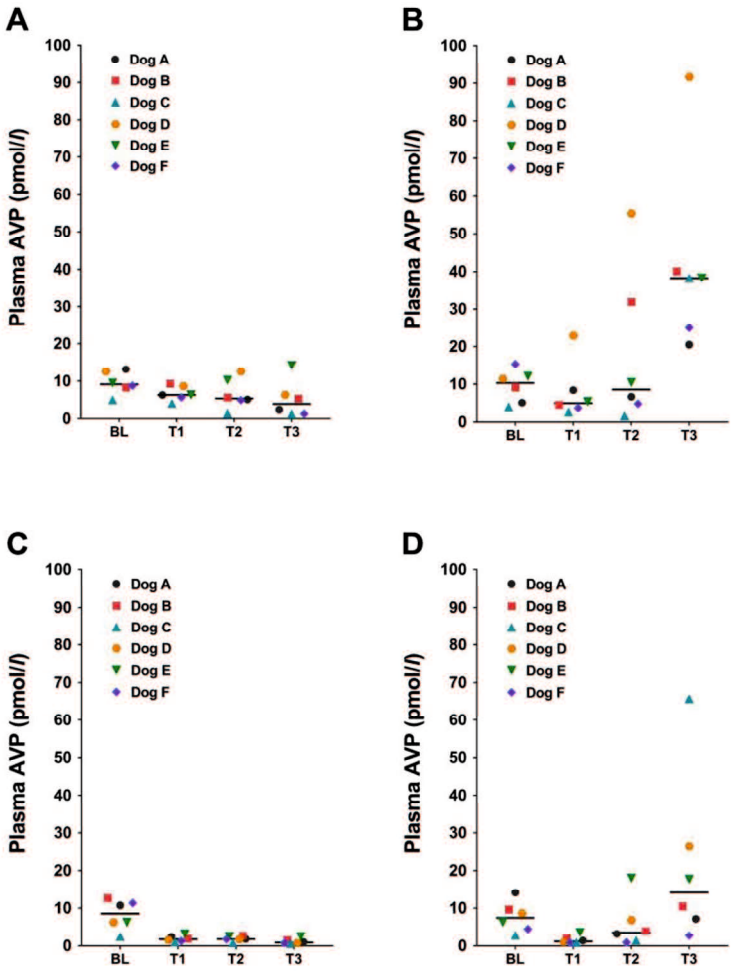

Figure 6. The median (bar) and individual values of plasma arginine vasopressin (AVP) concentrations in six Beagle dogs that before (baseline: BL) and after an infusion of (A) saline (C treatment), (B) remifentanil at incremented doses of 0.15 (T1), 0.60 (T2) and 2.40 (T3) μg/kg/min (R treatment), (C) dexmedetomidine at 0.5 μg/kg/hr consistently through T1-T3 (D treatment), and (D) the combination of dexmedetomidine and remifentanil (RD treatment) under sevoflurane anesthesia equipotent to 1.5 times of minimum alveolar concentration.

Discussion

In the present study, we outlined the changes in cardiovascular and renal functions, along with evaluations of AVP secretion with remifentanil and dexmedetomidine infusion, alone or in combination, in sevoflurane-anesthetized dogs. In this study, the remifentanil infusion led to a dose-dependent decrease in HR, although CI was maintained because of increased SVI, compared with BL. These properties were not consistent with results reported previously with remifentanil CRI at 0.15 to 0.90 μg/kg/min in mixed-breed dogs under isoflurane anesthesia equipotent to 1.3 MAC [42], as the previous study has demonstrated a significant decrease in CI with greater decreases in HR than those observed in the current study. The cardiovascular changes with dexmedetomidine, HR-dependent decrease in CI with increased SVRI and blood pressure, were typical to α_2-adrenoceptor agonists as reported previously [31, 36, 44, 53], but CI reduction in this study slightly varied from that in previous reports showing reduction values of 1 to 17% with dexmedetomidine CRI at 0.5 μg/kg/hr [53] and of 27.1% with medetomidine at 1.0 μg/kg/hr (equivalent to 0.5 μg/kg/hr of dexmedetomidine) [31], in isoflurane-anesthetized dogs. The differences in CI changes between the present and previous studies may be attributed to individual differences in dogs or variation in the inhaled anesthetics.

In the study reported here, the decreases in HR and CI with dexmedetomidine alone were not significantly augmented by an additional administration of remifentanil. These results coincided with previous reports that did fentanyl CRI combined with dexmedetomidine (0.1 to 3.0 μg/kg IV) in enflurane-anesthetized dogs [65] and that used fentanyl IV bolus in dogs sedated with medetomidine (1.5 μg/kg/hr) [19]. Both remifentanil and dexmedetomidine can

decrease HR and increase vagal tone; these changes are resulted by vagotonic effect with remifentanil through the stimulation of central and peripheral μ-opioid receptors [67]; however, they are associated with the baroreceptor reflex with dexmedetomidine in response to peripheral vasoconstriction resulting from the activation of α_{2b} adrenoceptors in vascular smooth muscle, or the sympatholytic effect through central α_2 adrenoceptor in the brainstem [47]. It is suggested that the influences in cardiovascular system with dexmedetomidine are more direct and are greater than those with remifentanil.

The highest infusion rate (2.4 μg/kg/min) of remifentanil was included in this study to clarify the effects when the rate was excessively high above the clinically used range (0.15 and 0.60 μg/kg/min). In RD treatment, SVRI was significantly elevated according to increased remifentanil rates particularly at T3, with concurrent increases in RAP and PAOP. The sympatholytic activity with dexmedetomidine can reduced inotropy and thus limit stroke volume in the face of increased afterload [47]. Although there was no statistically significant difference in CI between D and RD treatments, the increased RAP and PAOP are likely to represent blood stasis in the heart as a result of increasingly enhanced vasoconstriction by the combined CRI of remifentanil and dexmedetomidine. Moreover, pHa was significantly decreased at T3 in RD treatment; this was similar to the reported effects of remifentanil alone accompanied with significant decreases in DO_2I and increases in SVRI [42]. We did not measure the lactate concentration in blood, but the concomitant decrease in HCO_3^- and BE at a relatively low pHa indicated metabolic acidosis, suggesting decreased oxygenation in some tissues. These results suggested that the remifentanil particularly at excessively high rates during a dexmedetomidine infusion causes undesirable effects that result from excessive vasoconstriction and thus should be avoided.

