Effects of medetomidine, midazolam, ketamine and alfaxalone on stress-related neuroendocrine, metabolic and cardiovascular responses in isoflurane-anesthetized cats undergoing surgery and their antagonism by potential antagonists

(ネコにおけるイソフルラン麻酔と手術時のストレス関連性神経内分泌、代謝および循環器反応に及ぼすメデトミジン、ミダゾラム、ケタミンおよびアルファキサロンの影響と拮抗薬の効果)

The United Graduate School of Veterinary Science

Yamaguchi University

Hirokazu KAMOHARA

March 2022

Table of Contents

General introduction

The alpha(α)$_2$-adrenoceptor agonist, medetomidine (Me) is widely used in feline practice as an excellent analgesic and muscle relaxant. However, it induces undesirable effects like hyperglycemia, hypoinsulinemia, emesis and bradyarrhythmias in cats [7,24,25,37,40,44]. A combination of Me with midazolam (Mi) and/or ketamine (Ke) produces good anesthesia in cats, with a reduction of adverse effects or the potentiation of analgesia [5,8,9,15,17,28,39,43,48].

Alfaxalone (Af; 3α-hydroxy-5α-pregnane-11, 20-dione) is a newly developed anesthetic agent that is useful for the sedation, induction, and maintenance of anesthesia in cats [52]. However, during the recovery period, this agent produces more adverse events than propofol, including ataxia and muscular tremors [34,45]. The quality of recovery from anesthesia with Af is reported to be improved with the use of other sedative and/or inhalant anesthetic agents [52]. Therefore, there is a need to investigate the effects of using Af instead of ketamine in combination with Me–Mi as a preanesthetic medication in feline veterinary practice.

Stress consists of the biological responses of an animal in an attempt to cope with a disruption or threat to homeostasis [12]. Stressors such as anxiety, excitement, pain and anesthesia induce neurohormonal and metabolic changes in animals [11,22,49]. These changes are characterized by elevations in the levels of blood cortisol, catecholamines, glucose and non-esterified fatty acid (NEFA) and a reduction in blood insulin levels [15,22,49]. Actions mediated by α_2-adrenoceptors are closely coordinated with these events. In cats, Me suppresses catecholamine release, insulin release and lipolysis and induces hyperglycemia [25].

Conversely, it has been reported that premedication with Me prevents or delays the stress response induced by ovariohysterectomy in isoflurane-anesthetized dogs [6]. In halothane-anesthetized dogs undergoing ovariohysterectomy, treatment with Me prevents an increase in plasma cortisol concentrations during the surgery and early-recovery process [29]. It has also been

reported that Me may offer some advantages over acepromazine by decreasing perioperative concentrations of stress-related hormones including plasma catecholamine and cortisol [51]. However, to the best of our knowledge, there are no published reports on the effects of pretreatment with Me alone and in combination for stress responses in anesthetized cats undergoing surgery.

On the other hand, antagonism may be required when anesthetized animals show a profound depression of vital signs, adverse effects, and/or delayed recovery from anesthesia. Atipamezole (Ati), flumazenil (Flu) and 4-aminopyridine (Ap) either completely or partially antagonize the effects of the combination with medetomidine, midazolam and ketamine (MMK) in cats [13,20,23,42,47,48,50]. After surgery and when combined with MMK, these antagonists can accelerate awakening from anesthesia and are useful in anesthesia-associated emergency and critical care. In cats, Ati, both alone and in combination with Flu administered intravenously (IV), effectively antagonizes the anesthetic and neurohormonal effects induced by MMK [47,48]. Compared to Ati alone, Ati in combination with Flu leads to a similar recovery time from MMK anesthesia but improves the quality of recovery by reducing excitation and hyperaesthesia [13]. The antagonistic effects of Ati and Ati–Flu also differ depending on the route and timing of administration in MMK-anesthetized normal and castrated cats [9,13]. It is important to evaluate cats' stress-related hormonal and metabolic responses due to the post-operative administration of potential antagonists in clinical practice. In terms of the overall effect of these antagonists on general anesthesia and post-surgical recovery, the use of potential antagonists may be advantageous to recovery from anesthesia if the sympathoadrenal system is adequately but not excessively activated. However, to the best of our knowledge, there are no published reports on the effects of Ati and Flu, both alone and in combination, on the stress responses of anesthetized cats undergoing surgery.

Although the triple combination (Ati, Flu, and Ap) was the most effective in terms of accelerating recovery from anesthesia induced by MMK, it was unsuitable for smooth antagonism, because of adverse reactions such as tachycardia, tachypnoea, excitement, muscle tremors, and excessive stress-related hormonal responses [47,48]. However, when evaluating the clinical usefulness of the triple antagonist regimen it would appear insufficient to conclude only on aspects of behavioral and hormonal effects. In previous studies, it has been reported that Ati reversed the increase in blood pressure induced by dexmedetomidine but induced short-acting hypotensive effects, and did not noticeably improve dexmedetomidine-induced bradycardia in isoflurane-anesthetized cats [33,54]. Therefore, it is also important to examine the effect of the reversals of arterial blood pressure to ensure the appropriate use of these antagonists during an anesthetic emergency when using MMK.

In chapter 1, the effects of Me, Mi, Ke alone and in combination on the key stress-related neurohormonal and metabolic variables were investigated in isoflurane-anesthetized cats undergoing ovariohysterectomy and castration. This study was designed to assess stress responses focusing on clinically important perioperative stages in feline practice.

In chapter 2, the effects of the intramuscular (IM) and IV administrations of Af, both alone and in combination with Me and Mi, on key stress-related neurohormonal and metabolic changes were evaluated in isoflurane-anesthetized cats undergoing ovariohysterectomy or castration. This study was also designed to assess stress responses during clinically important perioperative stages in feline veterinary practice.

In chapter 3, this study evaluated the effects of IM and IV administrations of Ati and Flu, both alone and in combination and at different administration times, on key stress-related neurohormonal and metabolic changes in MMK–isoflurane-anesthetized cats undergoing ovariohysterectomy and castration.

In chapter 4, this study investigated the effects of Ati, Flu, and Ap, both alone and in combination, on arterial blood pressure and heart rate in healthy cats administered MMK under isoflurane and oxygen anesthesia.

Chapter 1

Effects of pretreatment with medetomidine, midazolam, ketamine, and their combinations on stress-related hormonal and metabolic responses in isoflurane-anesthetized cats undergoing surgery

Introduction

The alpha(α)$_2$-adrenoceptor agonist, medetomidine (Me) is widely used in feline practice as an excellent analgesic and muscle relaxant. However, it induces undesirable effects like hyperglycemia, hypoinsulinemia, emesis and bradyarrhythmias in cats [7,24,25,37,40,44]. A combination of Me with midazolam (Mi) and/or ketamine (Ke) produces good anesthesia in cats, with a reduction of adverse effects or the potentiation of analgesia [5,8,9,15,17,28,39,43,48].

Stress consists of the biological responses of an animal in an attempt to cope with a disruption or threat to homeostasis [12]. Stressors such as anxiety, excitement, pain and anesthesia induce neurohormonal and metabolic changes in animals [11,22,49]. These changes are characterized by elevations in the levels of blood cortisol, catecholamines, glucose and non-esterified fatty acid (NEFA) and a reduction in blood insulin levels [11,22,49]. Actions mediated by α_2-adrenoceptors are closely coordinated with these events. In cats, Me suppresses catecholamine release, insulin release and lipolysis and induces hyperglycemia [25].

Conversely, it has been reported that premedication with Me prevents or delays the stress response induced by ovariohysterectomy in isoflurane-anesthetized dogs [6]. In halothane-anesthetized dogs undergoing ovariohysterectomy, treatment with Me prevents an increase in plasma cortisol concentrations during the surgery and early-recovery process [16]. It has also been reported that Me may offer some advantages over acepromazine by decreasing perioperative concentrations of stress-related hormones including plasma catecholamine and cortisol [51]. However, to the best of our knowledge, there are no published reports on the effects of pretreatment with Me alone and in combination for stress responses in anesthetized cats undergoing surgery. Therefore, this study aimed to compare the effects of Me, Mi, Ke alone and in combination on the key stress-related neurohormonal and metabolic variables in isoflurane-anesthetized cats undergoing ovariohysterectomy and castration. This study was designed to assess stress responses focusing on clinically important perioperative stages in feline practice.

Materials and methods

Ethical approval

This study involved the use of client-owned animals, and followed established international recognized high standards ('best practice') of individual veterinary clinical patient care. Ethical approval from a committee was not necessarily required. Informed consent was obtained from the owner of all animals described in this study for the procedure undertaken.

Animals

One-hundred and twelve, client-owned mixed-breed cats (56 males, 56 females) were prospectively recruited at the Kamohara Animal Hospital for ovariohysterectomy or castration. They were clinically healthy and ranged in age from 6 months to 1 year weighing 3.5 ± 0.8 kg (mean ± standard deviation; SD). Informed consent was obtained from every cat owner to collect data. Physical and routine hematological examinations before the study revealed that all values were within normal physiological ranges. All cats were fasted for 12 h but water was available *ad libitum*. The owner brought the cat to our hospital early in the morning on the day of surgery. Then, preparations for surgery and anesthesia were performed. After preparation, each cat was rested in a darkened cage for 2–3 h before anesthesia. After complete-recovery from anesthesia, cats were allowed to freely drinking water and were fed.

Study protocol

The cats were randomly assigned to one of seven treatment groups (eight cats in each group) in both ovariohysterectomy and castration. Each cat was given an intramuscular injection of the following pretreatment: physiological saline solution (0.5 mL), 50 μg/kg Me (Dorbene, Kyoritsu Seiyaku; 1 mg/mL), 0.5 mg/kg Mi (Fuji Pharma; 5 mg/mL), 5 mg/kg Ke (Fujita Pharmaceutical; 50 mg/mL), 50 μg/kg Me + 0.5 mg/kg Mi, 50 μg/kg Me + 5 mg/kg Ke and 50 μg/kg Me + 0.5

mg/kg Mi + 5 mg/kg Ke. The groups will be referred to as control, Me, Mi, Ke, MM, MK and MMK, respectively. For drug combination, all drugs were mixed in a syringe immediately before injection. Twenty minutes (mins) after each premedication, anesthesia was induced by an intravenous injection of 5 mg/kg propofol (Mylan Pharmaceutical; 10 mg/mL) in the control and Mi groups. In other groups, anesthesia was induced with 4% isoflurane in oxygen at a total gas flow rate of 1.5 L/min using a face mask attached to ADS 1000 veterinary anesthesia delivery system (Engler, Hialeah, FL). After induction of anesthesia, a cuffed endotracheal tube was inserted. The cats were placed in supine position and maintained at a surgical depth of isoflurane anesthesia through the non-rebreathing system under controlled ventilation. Castration or ovariohysterectomy was performed using standard methods. Pre-operatively, an analgesic (0.3-mg/kg meloxicam; Inflacam, Chanelle Pharmaceuticals Manufacturing Ltd, Ireland) was injected subcutaneously in all cats, followed by once daily for several days after surgery if necessary. Lactated Ringer's solution was infused intravenously at 10 mL/kg/h during anesthesia. Inhalation of isoflurane was completely stopped approximately 5 mins after the end of each operation and the endotracheal tube was extubated after the laryngeal reflex was observed. During the recovery process, cats were kept in separate cages in a room with the air temperature set at 25°C. General postoperative management and care were performed in all cats. Specific attention to arousal behavior was paid in animals of the control group. After complete-recovery, another analgesic, butorphanol (0.1–0.4 mg/kg; Vetorphale, Meiji Seika, Tokyo, Japan) was injected intramuscularly especially in the control, to cats with signs of pain such as vocalization, anorexia and posture. No life-threatening events in surgery, anesthesia and postoperative management occurred in all cats.

Anesthesia and intraoperative monitoring

An agent-specific precision vaporizer was used to administer isoflurane. Gas samples were drawn from the breathing circuit through a tube attached to an adapter positioned at the oral end of

an endotracheal tube. Expired end-tidal isoflurane (EtIso) and carbon dioxide ($EtCO_2$) concentrations, arterial oxygen saturation of pulse oximetry (SpO_2), heart rate (HR), respiration rate (RR), rectal temperature and mean blood pressure (MBP) were measured using the oscillometric method continuously or intermittently using a multi-parameter monitor (BSM-5192; Nihon Kohden, Tokyo, Japan) during anesthesia. During controlled ventilation, RR was adjusted to a range of approximately 25–35 mmHg $EtCO_2$. The mean duration of anesthesia in castrated and ovariohysterectomized groups ranged from 24.8–31.3 mins and 48.1–60.0 mins, respectively. SpO_2 was > 98% in all cats. The mean RR in castrated and ovariohysterectomized groups ranged from 11.3–14.4 and 9.3–11.1 breaths/min, respectively. Mean rectal temperature during surgery ranged from 37.8 °C to 36.8 °C in castrated groups, and from 36.9 °C to 35.5 °C in ovariohysterectomized groups. There were no significant differences in duration of anesthesia, RR, $EtCO_2$, SpO_2 and rectal temperature values across groups in both surgeries.

Scoring of behavioral changes during recovery

The overall quality of recovery from anesthesia was assessed using the previously published scoring methods [47] as follows: score 1 = excellent; score 2 = good; score 3 = moderate; score 4 = poor; score 5 = extremely poor. The observer was blinded to each treatment.