Sevoflurane also induces dose-dependent cardiopulmonary depression and decreases arterial pressure primarily via systemic vasodilation [49]. In this study, sevoflurane doses in each treatment or time point were reduced to be equipotent to 1.5 MAC according to the MAC-sparing effects of remifentanil and dexmedetomidine reported in chapter 1. Therefore, the increased SVRI during administrations of remifentanil and dexmedetomidine CRI would be also induced by the modulation of peripheral vasodilation associated with reduced sevoflurane doses, similar to those in a previous study using remifentanil CRI during isoflurane anesthesia equipotent to 1.3 MAC in dogs [42].

This study showed that dexmedetomidine CRI obviously increased Hct and Hb by approximately 5% and 2 g/dL, respectively, in mean values in both alone and combined with remifentanil in dogs. Although the mechanisms have not been elucidated, the increased Hct and Hb were similarly reported in some studies with dexmedetomidine CRI in anesthetized dogs [37, 42]. The recruitment of red blood cells from the spleen could cause these increases; however, this idea has been controversial because the dexmedetomidine administration did not induce marked splenic contraction in dogs [5]. Another possible cause is a blood concentrating activity resulted by the movement of intravascular fluid to the interstitium because of increased hydrostatic pressure in micro circulation related to vasoconstriction.

The present study demonstrated that DO_2I and O_2ER values were unchanged with dexmedetomidine, either alone and in combination with remifentanil, despite the decreased CI, presumably because of an increase in oxygen content (Hct and Hb). Further, changes in $PmvO_2$ and $SmvO_2$ were within the physiologically normal ranges [22] although these values were decreased in D and RD treatments in parallel with the CI changes. Thus, we considered that the

cardiovascular changes are acceptable for healthy dogs in terms of maintenance of global oxygenation during combined infusions of remifentanil and dexmedetomidine.

The present study showed that despite the effect on CI due to the administration of remifentanil and dexmedetomidine, GFR and RBF were not significantly altered. These results were consistent with those in a previous study that dexmedetomidine alone were infused at 1.0 and 2.0 μg/kg/hr in isoflurane-anesthetized dogs [76]. Also, it has been demonstrated that GFR and RBF were not decreased by fentanyl administration even when the doses were sufficiently high to decrease blood pressure in dogs [10]. Dexmedetomidine decreases CO but did not eventually cause a reduction in the perfusion of vital organs, including the kidneys because functional redistribution of blood flow may occur [35]. Our results indicated that the hemodynamic changes with dexmedetomidine at 0.5 μg/kg/hr were enough to preserve functional renal perfusions even when used in combination with remifentanil.

In clinical relevance, the use of anticholinergic drugs to recover the decreased HR is debatable with the combined administration of remifentanil and dexmedetomidine during sevoflurane anesthesia. We suggest that aggressive recovery of HR in healthy dogs is not required when arterial blood pressure is maintained, because of global oxygenation and renal function as shown in the current study. In addition, anticholinergic drugs may lead to further hypertension particularly during dexmedetomidine administration [64]. Drugs that can attenuate systemic vasoconstriction, such as a peripheral α_2-adrenoceptor antagonist, would be an alternative for alleviating the bradycardia [64].

The plasma AVP levels at BL were higher than the physiologic reference range of 1 to 6 pmol/l in dogs [77]; possibly due to anesthetic conditions [23] or noxious stimuli such as intubation and catheter instrumentation. Remifentanil increased the plasma AVP concentrations

only at a relatively high infusion rate of 2.4 μg/kg/min compared from BL. The magnitude of the increase in plasma AVP concentrations with remifentanil was less, compared with those in a previous study reporting that remifentanil CRI at 0.15 to 0.90 μg/kg/min significantly increased the plasma AVP concentrations by 10- to 20-fold from that at baseline [42]. Monteiro et al. [42] also reported that the increase in plasma AVP levels correlated well with the significant increase in SVRI accompanied by CI reductions. In the present study, we did not find any significant changes in SVRI and CI with remifentanil alone. The increase in AVP secretion with pure μ opioid agonists possibly occurs as a compensatory response to a decrease in blood pressure secondary to bradycardia [61] or as a direct stimulation via μ opioid receptors in the neurohypophysis [26]. Simultaneously, the increase in SVRI with opioids was reported as part of the physiologic compensation for decreased blood pressure by bradycardia [30]. Therefore, the less increase in SVRI and plasma AVP concentrations in this study than in the previous report might have been related to the small CI changes. Interestingly, in this present study, 2.4 μg/kg/min of remifentanil significantly increased plasma AVP levels despite maintained CI. The result suggests the direct effect of the at least high rate of remifentanil on AVP secretion.

In contrast, plasma AVP levels were lowered by dexmedetomidine at 0.5 μg/kg/hr in accordance with previously published results on isoflurane-anesthetized dogs that received IV medetomidine at 20 and 40 μg/kg [63] and dexmedetomidine CRI at 1.0 to 2.0 μg/kg/hr [76]. Furthermore, the present study demonstrated that dexmedetomidine attenuated AVP secretion, even when given in combination with low-dose (0.15 μg/kg/min) remifentanil, whereas the attenuation disappeared under remifentanil infusion above 0.6 μg/kg/min. These results indicated dose-dependent interference activity between remifentanil and dexmedetomidine on AVP secretion. The mechanisms responsible for the decrease in AVP secretion with dexmedetomidine

were not addressed in the current study, but it was presumably secondary to increased blood pressure [28] or was the result of the direct inhibition of neurosecretory cells in the hypothalamus [9, 32].

The UO significantly increased only with D treatment but was unchanged in C, R and RD treatments as compared to respective baseline values. UO with remifentanil CRI did not significantly differ from baseline, although the antidiuretic effects of opioids have been recognized in dogs [1, 60]. Anderson et al. [1] reported that the UO was significantly reduced to 0.012 ml/kg/min with a fentanyl CRI in healthy dogs compared from the control value of 0.021 ml/kg/min with an infusion of lactated Ringer's solution, which was higher than our baseline values. The relatively lower UO values at BL in the present study might cause ambiguity in the antidiuretic effect.

Previous reports have shown that α_2-adrenoceptor agonists produced diuretic effects in dogs, regardless of whether or not they received general anesthesia, including both aquaretic and natriuretic conditions [8, 9, 63, 68, 70, 76]. In our experiment, dexmedetomidine CRI induced diuresis that was concomitant with increased CL_{Osm} and FE_{Na}, which represents the natriuresis. The diuretic condition was different from results in previous studies demonstrating that dexmedetomidine CRI (1.0 and 2.0 μg/kg/hr) and medetomidine IV (20 and 40 μg/kg) resulted in aquaresis (increases in UO and CL_{H2O}) in isoflurane-anesthetized dogs, although concurrent decreases in plasma AVP levels was consistently observed in the present and previous studies [63, 76]. The cause of variations in diuretic conditions between the present and previous studies were still unknown but might have included the differences in administration doses or underlying hydration state of dogs. Considerable individual variation between dogs in this study also might

have been caused by a lower dose rate (0.5 μg/kg/hr) of dexmedetomidine that was presumably too low to reveal the effect eventually in all dogs.

The concurrent administration of remifentanil seemed to make the dexmedetomidine-induced diuresis unclear in our experiment; to our knowledge, this was the first report representing the interference activity of an μ-opioid of remifentanil on the diuretic properties of dexmedetomidine. Although the experimental study has exhibited the diuretic effect with α_2-adrenoceptor agonists, clinically it has never been well demonstrated in veterinary practice. In human medicine, few studies have reported dexmedetomidine-induced polyuria including both aquaresis and natriuresis; however, the occurrence is likely to be rare despite its widespread use [33, 55]. Our study suggests that the existence of co-administered opioid analgesics in clinical setting could be a cause for the rare appearance of diuresis with α_2-adrenoceptor agonists. Further studies on mechanisms and the clinical significance for its diuretic effect are needed.

In the present study, it is controversial whether the changes in plasma AVP levels predominantly determined the effects on urine production with a CRI of remifentanil and/or dexmedetomidine. The decreased plasma AVP concentrations may contribute to diluted urine (decreased U_{Osm} and USG) in D treatment, but the natriuretic condition represents functional alternation in mechanisms that are related to sodium reabsorption other than water reabsorption. Moreover, our data on individual dogs indicated that UO increased alongside decreased plasma AVP concentrations in some dogs but not in other dogs even with similar plasma AVP levels with D treatment. This independence was supported by a previous study on human patients according to which oral administration of 2.5 or 5.0 μg/kg clonidine apparently induced natriuresis without significant changes in the plasma AVP levels during isoflurane-nitrous oxide anesthesia [21]. In addition, it is difficult to explain using the changes in AVP secretion that UO

did not increase at T1 in RD treatment despite there was low plasma AVP concentrations (<5 pg/ml), similar to those in D treatment that represented diuresis. A previous study has reported the absence of a relationship between AVP secretion and lower urine production after administration of morphine as a preanesthetic agent in dogs that underwent surgery [60]. Considering these results, we assumed that the changes in plasma AVP concentrations might not be the only factor responsible for both diuretic and antidiuretic properties with dexmedetomidine and remifentanil, respectively.

This study has several limitations. First, sevoflurane doses were altered to prioritize simulating the surgical anesthetic depth expected to be used clinically, with reference to previous studies evaluating cardiovascular effects of remifentanil alone and dexmedetomidine alone in isoflurane-anesthetized dogs [42, 53]. To clarify the intrinsic effects of each drug, especially the complicated interactions on urine production and AVP secretion, another study with a constant sevoflurane dose is required. Second, we did not measure the drug concentrations in blood; these measurements could have given information on the pharmacokinetic interactions of both drugs and the adequacy of the equilibration periods of the drugs. Third, the current study did not evaluate other hormones that are strongly associated with AVP secretion and renal functions. Talukder and Hikasa [70] reported that medetomidine increased atrial natriuretic peptide along with a decrease in plasma AVP levels in dogs. Francis et al. [15] demonstrated a concomitant increase in angiotensin II and AVP by the administration of remifentanil in isoflurane-anesthetized dogs. The changes in these hormones might have influenced our data, and these measurements would have helped us understand the mechanisms.

In conclusion, the present study for the first time demonstrated the following: (1) remifentanil co-administered with dexmedetomidine under sevoflurane anesthesia in dogs, was

acceptable from the viewpoint of its cardiovascular effects, oxygenation, acid-base balance, and renal function, (2) 0.5 μg/kg/hr of dexmedetomidine significantly decrease plasma AVP concentrations and can elicit natriuresis, (3) remifentanil may interfere with the dexmedetomidine-induced diuresis and inhibition of AVP secretion.

General Conclusion

In chapter 1, the present study evaluated sevoflurane MAC-sparing effects resulting from infusions of dexmedetomidine and remifentanil, alone and in combination, in healthy dogs. Both dexmedetomidine and remifentanil in alone reduced sevoflurane MAC in dose-dependent manner. A combination of dexmedetomidine and remifentanil enhanced their dose-dependent sevoflurane MAC-sparing effects through clear synergistic interactions when dexmedetomidine was administered at 0.5 and 1.0 μg/kg/hr. Further, the results of this study showed that the required remifentanil dose rates for ED_{50} (0.12 and 0.076 μg/kg/min) of the MAC-sparing effect during dexmedetomidine CRI at 0.5 and 1.0 μg/kg/hr, respectively, were decreased compared to that for remifentanil alone (0.54 μg/kg/min). The combination of dexmedetomidine and remifentanil significantly reduced the requirement of sevoflurane for blunting response to noxious stimuli and may contribute to improve the efficiency of antinociception during surgical general anesthesia in dogs.