Blood sample collection

Blood sample collection was focused on clinically important perioperative stages. Blood samples (2 mL) were collected from a 24-gauge catheter inserted into the cephalic vein five times; immediately before pretreatment (baseline), 5 mins after induction of anesthesia and before the surgical procedure during anesthesia (preoperatively), after completing the surgical procedure during anesthesia (postoperatively), at head-up motion after the removal of the tracheal tube after discontinuation of anesthesia (early-recovery) and at recovery to normal behavior similar to that

before pretreatment (complete-recovery). Blood sampling time to post-operation, early- and complete-recovery is listed in Table 1. There were no significant differences in blood sampling times to post-operation and complete-recovery across groups in both surgeries.

Sample processing and analysis

Blood was mixed with ethylenediaminetetraacetic acid to prevent clotting. Samples were immediately centrifuged; the plasma was separated and frozen at −76°C until analysis. Glucose, NEFA, insulin, cortisol, adrenaline and noradrenaline levels were measured according to previously published methods [25,47] as follows. Glucose and NEFA levels were determined by an enzyme assay and a spectrophotometer. Insulin and cortisol levels were measured by a solid phase two-site enzyme immunoassay with a feline kit and a solid phase-antibody radioimmunoassay, respectively. Catecholamines were extracted on activated alumina and measured using high-performance liquid chromatography and an electrochemical detector.

Statistical analysis

All data obtained were analyzed using a statistical software (Prism 7.0; GraphPad, CA). Data are presented as the mean ± SD. All data except for score data were tested for normality using the Shapiro-Wilk test. The repeated measures of one-way analysis of variance (ANOVA) was used to examine the differences for changes in variables within each group. The *post hoc* Dunnett's multiple comparisons test was used to identify differences from baseline within the group. One-way ANOVA and *post hoc* Tukey's multiple comparisons test were used to determine differences among the groups. In both tests, the significance level was set at $P < 0.05$. Score data were analyzed using the Wilcoxon–Mann–Whitney test for treatment comparisons, and a $P < 0.00714$ was considered significant by Bonferroni correction.

Results

EtIso, HR and MBP during anesthesia

In both surgeries, the mean EtIso concentration was significantly lower in Me and Me-combined groups (1.43 ± 0.33, 1.18 ± 0.39, 1.30 ± 0.37 and 0.80 ± 0.23 % in castration and 1.65 ± 0.39, 1.44 ± 0.36, 1.45 ± 0.49 and 1.20 ± 0.18 % in ovariohysterectomy of Me, MM, MK and MMK group, respectively) than in the control group (2.09 ± 0.25 and 2.08 ± 0.29 %) and was lowest in the MMK group (0.80 ± 0.23 and 1.20 ± 0.18 %). The mean HR was significantly lower in Me-treated groups than in non-Me groups in either castrated or ovariohysterectomized cats. The mean MBP was significantly higher in MM and MMK groups than in non-Me-treated groups in castrated cats. The results are shown in Figure 1.

Adrenaline

The effects of pretreatments with Me, Mi, Ke alone and in combinations on the changes of adrenaline concentration are shown in Figure 2. In castrated cats, adrenaline concentration in all groups decreased significantly or tended to decrease pre- and postoperatively compared with the baseline values (ranged 0.20 ± 0.15 to 0.31 ± 0.20 ng/mL). A significant decrease in adrenaline concentration was also observed at early-recovery in MK group. In ovariohysterectomized cats, adrenaline concentration decreased significantly or tended to decrease pre- and postoperatively compared with baseline in all groups. Adrenaline concentration during anesthesia and early-recovery (0.23 ± 0.15, 0.14 ± 0.12 and 0.23 ± 0.26 ng/mL in castration; 0.24 ± 0.16, 0.20 ± 0.13 and 0.26 ± 0.16 ng/mL in ovariohysterectomy) in the MM group did not significantly change from the baseline (0.22 ± 0.14 ng/mL; 0.33 ± 0.17 ng/mL) in both surgeries.

Noradrenaline

The effects of pretreatments with Me, Mi, Ke alone and in combinations on the changes of noradrenaline concentration are shown in Figure 3. In castrated cats, noradrenaline concentration in control, Me, MK and MMK groups decreased significantly at pre- and post-operation and/or early-recovery compared with baseline, but that in Mi, Ke and MM groups did not significantly change at any phase compared with baseline (0.68 ± 0.41, 0.89 ± 0.31 and 0.73 ± 0.39 ng/mL). In ovariohysterectomized cats, noradrenaline concentration decreased significantly pre- and/or postoperatively compared with baseline in control, Me, Ke and MMK groups. Postoperative noradrenaline concentration was significantly higher in the MM group (0.81 ± 0.47 ng/mL) than in the control group (0.27 ± 0.31 ng/mL).

Cortisol

The effects of pretreatments with Me, Mi, Ke alone and in combinations on the changes of cortisol concentration are shown in Figure 4. In castrated cats, cortisol concentration in the control group did not significantly change during anesthesia and recovery compared with baseline, whereas that in the Me, MK and MMK groups decreased significantly pre- and postoperatively and at early-recovery compared with baseline. Cortisol concentration postoperatively and/or at early-recovery was significantly lower in Me-treated groups (Me, 2.0 ± 2.6 μg/dL; MM, 1.5 ± 1.3 μg/dL; MK, 2.3 ± 1. 5 μg/dL; and MMK, 2.0 ± 1.9 μg/dL at post-operation) than in the control group (8.9 ± 5.8 μg/dL at post-operation). In ovariohysterectomized cats, cortisol concentration in the control and Mi groups increased significantly postoperatively and at early- and/or complete-recovery compared with baseline, whereas that in the Me and MMK groups decreased significantly postoperatively. Cortisol concentration postoperatively and/or at early-recovery was significantly lower in Me-treated groups than in non-Me groups. Cortisol concentration postoperatively and at the recovery phase tended to be greater in ovariohysterectomy than castration in each group. A significant difference ($P < 0.05$) in cortisol concentration between

surgeries was observed at early-recovery and complete-recovery in the Mi and MM groups, respectively (4.4 ± 4.3 μg/dL in castration and 10.8 ± 4.6 μg/dL in ovariohysterectomy of Mi group; 6.3 ± 3.8 μg/dL in castration and 12.3 ± 5.2 μg/dL in ovariohysterectomy of MM group).

Insulin

The effects of pretreatments with Me, Mi, Ke alone and in combinations on the changes of insulin concentration are shown in Figure 5. In castrated cats, insulin concentration in all groups except for MM group decreased significantly pre- and/or postoperatively (0.54 ± 0.37, 0.32 ± 0.27, 0.43 ± 0.33, 0.57 ± 0.42, 0.32 ± 0.27, 0.34 ± 0.29 and 0.31 ± 0.25 ng/mL in control, Me, Mi, Ke, MK and MMK, respectively) compared with baseline (ranged 0.78 ± 0.11 to 1.37 ± 0.92 ng/mL). Insulin concentration in the Me and MMK groups was also significantly lower in early-recovery compared with baseline. Insulin concentration at early-recovery was significantly lower in the Me and MMK groups (0.48 ± 0.40 and 0.41 ± 0.31 ng/mL) than in the control group (1.69 ± 1.34 ng/mL). In ovariohysterectomized cats, insulin concentration in the Me, Mi, Ke and MMK groups decreased significantly pre- and/or postoperatively compared with baseline. Insulin concentration in the control, MM and MK groups (0.59 ± 0.39, 0.62 ± 0.71 and 0.46 ± 0.34 ng/mL) tended to decrease insignificantly pre- and/or postoperatively compared with baseline (0.95 ± 0.70, 0.96 ± 0.62 and 1.27 ± 0.91 ng/mL).

Glucose

The effects of pretreatments with Me, Mi, Ke alone and in combinations on the changes of glucose concentration are shown in Figure 6. In castrated cats, glucose concentration in all groups except for the Ke group increased significantly pre- and postoperatively and/or at early-recovery (162 ± 36, 186 ± 35, 135 ± 38, 212 ± 60, 203 ± 30 and 222 ± 31 mg/dL in control, Me, Mi, MM, MK and MMK at post-operation, respectively) compared with baseline (ranged 96 ±13 to 111 ±

39 mg/dL). Glucose concentration pre- and postoperatively and during early-recovery was significantly higher in the Me-treated groups than in non-Me groups. In ovariohysterectomized cats, glucose concentration increased significantly pre- and postoperatively and at early- and/or complete-recovery compared with baseline in all groups. Glucose concentration pre- and postoperatively and/or during early-recovery was significantly higher in Me-treated groups than in non-Me groups.

NEFA

The effects of pretreatments with Me, Mi, Ke alone and in combinations on the changes of NEFA concentration are shown in Figure 7. In castrated cats, NEFA concentration in the control group increased significantly preoperatively compared with baseline, whereas that in Me-treated groups decreased significantly pre- and postoperatively and/or during early-recovery. NEFA concentration pre- and postoperatively and at early-recovery was significantly lower in the Me, Mi, Ke and Me-combined groups than in the control group. In ovariohysterectomized cats, NEFA concentration in all groups except for the Ke group decreased significantly pre- and postoperatively and/or at early-recovery compared with baseline. NEFA concentration pre- and postoperatively and/or during early-recovery was significantly or insignificantly lower in Me-treated groups than in non-Me groups.

Recovery score

The results on scoring of behavioral changes during recovery are shown in Table 2. In both castrated and ovariohysterectomized cats, score data were significantly lower in Me, MK and MMK groups than in non-Me groups. No significant difference in score among Me-treated groups was observed, except for the fact that it was lower in the MMK group than in the MM group.

Table 1. Blood sampling time to post-operation, early-recovery and complete-recovery phases in isoflurane-anesthetized cats premedicated with medetomidine (Me; 50 μg/kg), midazolam (Mi; 0.5 mg/kg) and ketamine (Ke; 5 mg/kg), either alone or in combination.

Surgery	Group	During anesthesia (mins)	After discontinuing anesthesia (mins)	
		Post-operation	Early-recovery	Complete-recovery
Castration	Control	21.5 ± 2.6	17.8 ± 15.7	323 ± 29
	Me	23.3 ± 5.4	25.0 ± 9.7	323 ± 46
	Mi	27.9 ± 8.9	14.8 ± 5.0	342 ± 44
	Ke	21.0 ± 3.3	16.0 ± 14.1	331 ± 54
	MM	23.1 ± 7.6	35.0 ± 18.9	301 ± 45
	MK	31.3 ± 9.6	32.0 ± 12.9	324 ± 32
	MMK	30.6 ± 5.6	43.3 ± 23.9	335 ± 37
OHE	Control	48.0 ± 7.3	15.0 ± 7.0	312 ± 34
	Me	50.9 ± 4.0	34.0 ± 30.4	338 ± 17
	Mi	52.5 ± 6.1	19.3 ± 7.3	308 ± 27
	Ke	46.8 ± 4.1	14.1 ± 4.3	295 ± 23
	MM	58.8 ± 11.0	39.0 ± 19.1	307 ± 22
	MK	60.0 ± 7.8	30.0 ± 9.6	312 ± 21
	MMK	54.3 ± 14.2	47.6 ± 35.0	312 ± 30

Values represent mean ± SD of eight cats. OHE = Ovariohysterectomy; MM = Me and Ke; MK = Me and Ke; MMK = Me, Mi and Ke.

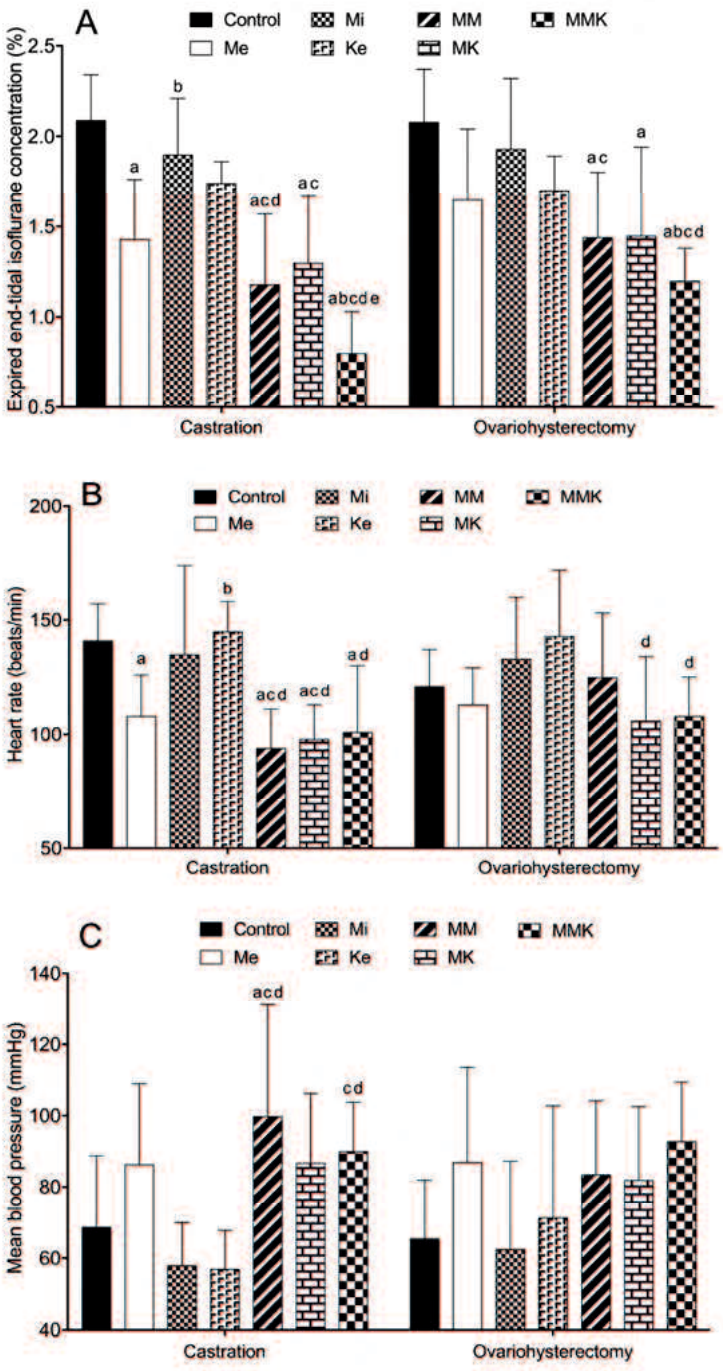


Figure 1. Mean expired end-tidal isoflurane concentration (A), heart rate (B) and mean blood pressure (C) during isoflurane anesthesia and surgery in cats premedicated with medetomidine (Me; 50 µg/kg), midazolam (Mi; 0.5 mg/kg) and ketamine (Ke; 5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of eight cats. MM = Me and Ke, MK = Me and Ke; MMK = Me, Mi and Ke; [a] significantly different from control; [b] significantly different from Me; [c] significantly different from Mi; [d] significantly different from Ke; [e] significantly different from MK; the significance level is $P < 0.05$.

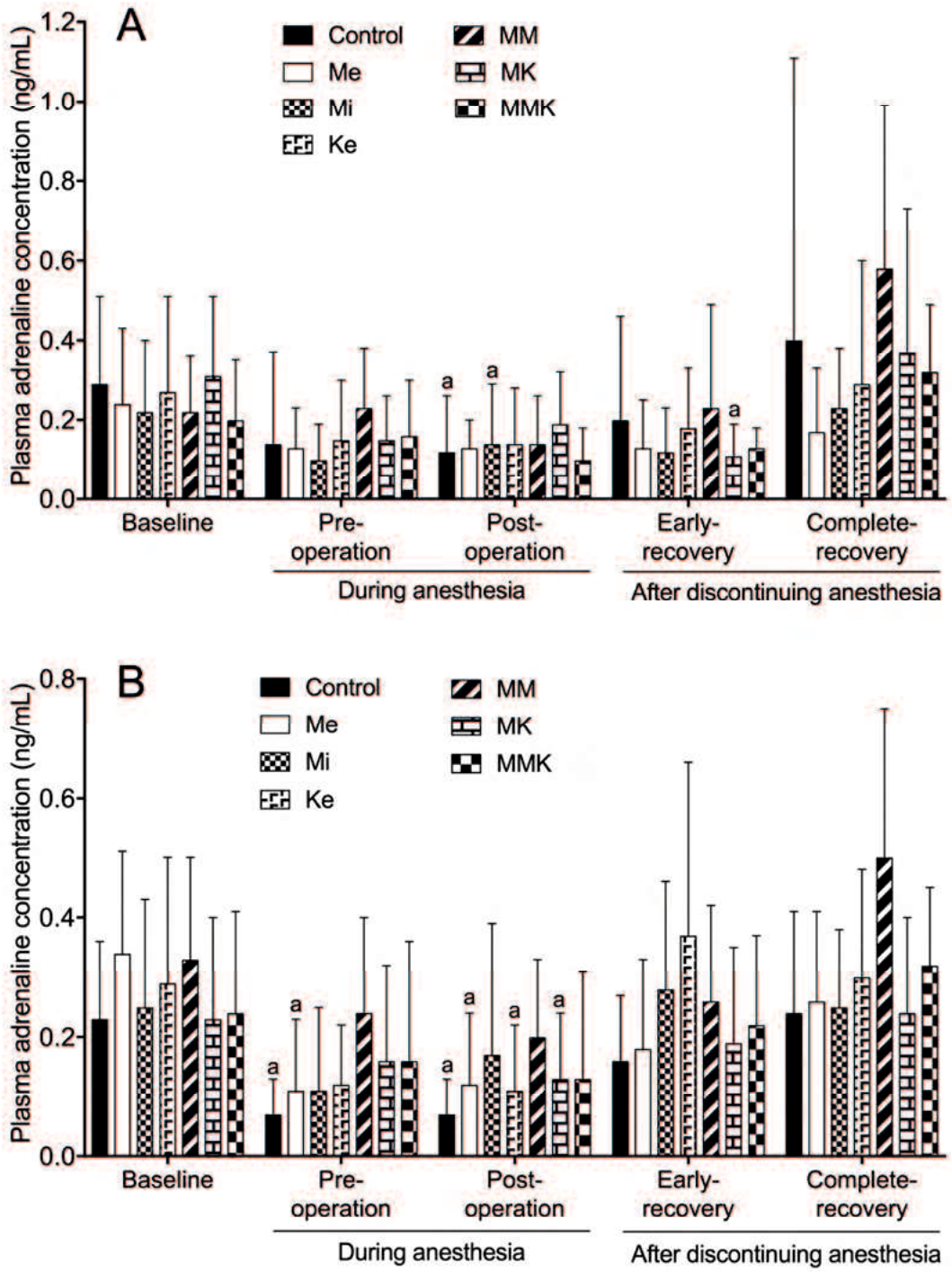


Figure 2. Plasma adrenaline concentration during isoflurane anesthesia and recovery phase in castrated (A) or ovariohysterectomized (B) cats premedicated with medetomidine (Me; 50 µg/kg), midazolam (Mi; 0.5 mg/kg) and ketamine (Ke; 5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of eight cats. MM = Me and Ke, MK = Me and Ke; MMK = Me, Mi and Ke; [a] significantly different from baseline; the significance level is $P < 0.05$.

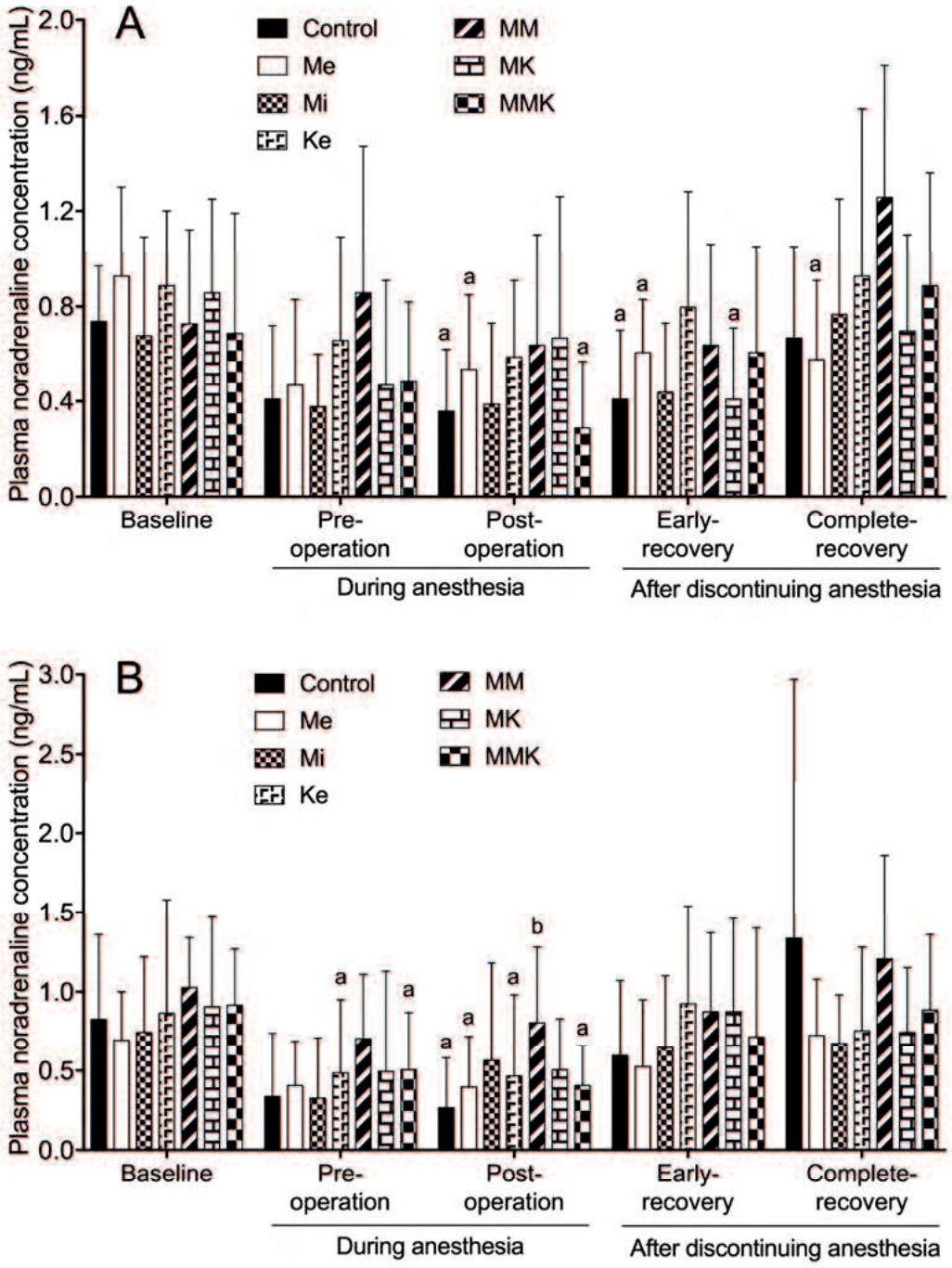


Figure 3. Plasma noradrenaline concentration during isoflurane anesthesia and recovery phase in castrated (A) or ovariohysterectomized (B) cats premedicated with medetomidine (Me; 50 µg/kg), midazolam (Mi; 0.5 mg/kg) and ketamine (Ke; 5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of eight cats. MM = Me and Ke; MK = Me and Ke; MMK = Me, Mi and Ke; [a] significantly different from baseline; [b] significantly different from control; the significance level is $P < 0.05$. different from control; the significance level is $P < 0.05$.

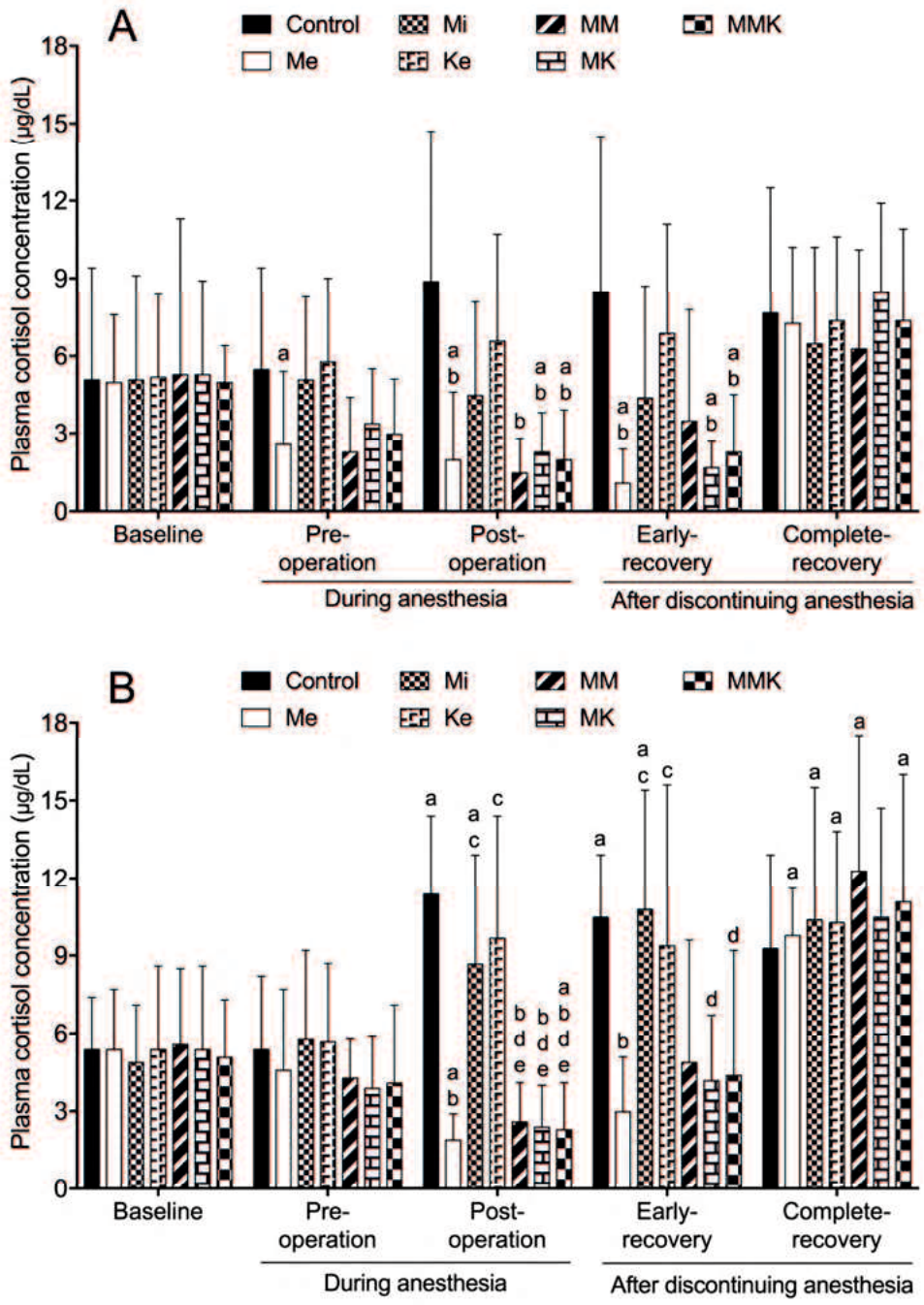


Figure 4. Plasma cortisol concentration during isoflurane anesthesia and recovery phase in castrated (A) or ovariohysterectomized (B) cats premedicated with medetomidine (Me; 50 µg/kg), midazolam (Mi; 0.5 mg/kg) and ketamine (Ke; 5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of eight cats. MM = Me and Ke; MK = Me and Ke; MMK = Me, Mi and Ke; [a] significantly different from baseline; [b] significantly different from control; [c] significantly different from Me; [d] significantly different from Mi; [e] significantly different from Ke; the significance level is $P < 0.05$.