In chapter 2, the present study outlined the changes in cardiovascular and renal functions, along with evaluations of AVP secretion with remifentanil and dexmedetomidine infusion, alone or in combination, in sevoflurane-anesthetized dogs. Cardiovascular effects of remifentanil CRI (0.15 to 2.40 μg/kg/min) combined with dexmedetomidine CRI (0.5 μg/kg/hr) were acceptable for maintaining the parameters of global oxygenation, GFR and RBF under general anesthesia with sevoflurane in dogs. We also revealed that 0.5 μg/kg/hr of dexmedetomidine significantly decrease plasma AVP concentrations and can elicit natriuresis. Further, remifentanil CRI at > 0.6

μg/kg/min interfered with those dexmedetomidine-induced diuresis and inhibition of AVP secretion, indicating the interference activity of an μ opioid of remifentanil on the diuretic properties of dexmedetomidine.

The present study found that the cardiovascular effects with a combined CRI of dexmedetomidine at 0.5 μg/kg/hr and remifentanil were within acceptable range while synergistically reducing the anesthetic requirement in healthy dogs anesthetized with sevoflurane. This study provided the evidence for clinical application of the combination of dexmedetomidine and remifentanil as an anesthetic adjunct and contributed to pave the way for its widespread utilization in small animal practice.

Abstract

Opioids and α_2-adrenoceptor agonists are useful adjunctive drugs for general anesthesia mainly due to potent analgesic properties, and their combination is known to be potentially beneficial in enhancing analgesic utility. Dexmedetomidine is a highly selective α_2-adrenoceptor agonist with useful sedative and analgesic properties as an anesthetic adjunct. In recent years, constant rate infusion (CRI) method with dexmedetomidine has been applied to minimize cardiovascular side effects and to consistently provide its efficacy through the anesthetic period. Remifentanil is a very-short acting μ-opioid analgesic that are characterized by a rapid onset and elimination from circulation. Remifentanil brings certain advantages in facilitating rapid adjustments of its analgesic effect and in making recovery times brief, compared with other opioids. However, no study was found in the literature that investigated the combined effects of dexmedetomidine and remifentanil in dogs. To verify the clinical efficacy and adaptability of a concurrent CRI of dexmedetomidine and remifentanil, this study aimed to reveal both anesthetic potency and simultaneous influences on cardiovascular side effects with a combination of these two drugs in anesthetized dogs with sevoflurane.

In chapter 1, we evaluated the effects of CRI of dexmedetomidine and remifentanil alone and their combination on minimum alveolar concentration (MAC), which is a standard measure of inhaled anesthetic potency, of sevoflurane in dogs. A total of six healthy, adult neutered Beagle dogs (three males, three females) were recruited. Anesthesia was induced with sevoflurane in oxygen until endotracheal intubation was possible and anesthesia maintained with sevoflurane using positive pressure ventilation. Each dog was anesthetized five times and was

administered each of the following treatments: saline (1 m*l*/kg/hr) or dexmedetomidine at 0.1, 0.5, 1.0 or 5.0 μg/kg loading dose intravenously over 10 min followed by a CRI at 0.1, 0.5, 1.0 or 5.0 μg/kg/hr, respectively. Following 60 min of CRI, sevoflurane MAC was determined in duplicate using an electrical stimulus (50 V, 50 Hz, 10 ms). Then, a CRI of successively increasing doses of remifentanil (0.15, 0.60 and 2.40 μg/kg/min) was added to each treatment. MAC was also determined after 30 min equilibration at each remifentanil dose. Isobolographic analysis determined interaction from the predicted doses required for a 50% MAC reduction (ED_{50}) with remifentanil, dexmedetomidine and remifentanil combined with dexmedetomidine, with the exception of dexmedetomidine 5.0 μg/kg/hr, obtained using log-linear regression analysis. The sevoflurane MAC decreased dose-dependently with increasing infusion rates of dexmedetomidine and remifentanil. The remifentanil ED_{50} values were lower when combined with dexmedetomidine than those obtained during saline–remifentanil. Synergistic interactions between dexmedetomidine and remifentanil for MAC reduction occurred with dexmedetomidine at 0.5 and 1.0 μg/kg/hr. Combined CRIs of dexmedetomidine and remifentanil synergistically resulted in sevoflurane MAC reduction. The combination of dexmedetomidine and remifentanil effectively reduced the requirement of sevoflurane during anesthesia in dogs.

In chapter 2, we evaluated changes in cardiovascular and renal functions as well as arginine vasopressin (AVP) secretion, with remifentanil and dexmedetomidine administration alone or in combination in sevoflurane-anesthetized dogs. Six healthy adult Beagle dogs received one of the following four treatments in a randomized crossover study: saline (C), remifentanil alone at successively increasing doses (R; 0.15, 0.60, and 2.40 μg/kg/min), dexmedetomidine alone (D; 0.5 μg/kg intravenously for initial 10 min followed by a constant rate infusion at 0.5 μg/kg/hr), and a combination of remifentanil and dexmedetomidine at the above-mentioned

doses (RD). Sevoflurane doses were adjusted to 1.5 times of MAC equivalent according to MAC-sparing effects with remifentanil and dexmedetomidine as previously reported. Cardiovascular measurements, renal function data, and plasma AVP concentrations were determined before and every 60 min until 180 min after drug administration as per each treatment. In the R, D and RD, heart rate significantly decreased and mean arterial pressure significantly increased from baseline or with C. Cardiac index significantly decreased and systemic vascular resistance index increased with D and RD. Oxygen extraction ratio, renal blood flow, and glomerular filtration rate were not affected. The plasma AVP concentrations significantly decreased in D and RD, but increased in R. Only in D, the natriuresis was elicited. The combination of remifentanil and dexmedetomidine in sevoflurane-anesthetized dogs was acceptable in terms of the hemodynamics, oxygenation, and renal function. Remifentanil may interfere with dexmedetomidine-induced diuresis and inhibition of AVP secretion.