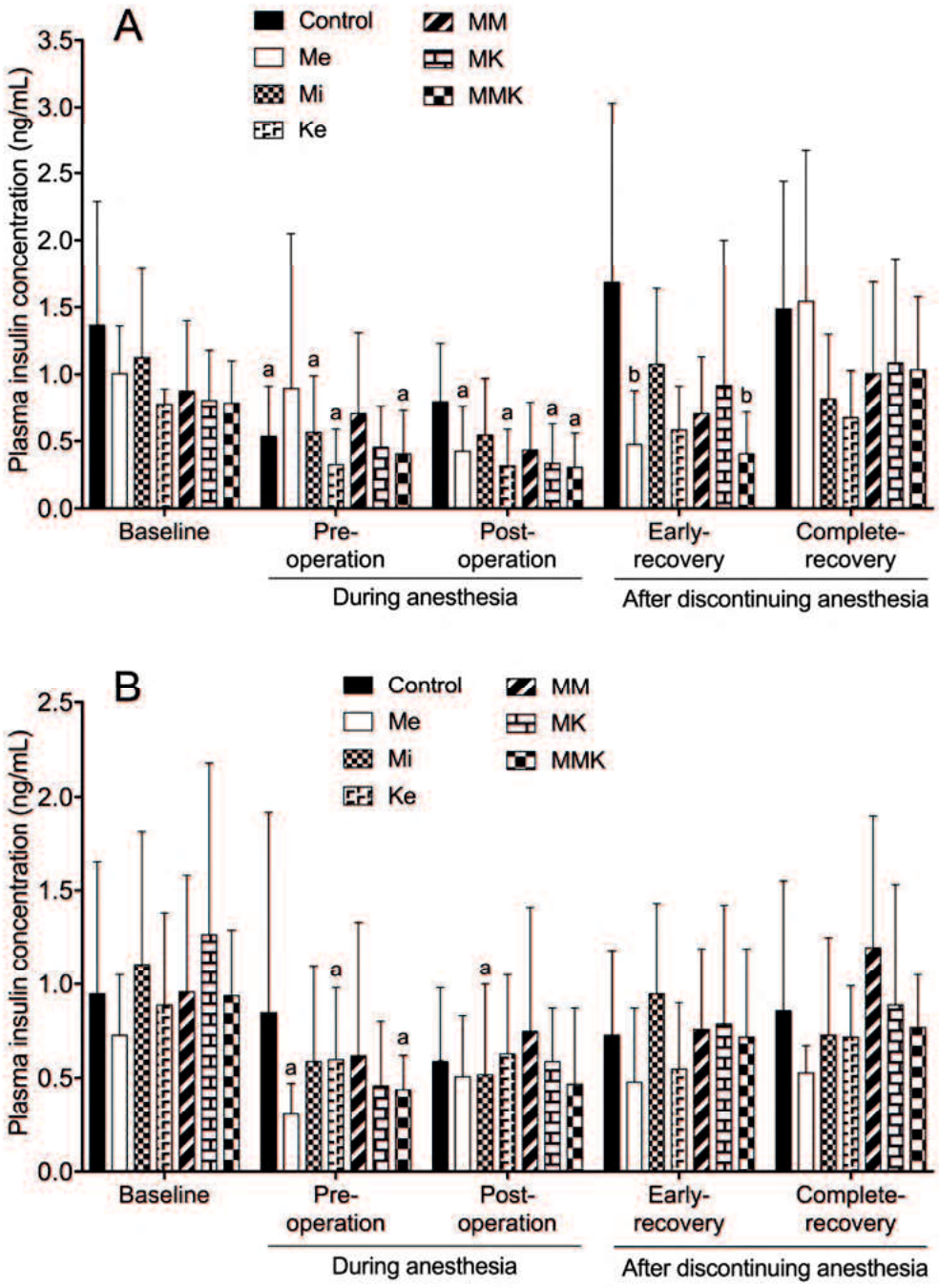


Figure 5. Plasma insulin concentration (ng/mL) during isoflurane anesthesia and recovery phase in castrated (A) or ovariohysterectomized (B) cats premedicated with medetomidine (Me; 50 μg/kg), midazolam (Mi; 0.5 mg/kg) and ketamine (Ke; 5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of eight cats. MM = Me and Ke; MK = Me and Ke; MMK = Me, Mi and Ke; [a] significantly different from baseline; [b] significantly different from control; the significance level is $P < 0.05$.

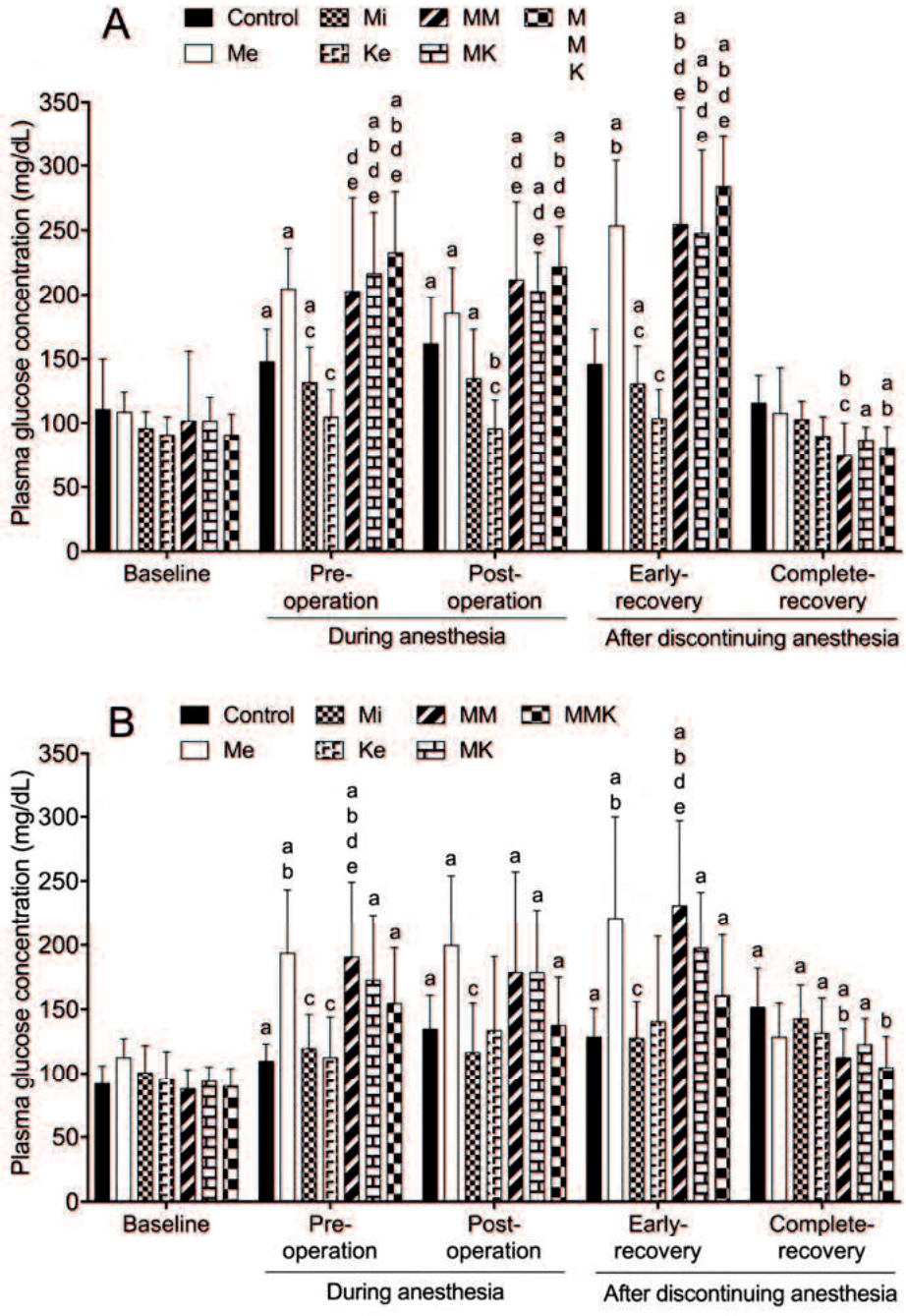


Figure 6. Plasma glucose concentration during isoflurane anesthesia and recovery phase in castrated (A) or ovariohysterectomized (B) cats premedicated with medetomidine (Me; 50 µg/kg), midazolam (Mi; 0.5 mg/kg) and ketamine (Ke; 5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of eight cats. MM = Me and Ke; MK = Me and Ke; MMK = Me, Mi and Ke; [a] significantly different from baseline; [b] significantly different from control; [c] significantly different from Me; [d] significantly different from Mi; [e] significantly different from Ke; the significance level is $P < 0.05$.

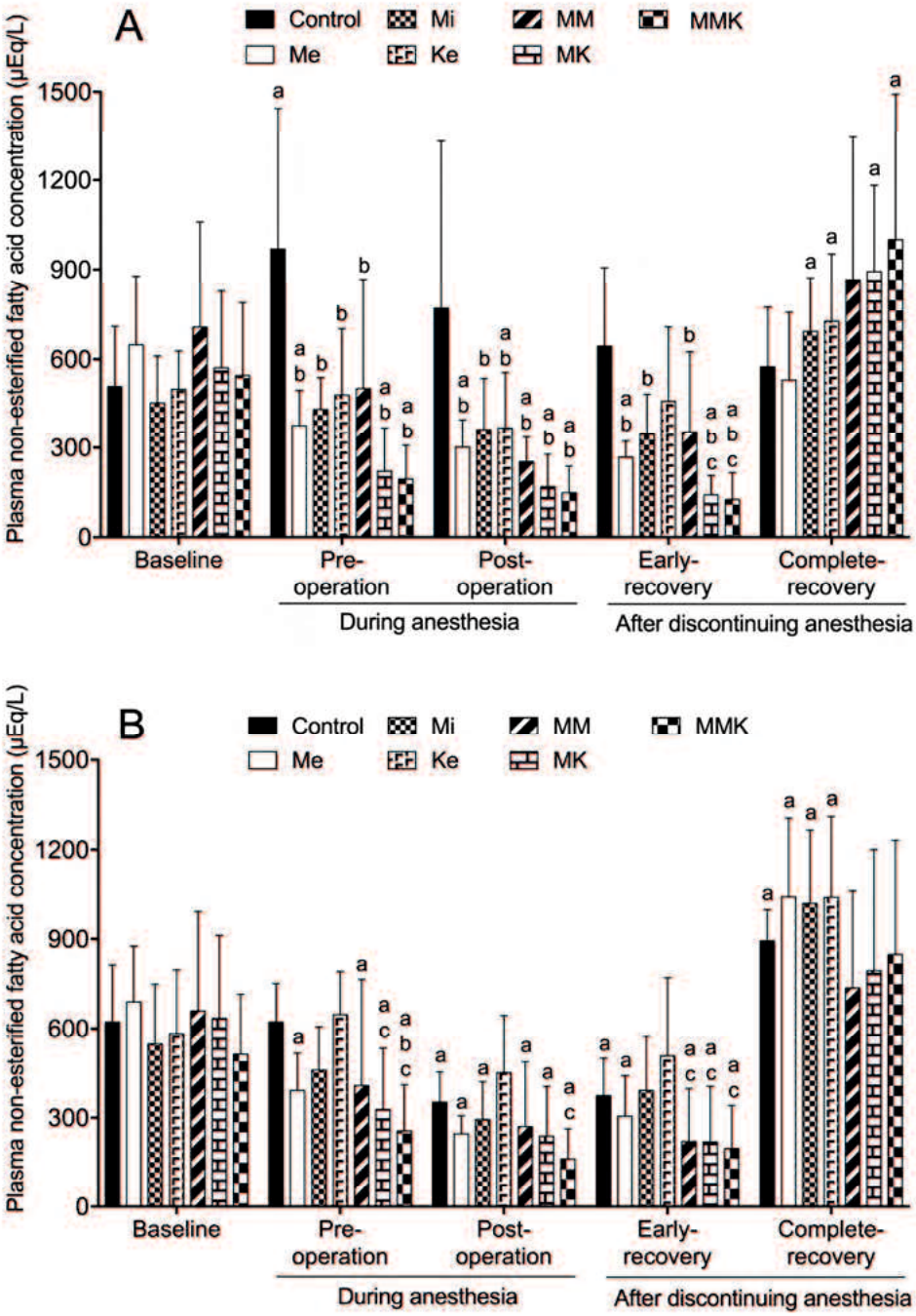


Figure 7. Plasma non-esterified fatty acid concentration during isoflurane anesthesia and recovery phase in castrated (A) or ovariohysterectomized (B) cats premedicated with medetomidine (Me; 50 μg/kg), midazolam (Mi; 0.5 mg/kg) and ketamine (Ke; 5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of eight cats. MM = Me and Ke; MK = Me and Ke; MMK = Me, Mi and Ke; [a] significantly different from baseline; [b] significantly different from control; [c] significantly different from Ke; the significance level is $P < 0.05$.