In conclusion, the present study found that the cardiovascular effects with a combined CRI of dexmedetomidine at 0.5 μg/kg/hr and remifentanil were within acceptable range while synergistically reducing the anesthetic requirement in healthy dogs anesthetized with sevoflurane. This study provided the evidence for clinical application of the combination of dexmedetomidine and remifentanil as an anesthetic adjunct and contributed to pave the way for its widespread utilization in small animal practice.

Acknowledgements

I would like to thank my doctoral course supervisor Professor Dr. Tomohiro Imagawa (Tottori University) for his guidance and support throughout my graduate student years, for the critical reviewing of the manuscript and escorting me to study in his Laboratory. He is a talented and brilliant scientist and a great mentor, from whom I have learned a great deal. I am grateful to Professor Dr. Yoshiaki Hikasa (Tottori University) and Assistant Professor Dr. Kazuhito Itamoto (Yamaguchi University) for their guidance as assistant supervisors during my study. I also wish to express my appreciation to Assistant Professor Dr. Yusuke Murahata (Tottori University) for his many important, irreplaceable assistance to my study. Special thanks to Professor Dr. Yoshiharu Okamoto (Tottori University), Associate Professor Dr. Norihiko Itoh (Tottori University), Associate Professor Dr. Tomohiro Osaki (Tottori University), Associate Professor Dr. Takeshi Tsuka (Tottori University) and Associate Professor Dr. Kazuo Azuma (Tottori University) for their kind and useful advises during my study.

I am especially grateful to the students of the Department of Veterinary Diagnostic Imaging, Tottori University including Sho Hayashi, Koki Shigematsu, Hikaru Kishida and Masahumi Hosokawa for their indispensable technical assistance during my experiments. Without their cooperation my research work could not have been completed.

I am greatly indebted to the Japan Student Services Organization for providing my scholarship.

References

1. Anderson, M. K., and Day, T. K. 2008. Effects of morphine and fentanyl constant rate infusion on urine output in healthy and traumatized dogs. *Vet. Anaesth. Analg.* **35**: 528-536.
2. Allweiler, S., Brodbelt, D.C., Borer, K., Hammond, R. A., and Alibhai, H. I. 2007. The isoflurane-sparing and clinical effects of a constant rate infusion of remifentanil in dogs. *Vet. Anaesth. Analg.* **34**: 388–393.
3. Antognini, J.F., and Schwartz, K. 1993. Exaggerated anesthetic requirements in the preferentially anesthetized brain. *Anesthesiology* **79**: 1244–1249.
4. Arvidsson, U., Riedl, M., Chakrabarti, S., Lee, J. H., Nakano, A. H., Dado, R. J., Loh, H. H., Law, P. Y., Wessendorf, M. W., and Elde, R. 1995. Distribution and targeting of a mu-opioid receptor (MOR1) in brain and spinal cord. *J. Neurosci.* **15**: 3328–3341.
5. Baldo, C. F., Garcia-Pereira, F. L., Nelson, N. C., Hauptman, J. G., and Shih, A. C. 2012. Effects of anesthetic drugs on canine splenic volume determined via computed tomography. *Am. J. Vet. Res.* **73**: 1715-1719.
6. Bloor, B.C., Frankland, M., Alper, G., Raybould, D., Weitz, J., and Shurtliff, M. 1992. Hemodynamic and sedative effects of dexmedetomidine in dog. *J. Pharmacol. Exp. Ther.* **263**: 690–697.
7. Burkle, H., Dunbar, S., and Van Aken, H. 1996. Remifentanil: a novel, short-acting, mu-opioid. Anesth. Analg. 83, 646-651.

8. Burton, S., Lemke, K. A., Ihle, S. L., and Mackenzie, A. L. 1998. Effects of medetomidine on serum osmolality; urine volume, osmolality and pH; free water clearance; and fractional clearance of sodium, chloride, potassium, and glucose in dogs. *Am. J. Vet. Res.* **59**: 756-761.
9. Cabral, A. M., Kapusta, D. R., Kenigs, V. A. and Varner, K. J. 1998. Central α2-receptor mechanisms contribute to enhanced renal responses during ketamine-xylazine anesthesia. *Am. J. Physiol.* **275**: R1867–1874.
10. Castiglia, Y. M. M., Braz, J. R. C., Vianna, P. T. G., Lemonica, L., and Vane, L. A. 1997. Effect of high-dose fentanyl on renal function in dogs. *Sao. Paulo. Med. J.* **115**: 1433-1439.
11. Chabot-Doré, A. J., Schuster, D. J., Stone, L. S., and Wilcox, G. L. 2015 Analgesic synergy between opioid and α2-adrenoceptors. *Br. J. Pharmacol.* **172**: 388–402.
12. Chism, J.P., and Rickert, D.E. 1996. The pharmacokinetics and extra-hepatic clearance of remifentanil, a short acting opioid agonist, in male beagle dogs during constant rate infusions. *Drug Metab. Dispos.* **24**: 34-40.
13. Ebner, L. S., Lerche, P., Bednarski, R. M., and Hubbell, J. A. 2013. Effect of dexmedetomidine, morphine-lidocaine-ketamine, and dexmedetomidine-morphine-lidocaine-ketamine constant rate infusions on the minimum alveolar concentration of isoflurane and bispectral index in dogs. *Am. J. Vet. Res.* **74**: 963–970.
14. Eger, E. I. 2nd., Saidman, L. J. and Brandstater, B. 1965 Minimum alveolar anesthetic concentration: a standard of anesthetic potency. *Anesthesiology* **26**: 756–763.
15. Francis, R. C., Reyle-Hahn, M. S., Höhne, C., Klein, A., Theruvath, I., Donaubauer, B., and Boemke, W. 2008. The haemodynamic and catecholamine response to xenon/remifentanil anaesthesia in Beagle dogs. *Lab. Anim.* **42**: 338-349.