Table 2. Behavioral recovery score after castration and ovariohysterectomy in isoflurane-anesthetized cats premedicated with medetomidine (Me; 50 μg/kg), midazolam (Mi; 0.5 mg/kg) and ketamine (Ke; 5 mg/kg), either alone or in combination.

Group	Castration	Ovariohysterectomy
Control	4 (3–5)	4 (3–5)
Me	2 (1–3) [a]	2 (1–4) [a]
Mi	4 (3–5) [b]	4 (3–5) [b]
Ke	4 (3–4) [b]	4 (3–4) [b]
MM	3 (2–4)	3 (2–4)
MK	2 (2–3) [acd]	2 (2–3) [acd]
MMK	2 (1–3) [acd]	2 (1–3) [acde]

Values represent the median (range) of eight cats. MM = Me and Ke; MK = Me and Ke; MMK = Me, Mi and Ke; [a] significantly different from control; [b] significantly different from Me; [c] significantly different from Mi; [d] significantly different from Ke; [e] significantly different from MM; the significance level is $P < 0.00714$.

Discussion

The present results revealed that both adrenaline and noradrenaline concentrations decreased during isoflurane anesthesia in cats undergoing castration or ovariohysterectomy. It has been reported that isoflurane inhibits the secretion of catecholamines in bovine adrenal chromaffin cells at concentrations within the range encountered during general anesthesia [38], showing that isoflurane anesthesia itself inhibits the release of catecholamines due to suppressing the sympathetic-adrenomedullary activity in cats. As Me is known to decrease plasma adrenaline and noradrenaline concentrations in cats [25], we postulated that Me treatment may significantly reduce plasma catecholamine levels during isoflurane anesthesia. However, the present results (Figures 2 and 3) demonstrated that Me treatment did not enhance the inhibition of catecholamine release compared to that in the control group during isoflurane anesthesia. This may be responsible for the decrease in isoflurane concentration by Me treatment. The present results also revealed that catecholamine concentrations during isoflurane anesthesia were higher in the MM group than in the control group in ovariohysterectomized cats (Figures 2 and 3). Plasma adrenaline and noradrenaline concentrations are increased by Mi in cats [26] and by Ke in dogs [4]. Ke-induced increases in plasma adrenaline and noradrenaline are mitigated by Mi [1] and even abolished by Me [4]. Therefore, both the reduction of isoflurane concentration by MM treatment and the effect of Mi on catecholamine release may be involved in preventing an excessive decrease in catecholamine concentrations during isoflurane anesthesia.

Previous studies have shown that Me alone or MM does not significantly affect plasma cortisol concentration in healthy cats without inhalant anesthesia and surgery [25,26]. In the present study, plasma cortisol concentration increased significantly in the control and non-Me groups postoperatively and at the early-recovery phase in isoflurane-anesthetized cats, whereas it decreased in Me-treated groups (Figure 4). These results in cats were similar to previous reports in dogs, which showed that Me premedication reduced or delayed the increase in cortisol

concentrations induced by ovariohysterectomy [6,29]. Ke alone increased cortisol concentration from sympathomimetic effects in dogs [4] and humans [27] and did not prevent further cortisol release from surgery in humans [18]. The present study also confirmed that Ke premedication did not diminish the increase in cortisol concentration during isoflurane anesthesia and surgery (Figure 4). Furthermore, cortisol concentration postoperatively and during the recovery phase tended to be greater in ovariohysterectomy than in castration in each group, supporting that cortisol release depends on the type of surgery, nociceptive stimulation and the degree of trauma [11]. Overall, the present findings indicate that pretreatment with Me alone and in combination is useful to suppress an excessive adrenocortical activity perioperatively in isoflurane-anesthetized cats. In the present study, the quality of recovery from anesthesia was better in Me-treated groups than in non-Me groups (Table 2), supporting that Me pretreatment reduces the adrenocortical response during the arousal from isoflurane anesthesia and surgery in cats.

Isoflurane itself inhibits insulin release from pancreatic ß cells and glucose tolerance independent of a dosage up to 1.5 minimal alveolar concentration in humans [46]. Anesthesia with low- or high-dose isoflurane reduces plasma insulin level in rats [41]. In the current study, the insulin concentration in the control group decreased during isoflurane anesthesia in either ovariohysterectomy or castration, supporting the hypothesis that isoflurane anesthesia can inhibit insulin release in cats as well as in humans. Me inhibits insulin secretion via α_2-adrenoceptors on ß cells of the pancreas [41]. In previous studies, an intramuscular Me at wide range of dosages decreased plasma insulin concentration in unanesthetized cats [25]. Mi alone did not significantly alter plasma insulin levels in healthy cats [26]. Ke alone did not affect basal plasma insulin concentration at initial stage of cesarean section in humans [18]. In the current study, the decrease in plasma insulin concentration tended to be greater in Me-treated groups than in the control group during isoflurane anesthesia and at early-recovery, suggesting that Me premedication enhances the inhibition of insulin release in isoflurane anesthesia and postoperatively in cats.

The present results revealed that blood glucose increased during isoflurane anesthesia and recovery in the control group. This increase in blood glucose may be responsible for several factors including surgical injuries, increased cortisol, decreased insulin and decreased peripheral use of glucose associated with inhalant anesthesia [11]. Me induces a dose-dependent hyperglycemia with inhibition of insulin release in healthy cats [25]. In the present study, glucose concentration during anesthesia and early-recovery was higher in Me-treated groups than in non-Me groups, showing that Me premedication facilitates the hyperglycemia during isoflurane anesthesia and surgery in cats. This enhancement of hyperglycemia by Me may be mainly due to Me-induced inhibition of insulin release via α_2-adrenoceptors on pancreatic ß-cells [16]. Conversely, an intravenous administration of 10 μg/kg dexmedetomidine induced hyperglycemia but did not significantly alter insulin concentration in healthy cats [7]. In the present study, the degree of hyperglycemia in Me-treated groups seemed not to be correlated with decreased insulin levels, suggesting that insulin is not the only factor affecting glucose levels in Me-treated cats. Nevertheless, this hyperglycemia may limit the use of Me in cats with metabolic and neurohormonal problems such as diabetes mellitus, ketosis and glycosuria. To overcome Me-induced hyperglycemia and possible risks, it may be necessary to consider early use of antagonists after surgery was completed.

The change in NEFA concentration is important as a metabolic index of the stress response because it is affected by hormones like cortisol and catecholamines [11]. Lipolytic activity is stimulated by cortisol and catecholamines and inhibited in the presence of insulin [11]. In the present study, NEFA concentration in the control group increased slightly preoperatively in castrated cats but decreased postoperatively and during early-recovery in ovariohysterectomized cats. These results may be attributed to the complicated effects of decreased insulin, decreased catecholamine and increased cortisol in isoflurane anesthesia and surgery. Me reduces plasma NEFA concentration; however, Mi does not reduce it in unanesthetized cats [25,26]. Ke alone

does not significantly alter NEFA concentrations in dogs [4]. In the present study, treatment with Me alone and in combination significantly reduced NEFA concentration during anesthesia and in early-recovery in cats. These results may be due to the suppression of lipolytic activity via α_2-adrenoceptors on adipose tissues as well as decreased cortisol and catecholamine concentrations [11,16]. Although the fluctuation of NEFA concentration may not be directly harmful to anesthetized cats, this change may indirectly reflect the sympathetic-adrenal activation associated with anesthesia and surgery. Therefore, the Me pretreatment-induced reduced NEFA concentration may be clinically significant as a metabolic index of inhibition of sympathetic-adrenal activation in isoflurane-anesthetized cats undergoing surgery.

In conclusions, isoflurane anesthesia itself inhibited decreased adrenaline and noradrenaline concentrations but increased cortisol concentrations and hyperglycemia in castrated and ovariohysterectomized cats. Pretreatment with Me alone and in combination reduced cortisol release during isoflurane anesthesia and in the early-recovery phase as well as the improvement of the quality of recovery. No remarkable differences in sympathetic-adrenal and metabolic responses were observed between Me-treated groups, except that MM treatment prevented an excessive decrease in catecholamine concentrations during isoflurane anesthesia. This study demonstrated newly that pretreatments with Me alone and in combination are useful for the prevention of stress responses induced by isoflurane anesthesia and surgery in feline practice.

Chapter 2

Effects of alfaxalone in combination with medetomidine and midazolam on the stress-related neurohormonal and metabolic responses of isoflurane-anesthetized cats undergoing ovariohysterectomy or castration

Introduction

Alfaxalone (Af; 3α-hydroxy-5α-pregnane-11, 20-dione) is a newly developed anesthetic agent that is useful for the sedation, induction, and maintenance of anesthesia in cats [52]. However, during the recovery period, this agent produces more adverse events than propofol, including ataxia and muscular tremors [34,45]. The quality of recovery from anesthesia with Af is reported to be improved with the use of other sedative and/or inhalant anesthetic agents [52]. The alpha(α)$_2$-adrenoceptor agonist medetomidine (Me) is widely used in feline veterinary practice as an excellent analgesic and muscle relaxant; however, it induces undesirable effects, such as bradyarrhythmia, hyperglycemia, and emesis [10,25,36]. A combination of Me with midazolam (Mi) and ketamine produces good analgesia and anesthesia in cats, with better analgesia potentiation [9, 39,43,48]. Therefore, there is a need to investigate the effects of using Af instead of ketamine in combination with Me–Mi as a preanesthetic medication in feline veterinary practice.

Stressors such as anxiety, excitement, pain, anesthesia, and surgery induce neurohormonal and metabolic changes in animals; these changes are characterized by elevated blood cortisol levels, catecholamines, glucose, and non-esterified fatty acids (NEFA) [11]. Actions mediated by α_2-adrenoceptors are closely coordinated with these stressors [25]. In cats, anesthesia with Me, Mi, and ketamine suppresses the release of catecholamine and cortisol, suppresses lipolysis, and induces hyperglycemia [48]. Thus, there is a need to evaluate cats' stress-related hormonal and metabolic responses during anesthesia and post-operatively in clinical practice.

Me prevents or delays the stress response induced by ovariohysterectomy in isoflurane-anesthetized dogs [6]. In halothane-anesthetized dogs undergoing ovariohysterectomy, Me prevents an increase in plasma cortisol concentrations both during the surgery and during early recovery [29]. Me appears to offer some advantages over acepromazine in terms of decreasing perioperative concentrations of stress-related hormones, including plasma catecholamine and

cortisol [51]. However, to the best of our knowledge, there are no published reports on the effects of pretreatment with Af alone, and in combination with other sedatives, on stress responses in anesthetized cats undergoing surgery. Therefore, this study aimed to evaluate the effects of the intramuscular (IM) and intravenous (IV) administrations of Af, both alone and in combination with Me and Mi, on key stress-related neurohormonal and metabolic changes in isoflurane-anesthetized cats undergoing ovariohysterectomy or castration. This study was designed to assess stress responses during clinically important perioperative stages in feline veterinary practice.

Materials and methods

Animals

Seventy-two client-owned, mixed-breed cats (36 males, 36 females) were prospectively recruited at the Kamohara Animal Hospital for ovariohysterectomy or castration. They were clinically healthy and ranged from 6 months to 1 year of age. On average, males weighed 3.76 ± 0.39 kg (mean ± standard deviation [SD]), and females weighed 3.13 ± 0.54 kg. Each cat owner provided informed consent for data collection.

Physical and routine hematological examinations prior to the study revealed that all values were within the normal physiological ranges. All cats fasted for 12 h but had *ad libitum* access to water. Owners brought their cats to our hospital early in the morning on the day of surgery. After preparation for surgery and anesthesia, each cat rested in a darkened cage for 2–3 h before anesthesia. After complete recovery from anesthesia, all cats received water *ad libitum* and food.

Study protocol

The cats were randomly assigned to one of six treatment groups (six cats in each group) for both ovariohysterectomy and castration. Each cat was given one of the following pretreatments: 1) physiological saline solution (0.5 mL) intramuscularly (IM) + 5 mg/kg Af intravenously (IV); 2) physiological saline solution IM + 5 mg/kg Af IM; 3) 50 μg/kg Me (1 mg/mL) IM + 5 mg/kg Af IV; 4) 50 μg/kg Me IM + 5 mg/kg Af IM; 5) 50 μg/kg Me + 0.5 mg/kg Mi (5 mg/mL) IM + 5 mg/kg Af IV; and 6) 50 μg/kg Me + 0.5 mg/kg Mi IM + 5 mg/kg Af IM. The groups will be referred to as Af IV, Af IM, Me + Af IV, Me + Af IM, MM + Af IV, and MM + Af IM, respectively.