16. Gellai, M. 1990. Modulation of vasopressin antidiuretic action by renal α2-adrenoceptors. *Am. J. Physiol.* **259**: F1–8.

17. Gessner, P. K. 1995. Isobolographic analysis of interactions: an update on applications and utility. *Toxicology* **105**: 161–179.

18. Gilsbach, R., Röser, C., Beetz, N., Brede, M., Hadamek, K., Haubold, M., Leemhuis, J., Philipp M., Schneider, J., Urbanski, M., Szabo, B., Weinshenker, D., and Hein, L. 2009. Genetic dissection of alpha2-adrenoceptor functions in adrenergic versus nonadrenergic cells. *Mol. Pharmacol.* **75**: 1160–1170.

19. Grimm, K. A., Tranquilli, W. J., Gross, D. R., Sisson, D. D., Bulmer, B. J., Benson, G. J., and Martin-Jimenez, T. 2005. Cardiopulmonary effects of fentanyl in conscious dogs and dogs sedated with a continuous rate infusion of medetomidine. *Am. J. Vet. Res.* **66**: 1222-1226.

20. Gutierrez-Blanco, E., Victoria-Mora, J. M., Ibancovichi-Camarillo, J. A., Sauri-Arceo, C. H., Bolio-González, M. E., Acevedo-Arcique, C. M., and Steagall, P. V. 2013. Evaluation of the isoflurane-sparing effects of fentanyl, lidocaine, ketamine, dexmedetomidine, or the combination lidocaine-ketamine-dexmedetomidine during ovariohysterectomy in dogs. *Vet. Anaesth. Analg.* **40**: 599-609.

21. Hamaya, Y., Nishikawa, T., and Dohi, S. 1994. Diuretic effect of clonidine during isoflurane, nitrous oxide, and oxygen anesthesia. *Anesthesiology* **81**: 811-819.

22. Haskins, S., Pascoe, P. J., Ilkiw, J. E., Fudge, J., Hopper, K., and Aldrich, J. 2005. Reference cardiopulmonary values in normal dogs. *Comp. Med.* **55**: 156-161.

23. Hauptman, J. G., Richter, M. A., Wood, S. L., and Nachreiner, R. F. 2000. Effects of anesthesia, surgery, and intravenous administration of fluids on plasma antidiuretic hormone concentrations in healthy dogs. *Am. J. Vet. Res.* **61**: 1273-1276.

24. Hector, R. C., Rezende, M. L., Mama, K. R., Steffey, E. P., Knych, H. K., Hess, A. M., and Vainio, O. M. 2017. Effects of constant rate infusions of dexmedetomidine or MK-467 on the minimum alveolar concentration of sevoflurane in dogs. *Vet. Anaesth. Analg.* **44**: 755–765.

25. Heiene, R., and Moe, L. 1998. Pharmacokinetic aspects of measurement of glomerular filtration rate in the dog: A review. *J. Vet. Intern. Med.* **12**: 401-414.

26. Hellebrekers, L. J., Van den Brom, W. E., and Mol, J. A. 1989. Plasma arginine vasopressin response to intravenous methadone and naloxone in conscious dogs. *J. Pharmacol. Exp. Ther.* **248**: 329-333.

27. Hoke, J. F., Cunningham, F., James, M. K., Muir, K. T., and Hoffman, W. E. (1997). Comparative pharmacokinetics and pharmacodynamics of remifentanil, its principle metabolite (GR90291) and alfentanil in dogs. *J. Pharmacol. Exp. Ther.* **281**: 226-232.

28. Humphreys, M. H., Reid, I. A., and Chou, L. Y. N. 1975. Suppression of antidiuretic hormone secretion by clonidine in the anesthetized dog. *Kidney Int.* **7**: 405-412.

29. Ilkiw, J. E. 1999. Balanced anesthetic techniques in dogs and cats. *Clin. Tech. Small. Anim. Pract.* **14**: 27-37.

30. Ilkiw, J. E., Pascoe, P. J., Haskins, S. C., Patz, J. D., and Jaffe, R. 1994. The cardiovascular sparing effect of fentanyl and atropine, administered to enflurane anesthetized dogs. *Can. J. Vet. Res.* **58**: 248-253.

31. Kaartinen, M. J., Pang, D. S. J., Moreau, M., Vainio, O., Beaudry, F., del Castillo, J., and Troncy, E. 2010. Hemodynamic effects of an intravenous infusion of medetomidine at six different dose regimens in isoflurane-anesthetized dogs. *Vet. Ther.* **11**: E1-E16.

32. Kimura, T., Share, L., Wang, B. C., and Crofton, J. T. 1981. The role of central adrenoreceptors in the control of vasopressin release and blood pressure. *Endocrinology* **108**: 1829-1836.

33. Kirschen, G. W., Kim, E., and Adsumelli, R. S. 2019. Dexmedetomidine-induced massive diuresis in a patient undergoing spinal fusion surgery: a case report and synthesis of the literature. *A A Pract* **12**: 112-114.

34. Kuusela, E., Raekallio, M., Anttila, M. Falck, I., Mölsä, S., and Vainio, O. 2000. Clinical effects and pharmacokinetics of medetomidine and its enantiomers in dogs. *J. Vet. Pharmacol. Ther.* **23**: 15–20.

35. Lawrence, C. J., Prinzen, F. W., and De Lange, S. 1996. The effect of dexmedetomidine on nutrient organ blood flow. *Anesth. Analg.* **83**: 1160-1165.

36. Li, X., Zhao, Z., Pan, H. L., Eisenach, J. C. and Paqueron, X. 2000. Norepinephrine release from spinal synaptosomes: auto-alpha2-adrenergic receptor modulation. *Anesthesiology* **93**: 164–172.

37. Lin, G. Y., Robben, J. H., Murrell, J. C., Aspegrén, J., McKusick, B. C., and Hellebrekers, L. J. 2008. Dexmedetomidine constant rate infusion for 24 hours during and after propofol or isoflurane anaesthesia in dogs. *Vet. Anaesth. Analg.* **35**: 141–153.