Me and Mi were mixed in the same syringe immediately prior to injection. Ten minutes after the Me and Mi injection, Af was administered IV or IM to induce anesthesia. In the Af IM and Me + Af IM groups, anesthesia was induced with 4% isoflurane in oxygen at a total gas flow rate of

1.5 L/min using a face mask attached to the ADS 1000 veterinary anesthesia delivery system (Engler, Hialeah, FL). Then, a cuffed endotracheal tube was inserted in all cats. In the Af IV and Me + Af IV groups, anesthetic induction using a face mask was assisted when the effect on tracheal intubation was insufficient.

All cats were placed supine and maintained at a surgical depth of anesthesia with isoflurane in oxygen through a non-rebreathing system under controlled ventilation. During isoflurane anesthesia, the cats underwent castration or ovariohysterectomy using standard methods. Pre-operatively, an analgesic (0.3 mg/kg meloxicam; Inflacam, Chanelle Pharmaceuticals Manufacturing Ltd., Ireland) was injected subcutaneously in all cats, followed by a subcutaneous injection once daily for 2–3 days after surgery, if necessary. Ringer's lactate solution was infused IV at 10 ml/kg/h during anesthesia and surgery. The duration of isoflurane anesthesia was 30 and 60 min in castrated and ovariohysterectomized cats, respectively, after which time isoflurane inhalation was completely stopped. The endotracheal tube was extubated once a laryngeal reflex was visible.

During the recovery process, the cats remained in separate cages in a room with an air temperature of 25°C. General postoperative management and care were performed for all cats. After complete recovery, another analgesic, butorphanol (0.1–0.4 mg/kg; Vetorphale, Meiji Seika, Tokyo, Japan) was injected IM to cats with signs of pain, such as those exhibiting vocalizations, anorexia, and pain-related postures. There were no issues with surgery or anesthesia in any of the cats.

Anesthesia and intraoperative monitoring

An agent-specific precision vaporizer was used to administer isoflurane. Gas samples were collected from the breathing circuit through a tube attached to an adapter positioned at the oral end of an endotracheal tube. During anesthesia, we assessed the expired end-tidal isoflurane (EtIso)

and carbon dioxide ($EtCO_2$) concentrations, arterial oxygen saturation (SpO_2) using pulse oximetry, heart rate (HR), respiration rate (RR), rectal temperature, and mean blood pressure (MBP) using the oscillometric method, either continuously or intermittently, with a multi-parameter monitor (BSM-5192; Nihon Kohden, Tokyo). During controlled ventilation, RR was adjusted to a range of approximately 25–35 mmHg $EtCO_2$. The mean RR during anesthesia in the castrated and ovariohysterectomized groups were 11.0–13.7 and 10.1–12.5 breaths/min, respectively. SpO_2 was >98% in all cats. Mean RT ranged from 38.2°C–37.4°C pre-operatively and 37.6°C–36.5°C post-operatively in the castration groups, and from 38.4°C–37.2°C pre-operatively and 36.8°C–35.8°C post-operatively in the ovariohysterectomy groups. There were no significant differences in RT, RR, $EtCO_2$, or SpO_2 values across groups for both types of surgery.

Behavioral scoring of recovery

The overall quality of recovery from anesthesia was assessed using previously published scoring methods [47], as follows: score 1 = excellent; score 2 = good; score 3 = moderate; score 4 = poor; and score 5 = extremely poor. The observer was blinded to treatment. Times to extubation and the head-up motion after discontinuing isoflurane anesthesia were also assessed in all groups.

Blood sample collection

Blood sample collection was conducted during clinically important perioperative stages. Blood samples (2 mL) were collected from a 24-gauge catheter inserted into the cephalic vein on five occasions: 1) immediately before pretreatment (baseline); 2) 5 min after induction of anesthesia and before the surgical procedure during anesthesia (pre-operatively); 3) after completing the surgical procedure during anesthesia (post-operatively); 4) at head-up motion after removal of the tracheal tube after discontinuation of anesthesia (early recovery); and 5) 180 min after the discontinuation of anesthesia (complete recovery). During isoflurane anesthesia,

postoperative blood samples were collected at 25 and 55 min in the castrated and ovariohysterectomized groups, respectively.

Sample processing and analysis

Blood was mixed with ethylenediaminetetraacetic acid to prevent clotting. Samples were immediately centrifuged; the plasma was separated and frozen at −76°C until analysis. Glucose, NEFA, cortisol, adrenaline, and noradrenaline levels, were measured according to previously published methods [25,47]. In brief, glucose and NEFA levels were determined via an enzyme assay and a spectrophotometer. Cortisol levels were measured via a solid phase-antibody radioimmunoassay. Catecholamines were extracted on activated alumina and measured using high-performance liquid chromatography and an electrochemical detector.

Statistical analysis

All data were analyzed using Prism 7.0 (GraphPad, CA). All data are presented as mean ± SD. All data (other than score data) were tested for normality using the Shapiro–Wilk test. A repeated-measures one-way analysis of variance (ANOVA) was used to examine differences across variables within each group. *Post hoc* Dunnett's multiple-comparisons tests were used to identify differences from baseline within each group. One-way ANOVA and *post hoc* Tukey's multiple-comparisons tests were used to determine differences across groups. In all tests, the significance level was set at $P < 0.05$. Score data were analyzed using the Wilcoxon–Mann–Whitney test for treatment comparisons; $P < 0.00833$ was considered significant by Bonferroni correction.

Results

EtIso, HR, and MBP during anesthesia

For both surgeries, mean EtIso concentrations were significantly lower in the Me-combined groups than in the non-Me groups and were lowest in the MM + Af groups (Figure 8). Similarly, for both surgeries, the mean HR was significantly lower in the Me-treated groups than in the non-Me groups. Also, for both surgeries, the MBP was significantly higher in the Me-treated groups than in the non-Me groups. In the Af, Me + Af, and MM + Af treatment groups, all variables were similar for the IV and IM routes of administration.

Adrenaline

In all treatment groups except for the MM + Af IM group, adrenaline concentration decreased pre- and/or post-operatively compared to baseline levels; this was true for both types of surgery (Figure 9). In ovariohysterectomized cats, postoperative adrenaline concentration was higher in the MM + Af IM group than in the Af IM group (Figure 9). Adrenaline concentration did not differ across groups during the early and complete recovery phases.

Noradrenaline

For both surgeries, noradrenaline concentration decreased pre- and/or post-operatively compared with baseline levels in all groups except for the MM + Af IM group, in which it did not change (Figure 10). In ovariohysterectomized cats, postoperative noradrenaline concentration was significantly higher in the MM + Af IM group than in the Af IV group. Noradrenaline concentration did not differ across groups during the early and complete recovery phases.

Cortisol

In castrated cats in the Af IV and Af IM groups, cortisol concentration did not differ from baseline during anesthesia and recovery. Conversely, in the Me + Af IM and MM + Af IM groups undergoing castration, cortisol decreased post-operatively compared to baseline levels (Figure 11). In ovariohysterectomized cats in the Af IV and Af IM groups, compared to baseline levels, cortisol concentration was similar during anesthesia but increased in the early recovery phase. On the other hand, in the MM + Af group, cortisol concentration decreased post-operatively compared with baseline levels. Post-operatively, cortisol concentration was lower in the Me + Af IM and MM + Af IV groups than in the Af IV group. Cortisol levels did not differ across the Me-combined groups in any phases (Figure 11).

Glucose

In castrated cats in the Af IM group, glucose concentration increased slightly pre- and/or post-operatively compared with baseline levels (Figure 12). On the other hand, glucose concentration in the Me-combined groups undergoing castration increased pre- and post-operatively compared with baseline. Pre- and postoperative glucose concentrations were higher in the Me + Af IV, Me + Af IM, and MM + Af IV groups than in the Af IV and Af IM groups. Glucose concentration during early recovery was also higher in the Me + Af IM group than in the Af IM group.

In ovariohysterectomized cats in the Af IV and Af IM groups, glucose concentration increased pre- and/or post-operatively and during early and/or complete recovery compared to baseline (Figure 12). Glucose concentration also increased pre- and/or post-operatively compared with baseline in the Me-combined groups. Pre-operative glucose concentration was higher in the Me + Af IV, Me + Af IM, and MM + Af IV groups than in the Af IV and Af IM groups.

NEFA

In castrated cats in the Af IV and Af IM groups, NEFA concentration decreased post-operatively compared with baseline but thereafter increased during early and complete recovery (Figure 13). In the Me-combined groups, NEFA concentration decreased pre- and/or post-operatively, compared to baseline. Pre-operative NEFA concentration was lower in the MM + Af IV group than in the Af IV group.

In ovariohysterectomized cats in the Af IV and Af IM groups, NEFA concentration did not significantly change pre- and/or post-operatively from baseline, and thereafter increased during the recovery phases in the Af IV group (Figure 13). In the Me-combined groups, NEFA concentration decreased pre- and/or post-operatively compared to baseline. Pre- and postoperative NEFA concentrations were lower in the Me + Af IM and Me + Af IV groups than in the Af IV group, respectively.

Recovery time and behavioral recovery scores

In both castrated and ovariohysterectomized cats, recovery times to extubation and head-up were significantly longer in the Me-combined groups than in the Af IV and Af IM groups (Table 3). Generally, there were no differences in recovery time and time to extubation between the Af IV and Af IM groups and between the Me-combined groups, other than in ovariohysterectomized cats, in which time to extubation was longer in the MM + Af IM group than in the Me + Af IV, and longer in both the MM + Af IV and MM + Af IM groups than in the Me + Af IM group.
For both castrated and ovariohysterectomized cats, behavioral scores were lower in the Me-combined groups than the Af IM group (Table 4). For all cats, behavioral scores were lower in the Me + Af IV, MM + Af IV, and MM + Af IM groups than in the Af IV group.

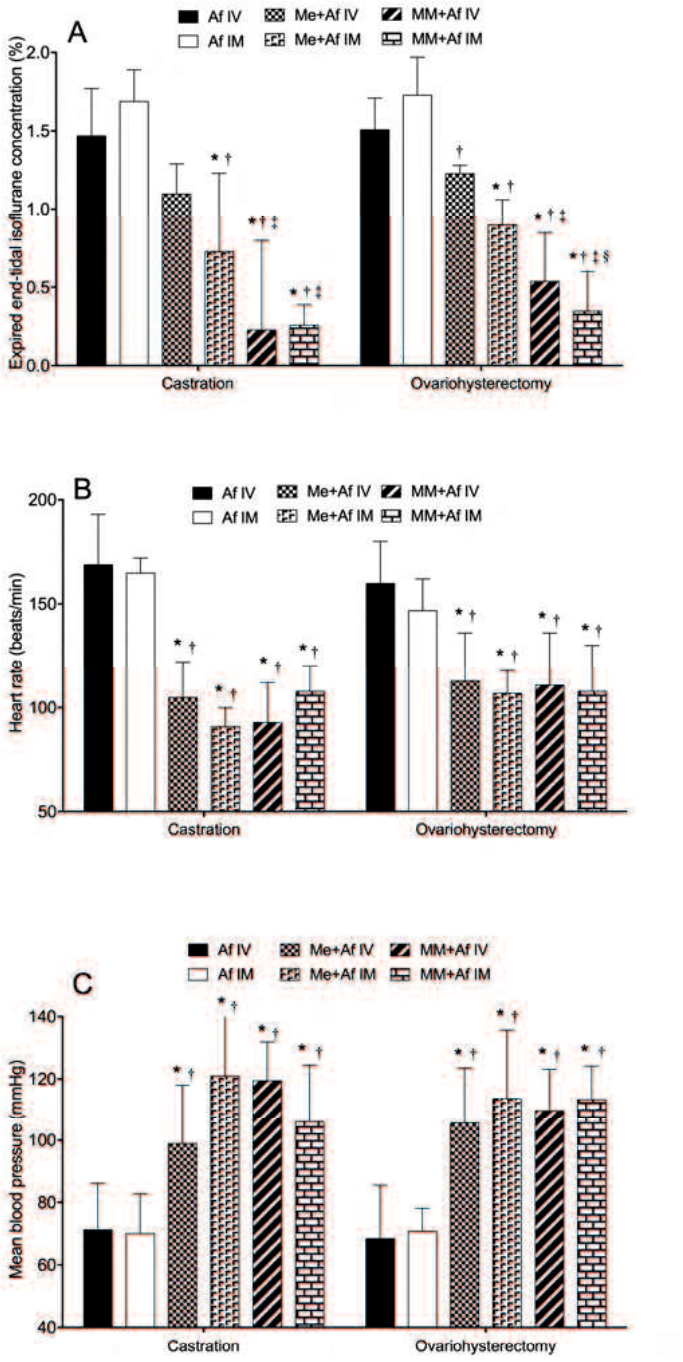


Figure 8. Mean expired end-tidal isoflurane concentration (A), heart rate (B) and mean blood pressure (C) during isoflurane anesthesia and surgery in cats premedicated with alfaxalone (Af; 5 mg/kg), medetomidine (Me; 50 µg/kg), and midazolam (Mi; 0.5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of six cats. IV = intravenously; IM = intramuscularly; MM = Me and Mi; * significantly different from Af IV; † significantly different from Af IM; ‡ significantly different from Me + Af IV; § significantly different from Me + Af IV; the significance level is $P < 0.05$.