38. Mann, W. A., and Kinter, L. B. 1993. Characterization of the renal handling of p-aminohippurate (PAH) in the beagle dog (Canis familiaris). *Gen. Pharmacol.* **24**: 367-372.

39. Matute, E., Rivera-Arconada, I., and López-Garcia, J. A. 2004 Effects of propofol and sevoflurane on the excitability of rat spinal motoneurones and nociceptive reflexes in vitro. *Br J Anaesth* **93**: 422–427.

40. Michelsen, L. G., Salmenperä, M., Hug, C. C., Szlam, F., and VanderMeer, D. 1996. Anesthetic potency of remifentanil in dogs. *Anesthesiology* **84**: 865–872.

41. Monteiro, E. R., Teixeira-Neto, F. J., Campagnol, D., Alvaides, R. K., Garofalo, N. A., and Matsubara, L. M. 2010. Effects of remifentanil on the minimum alveolar concentration of isoflurane in dogs. *Am. J. Vet. Res.* **71**: 150–156.

42. Monteiro, E. R., Neto, F. J. T., Campagnol, D., Garofalo, N. A., and Alvaides, R. K. 2010. Hemodynamic effects in dogs anesthetized with isoflurane and remifentanil-isoflurane. *Am. J. Vet. Res.* **71**: 1133-1141.

43. Moran-Muñoz, R., Ibancovichi, J. A., Gutierrez-Blanco, E., Acevedo-Arcique, J., Victoria Mora, M., Tendillo, F. J., Santos-Gonzalez, M., and Yamashita, K. 2014. Effects of lidocaine, dexmedetomidine or their combination on the minimum alveolar concentration of sevoflurane in dogs. *J. Vet. Med. Sci.* **76**: 847–853.

44. Moran-Muñoz, R., Valverde, A., Ibancovichi, J. A., Acevedo-Arcique, C. M., Recillas-Morales, S., Sanchez-Aparicio, P., and Chavez-Monteagudo, J. R. 2017. Cardiovascular effects of constant rate infusions of lidocaine, lidocaine and dexmedetomidine, and dexmedetomidine in dogs anesthetized at equipotent doses of sevoflurane. *Can. Vet. J.* **58**: 729-734.

45. Muir, W. W. 3rd., Wiese, A. J., and March, P. A. 2003. Effects of morphine, lidocaine, ketamine and morphine-lidocaine-ketamine drug combination on minimum alveolar concentration in dogs anesthetized with isoflurane. *Am. J. Vet. Res.* **64**: 1155–1160.

46. Murahata, Y., Hikasa, Y., Hayashi, S., Shigematsu, K., Akashi, N., Osaki, T., Tsuka, T., Okamoto, Y., and Imagawa, T. 2018. The effect of remifentanil on the minimum alveolar concentration (MAC) and MAC derivatives of sevoflurane in dogs. *J. Vet. Med. Sci.* **80**: 1086–1093.

47. Murrell, J.C., and Hellebrekers, L.J. 2005. Medetomidine and dexmedetomidine: a review of cardiovascular effects and antinociceptive properties in the dog. *Vet. Anaesth. Analg.* **32**: 117-127.

48. Murrell, J. C., van Notten, R. W., and Hellebrekers, L. J. 2005. Clinical investigation of remifentanil and propofol for the total intravenous anaesthesia of dogs. *Vet. Rec.* **156**: 804–808.

49. Mutoh, T., Nishimura, R., Kim, H.Y. Matsunaga, S., and Sasaki, N. 1997. Cardiopulmonary effects of sevoflurane, compared with halothane, enflurane, and isoflurane, in dogs. *Am. J. Vet. Res.* **58**: 885-890.

50. Nishida, M., Uechi, M., Kono, S., Harada, K., and Fujiwara, M. 2012. Estimating glomerular filtration rate in healthy dogs using inulin without urine collection. *Res. Vet. Sci.* **93**: 398-403.

51. Ossipov, M. H., Harris, S., Lloyd, P., Messineo, E., Lin, B. S., and Bagley, J. 1990. Antinociceptive interaction between opioids and medetomidine: systemic additivity and spinal synergy. *Anesthesiology* **73**: 1227–1235.

52. Pascal, M., Allison, A., and Kaartinen, J. 2020. Opioid-sparing effect of a medetomidine constant rate infusion during thoraco-lumbar hemilaminectomy in dogs administered a ketamine infusion. *Vet. Anaesth. Analg.* **47**: 61-69.

53. Pascoe, P. J. 2015. The cardiopulmonary effects of dexmedetomidine infusions in dogs during isoflurane anesthesia. *Vet. Anaesth. Analg.* **42**: 360-368.

54. Pascoe, P. J., Raekallio, M., Kuusela, E., McKusick, B., and Granholm, M. 2006. Changes in the minimum alveolar concentration of isoflurane and some cardiopulmonary measurements during three continuous infusion rates of dexmedetomidine in dogs. *Vet. Anaesth. Analg.* **33**: 97–103.

55. Pratt, A., Aboudara, M., and Lung, L. 2013. Polyuria related to dexmedetomidine. *Anesth. Analg.* **117**: 150-152.

56. Pypendop, B. H., and Verstegen, J. P. 1998. Hemodynamic effects of medetomidine in the dog: a dose titration study. *Vet. Surg.* **27**: 612-622.

57. Quasha, A. L., Eger, E. l. 2nd., and Tinker, J. H. 1980. Determination and application of MAC. *Anesthesiology* **53**: 315–334.

58. Reid, I. A., Nolan, P. L., Wolf, J. A., and Keil, L. C. 1979. Suppression of vasopressin secretion by clonidine: effect of alpha-adrenoceptor antagonists. *Endocrinology* **104**: 1403-1406.

59. Rioja, E., Gianotti, G., and Valverde, A. 2013. Clinical use of a low-dose medetomidine infusion in healthy dogs undergoing ovariohysterectomy. *Can. Vet. J.* **54**: 864-868.