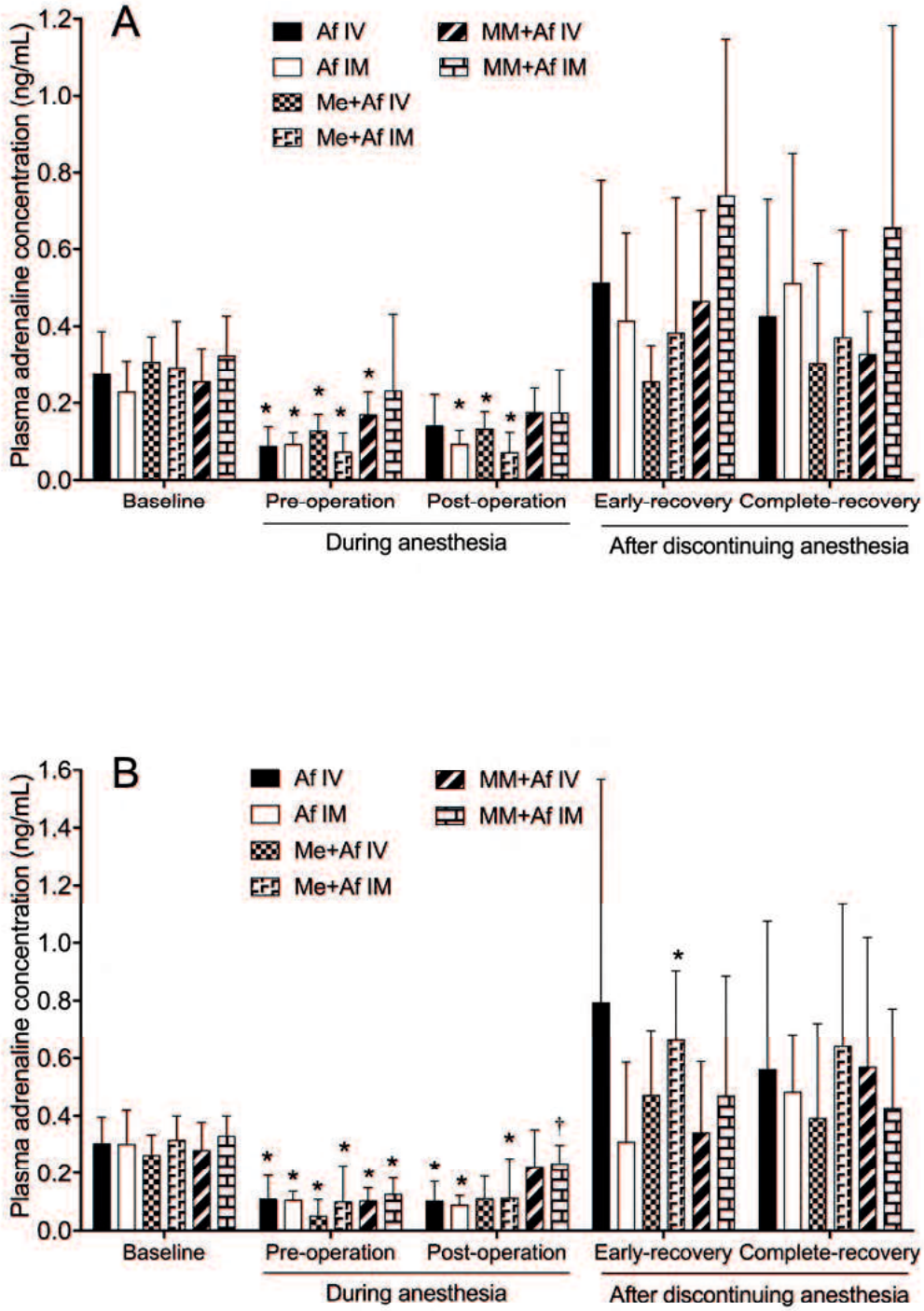


Figure 9. Plasma adrenaline concentration (ng/mL) during isoflurane anesthesia, surgery, and recovery in castrated (A) or ovariohysterectomized (B) cats premedicated with alfaxalone (Af; 5 mg/kg), medetomidine (Me; 50 µg/kg), and midazolam (Mi; 0.5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of six cats. IV = intravenously; IM = intramuscularly; MM = Me and Mi; * significantly different from baseline; † significantly different from Af IM; the significance level is $P < 0.05$.

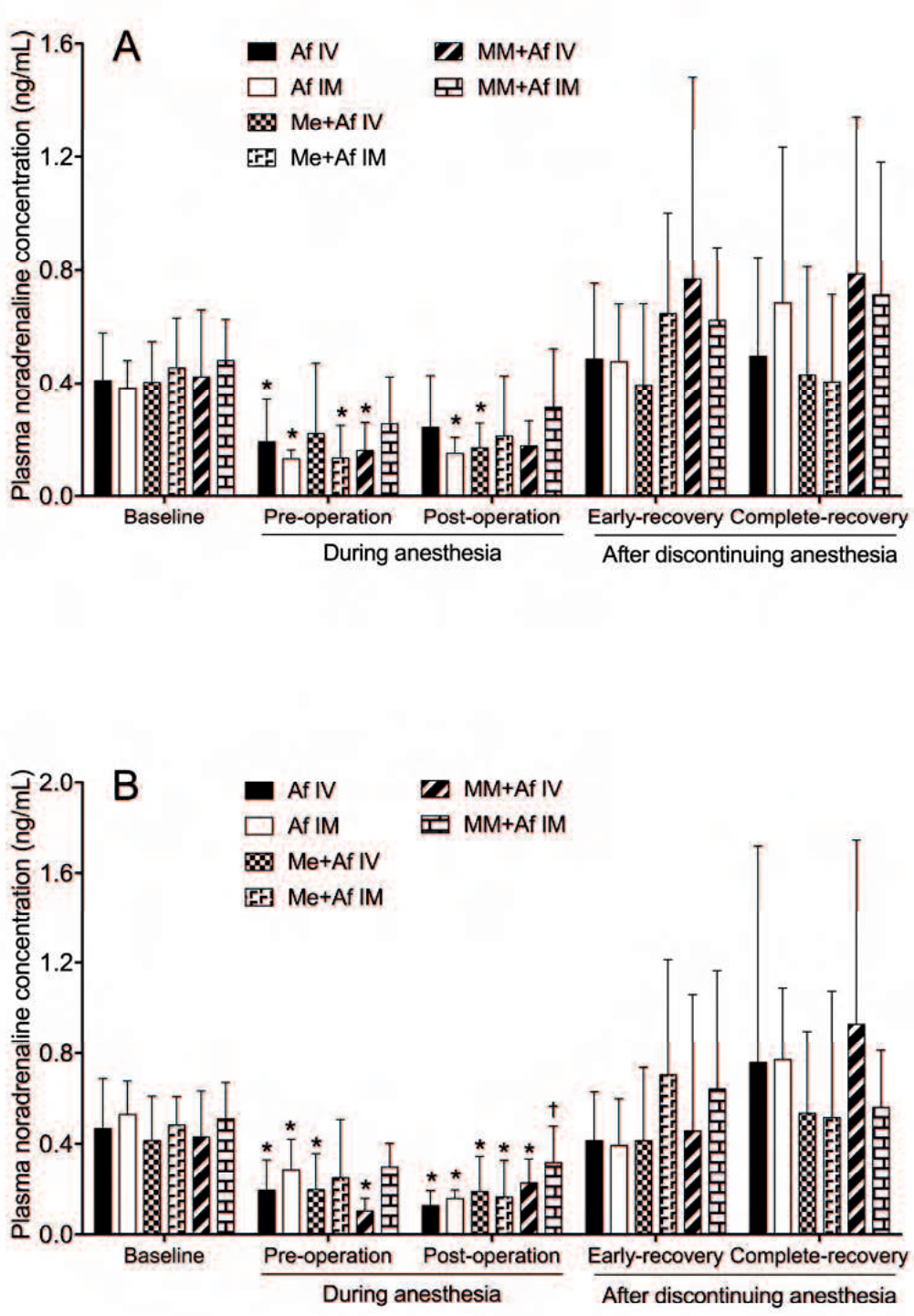


Figure 10. Plasma noradrenaline concentration (ng/mL) during isoflurane anesthesia, surgery, and recovery in castrated (A) or ovariohysterectomized (B) cats premedicated with alfaxalone (Af; 5 mg/kg), medetomidine (Me; 50 μg/kg), and midazolam (Mi; 0.5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of six cats. IV = intravenously; IM = intramuscularly; MM = Me and Mi; * significantly different from baseline; † significantly different from Af IV; the significance level is $P < 0.05$.

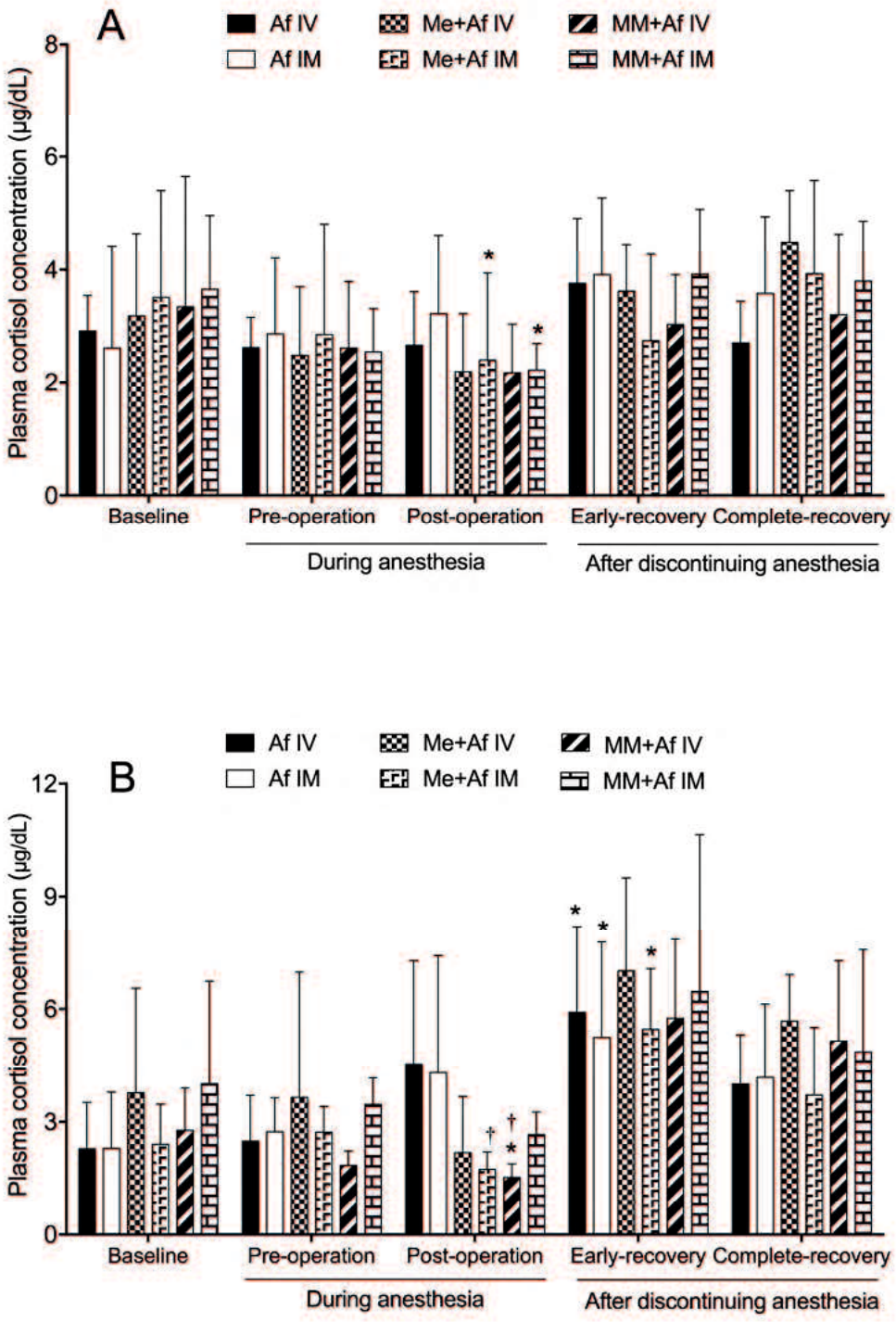


Figure 11. Plasma cortisol concentration (µg/dL) during isoflurane anesthesia, surgery, and recovery in castrated (A) or ovariohysterectomized (B) cats premedicated with alfaxalone (Af; 5 mg/kg), medetomidine (Me; 50 µg/kg), and midazolam (Mi; 0.5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of six cats. IV = intravenously; IM = intramuscularly; MM = Me and Mi; * significantly different from baseline; † significantly different from Af IV; the significance level is $P < 0.05$.