60. Robertson, S. A., Hauptman, J. G., Nachreiner, R. F., and Richter, M. A. 2001. Effects of acetylpromazine or morphine on urine production in halothane-anesthetized dogs. *Am. J. Vet. Res.* **62**: 1922-1927.

61. Rockhold, R.W., Crofton, J.T., Wang, B.C., and Share, L. 1983. Effect of intracarotid administration of morphine and naloxone on plasma vasopressin levels and blood pressure in the dog. *J. Pharmacol. Exp. Ther.* **224**: 386-390.

62. Roerig, S. C., and Howse, K. M. 1996. Omega-agatoxin IVA blocks spinal morphine/clonidine antinociceptive synergism. *Eur J Pharmacol* **314**: 293–300.

63. Saleh, N., Aoki, M., Shimada, T., Akiyoshi, H., Hassanin, A., and Ohashi, F. 2005. Renal effects of medetomidine in isoflurane-anesthetized dogs with special reference to its diuretic action. *J. Vet. Med. Sci.* **67**: 461-465.

64. Salla, K. M., Tuns, C. I., Bennett, R. C., Raekallio, M. R., Scheinin, M., Kuusela, E., and Vainio, O. M. 2017. Cardiovascular effects of premedication with medetomidine alone and in combination with MK-467 or glycopyrrolate in dogs subsequently anesthetized with isoflurane. *Am. J. Vet. Res.* **78**: 1245-1254.

65. Salmenperä, M. T., Szlam, F., and Hug Jr, C.C. 1994. Anesthetic and hemodynamic interactions of dexmedetomidine and fentanyl in dogs. *Anesthesiology* **80**: 837–846.

66. Schuster, D. J., Kitto, K. F., Overland, A. C., Messing, R. O., Stone, L. S., Fairbanks, C. A., and Wilcox, G. L. 2013. Protein kinase C (epsilon) is required for spinal analgesic synergy between delta opioid and alpha-2A adrenergic receptor agonist Pairs. *J Neurosci* **33**: 13538–13546.

67. Shinohara, K., Aono, H., Unruh, G.K., Kindscher, J. D. and Goto, H. 2000. Suppressive effects of remifentanil on hemodynamics in baro-denervated rabbits. *Can J Anaesth* **47**: 361–366.

68. Strandhoy, J. W., Morris, M. and Buckalew, V. M. 1982. Renal effects of the antihypertensive, guanabenz, in the dog. *J. Pharmacol. Exp. Ther.* **221**: 347–352.

69. Tallarida, R. J. 2012. Revisiting the isobole and related quantitative methods for assessing drug synergism. *J. Pharmacol. Exp. Ther.* **342**: 2–8.

70. Talukder, M. H. and Hikasa, Y. 2009. Diuretic effects of medetomidine compared with xylazine in healthy dogs. *Can. J. Vet. Res.* **73**: 224-236.

71. Tayari, H. and Bell, A. 2019. Dexmedetomidine infusion as perioperative adjuvant in a dog undergoing craniotomy. *Vet Rec Case Rep* **7**: e000727.

72. Tranquilli, W. J. and Grimm, K. A. 2015. Introduction: use, definitions, history, concepts, classification, and considerations for anesthesia and analgesia. pp. 3–10. In: Veterinary Anesthesia and Analgesia, 5th ed. (Grimm, K. A., Lamont, L. A., Tranquilli, W. J., Greene, S. A. and Robertson, S. A. eds.), Wiley-Blackwell, Hoboken.

73. Uilenreef, J. J., Murrell, J. C., McKusick, B. C., and Hellebrekers, L. J. 2008. Dexmedetomidine continuous rate infusion during isoflurane anaesthesia in canine surgical patients. *Vet. Anaesth. Analg.* **35**: 1–12.

74. Valtolina, C., Robben, J.H., Uilenreef, J., Murrell, J. C., Aspegrén, J., McKusick, B. C., and Hellebrekers, L. J. 2009. Clinical evaluation of the efficacy and safety of a constant rate infusion of dexmedetomidine for postoperative pain management in dogs. *Vet. Anaesth. Analg.* **36**: 369-383.

75. Valverde, A., Morey, T. E., Hernández, J., and Davies, W. 2003. Validation of several types of noxious stimuli for use in determining the minimum alveolar concentration for inhalation anesthetics in dogs and rabbits. *Am. J. Vet. Res.* **64**: 957–962.

76. Villela, N. R., Nascimento Júnior, P. D., Carvalho, L. R. D., and Teixeira, A. 2005. Effects of dexmedetomidine on renal system and on vasopressin plasma levels: experimental study in dogs. *Rev Bras Anestesiol* **55**: 429-440.

77. Van Vonderen, I. K., Wolfswinkel, J., Oosterlaken-Dijksterhuis, M. A., Rijnberk, A., and Kooistra, H. S. 2004. Pulsatile secretion pattern of vasopressin under basal conditions, after

water deprivation, and during osmotic stimulation in dogs. *Domest. Anim. Endocrinol.* **27**: 1-12.

78. Voigt, A. M., Bergfeld, C., Beyerbach, M., and Kästner, S. B. 2013. Effects of isoflurane with and without dexmedetomidine or remifentanil on heart rate variability before and after nociceptive stimulation at different multiples of minimum alveolar concentration in dogs. *Am. J. Vet. Res.* **74**: 665–671.
79. Wang, H-B., Zhao, B., Zhong, Y-Q., Li, K-C., Li, Z-Y., Wang, Q., Lu, Y-J., Zhang, Z-N., He, S-Q., Zheng, H-C., Wu, S-X, Tomas, G. M. H., Bao, L., and Zhang, X. 2010. Coexpression of delta- and mu-opioid receptors in nociceptive sensory neurons. PNAS 107: 13117–13122.
80. Wei, Z. Y. and Roerig, S. C. 1998. Spinal morphine/clonidine antinociceptive synergism is regulated by protein kinase C, but not protein kinase A activity. *J. Pharmacol. Exp. Ther.* **287**: 937–943.