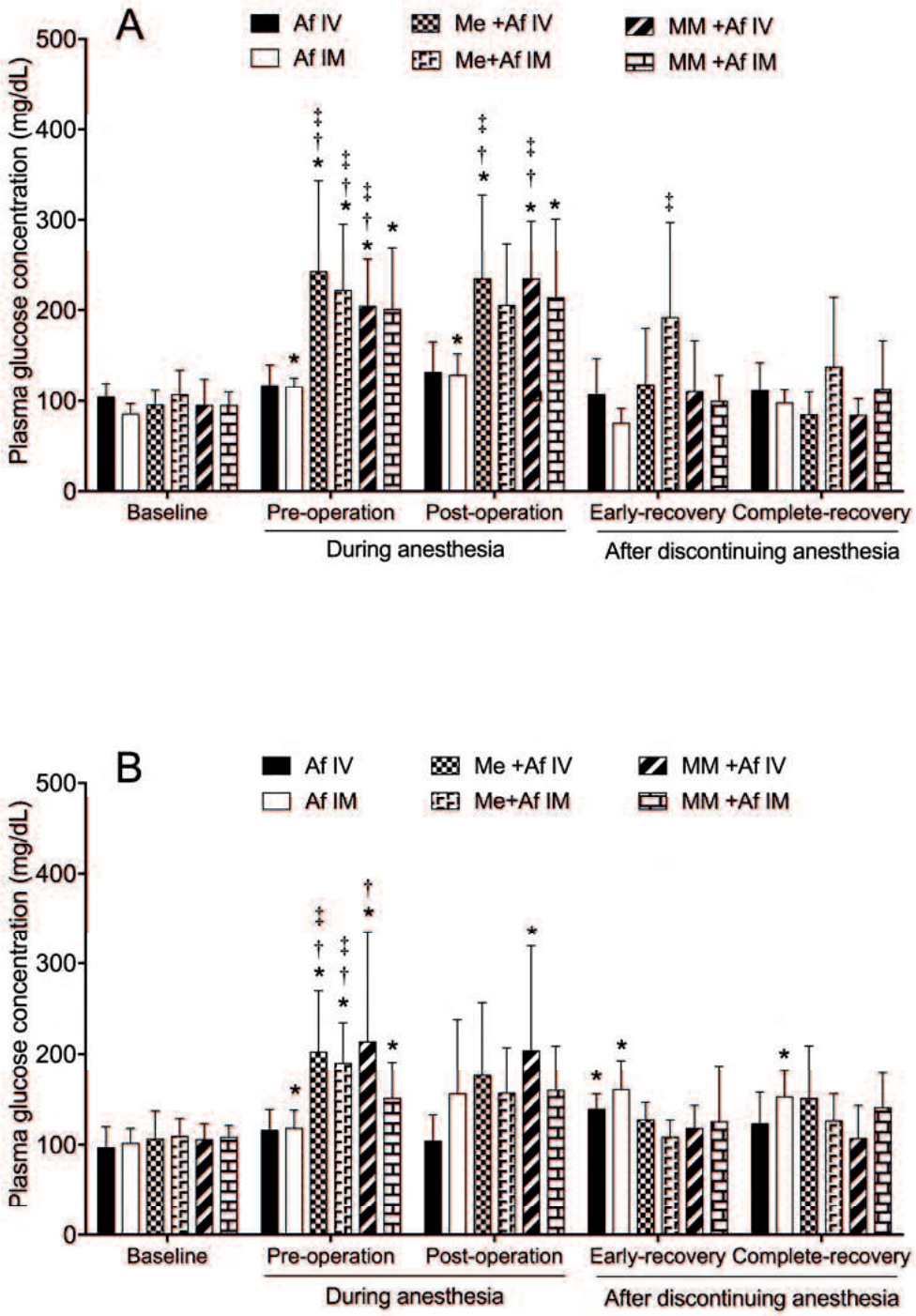


Figure 12. Plasma glucose concentration (mg/dL) during isoflurane anesthesia, surgery, and recovery in castrated (A) or ovariohysterectomized (B) cats premedicated with alfaxalone (Af; 5 mg/kg), medetomidine (Me; 50 μg/kg), and midazolam (Mi; 0.5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of six cats. IV = intravenously; IM = intramuscularly; MM = Me and Mi; * significantly different from baseline; † significantly different from Af IV; ‡ significantly different from Af IM; the significance level is $P < 0.05$.

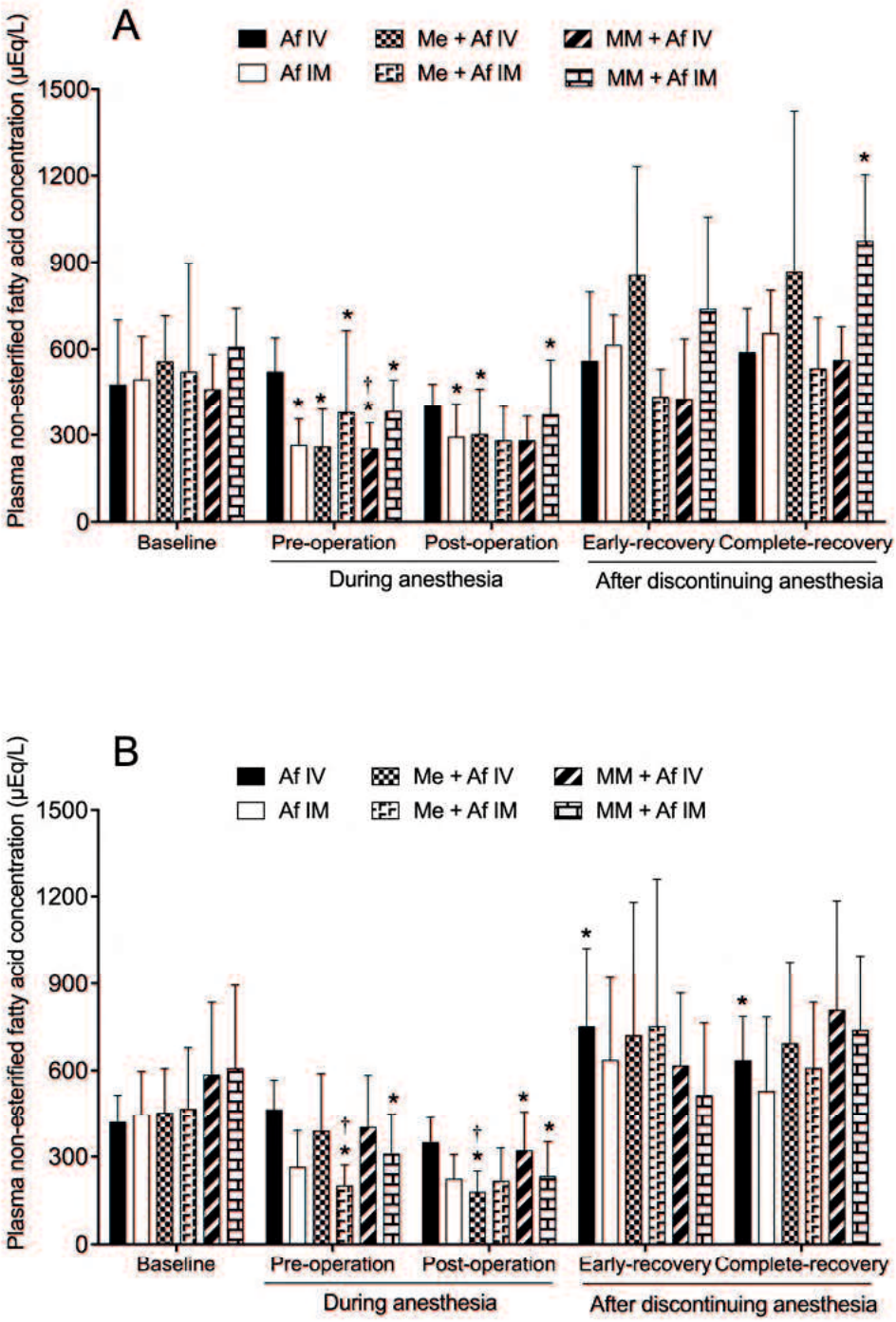


Figure 13. Plasma non-esterified fatty acid concentration (µEq/L) during isoflurane anesthesia, surgery, and recovery in castrated (A) or ovariohysterectomized (B) cats premedicated with alfaxalone (Af; 5 mg/kg), medetomidine (Me; 50 µg/kg), and midazolam (Mi; 0.5 mg/kg), either alone or in combination. Each vertical bar indicates the mean and SD of six cats. IV = intravenously; IM = intramuscularly; MM = Me and Mi; * significantly different from baseline; † significantly different from Af IV; the significance level is $P < 0.05$.

Table 3. Recovery time after castration or ovariohysterectomy in isoflurane-anesthetized cats premedicated with alfaxalone (Af; 5 mg/kg), medetomidine (Me; 50 μg/kg), and midazolam (Mi; 0.5 mg/kg), either alone or in combination.

Group	Time to extubation (min)		Time to head-up (min)	
	Castration	OHE	Castration	OHE
Af IV	8.3 ± 1.6	9.3 ± 2.0	24.2 ± 8.9	42.8 ± 13.2
Af IM	9.3 ± 3.2	12.5 ± 2.7	29.2 ± 9.0	40.0 ± 3.4
Me + Af IV	18.2 ± 4.6*†	28.8 ± 7.2*†	46.2 ± 7.6*†	56.7 ± 12.5
Me + Af IM	20.3 ± 6.2*†	26.2 ± 4.6*†	55.8 ± 7.4*†	60.5 ± 6.8*†
MM + Af IV	22.3 ± 4.5*†	36.7 ± 4.3*†§	48.8 ± 4.9*†	57.8 ± 8.5†
MM + Af IM	21.0 ± 3.7*†	38.2 ± 5.1*†‡§	58.3 ± 8.7*†	61.5 ± 11.7*†

Values represent mean ± SD of six cats; OHE = ovariohysterectomy; IV = intravenously; IM = intramuscularly; MM = Me and Mi; * significantly different from Af IV; † significantly different from Af IM; ‡ significantly different from Me + Af IV; § significantly different from Me + Af IM; the significance level is $P < 0.05$.

Table 4. Behavioral recovery score after castration or ovariohysterectomy in isoflurane-anesthetized cats premedicated with alfaxalone (Af; 5 mg/kg), medetomidine (Me; 50 μg/kg), and midazolam (Mi; 0.5 mg/kg), either alone or in combination.

Group	Castration (n=6)	Ovariohysterectomy (n=6)	Total (n=12)
Af IV	3 (2-4)	3 (2-4)	3 (2-4)
Af IM	4 (3-4)	4 (3-4)	4 (3-4)
Me + Af IV	2 (2-3) †	2 (1-3) †	2 (1-3) * †
Me + Af IM	3 (2-3)	2 (2-3) †	2.5 (2-3) †
MM + Af IV	2 (1-3) †	2 (1-2) †	2 (1-3) * †
MM + Af IM	1.5 (1-3) †	2 (1-3) †	2 (1-3) * †

Values represent mean ± SD of six cats; IV = intravenously; IM = intramuscularly; MM = Me and Mi; * significantly different from Af IV; † significantly different from Af IM; the significance level is $P < 0.00833$.

Discussion

The rationale for using fixed dosages of Af alone and in combination with Me or Mi has been outlined in earlier studies [30,32,45]. The present study revealed that, in both castrated and ovariohysterectomized cats, the combination of Me or MM with Af reduced isoflurane concentration and maintained blood pressure during anesthesia, indicating a minimized effect on the cardiovascular system. The present study further found that, in both castrated and ovariohysterectomized cats, the addition of Me or MM to Af compared with Af alone improved the quality of recovery from isoflurane anesthesia and surgery. These results support previous findings that the quality of recovery from anesthesia with Af is improved with the simultaneous use of other sedatives [52].

The present study revealed that both adrenaline and noradrenaline concentrations decreased during isoflurane anesthesia in castrated or ovariohysterectomized cats premediated with Af with and without Me, except for cats in the MM + Af IM group. Isoflurane inhibits the secretion of catecholamines in adrenal chromaffin cells at concentrations within the range encountered during general anesthesia [38], suggesting that isoflurane anesthesia itself inhibits the release of catecholamines due to a suppression of sympathetic-adrenomedullary activity in cats. As Me is known to decrease plasma adrenaline and noradrenaline concentrations in both cats [25] and dogs [3], it was postulated that the addition of Me to Af may reduce plasma catecholamine levels during isoflurane anesthesia. However, the present study revealed that, during isoflurane anesthesia, Me or MM premedication did not enhance the inhibition of catecholamine release compared to Af premedication alone; this may be responsible for the reduction of isoflurane concentration induced by the Me and MM treatments. The present results also revealed that postoperative catecholamine concentrations tended to be higher in the MM + Af IM group than in the Af IV and/or IM groups in ovariohysterectomized cats; this may be due to the effect of Mi on catecholamine release since plasma adrenaline and noradrenaline concentrations are increased by

Mi in cats [26]. Therefore, both the reduction of isoflurane concentration with the MM + Af treatment and the effect of Mi on catecholamine release may be useful in preventing excessive decreases in catecholamine concentrations during isoflurane anesthesia and surgery.

Previous studies have shown that Me alone or MM does not significantly affect plasma cortisol concentrations in healthy cats without inhalant anesthesia and surgery [25,26]. In the present study, plasma cortisol concentration increased during the early recovery phase in the Af IV and IM groups of ovariohysterectomized cats, whereas it was significantly lower post-operatively in the Me + Af IM and MM + Af IV groups than in the Af IV groups. These findings in cats are similar to those in previous reports in dogs, in which Me premedication reduced or delayed the increase in cortisol concentrations that is induced by ovariohysterectomy [6,29]. On the other hand, postoperative cortisol concentrations in the Af IV and IM groups and during the early recovery phase in all groups tended to be greater for ovariohysterectomy than for castration, supporting the belief that cortisol release depends on the type of surgery, nociceptive stimulation, and the degree of trauma [11]. Overall, the present findings indicate that combinations of Me or MM with Af are useful for suppressing excessive postoperative adrenocortical activity in isoflurane-anesthetized cats.

The present results revealed that blood glucose increased slightly during isoflurane anesthesia and the early recovery phase in the Af IV and IM groups undergoing either ovariohysterectomy or castration. This increase in blood glucose may be responsible for several side effects, including surgical injuries, increased cortisol, decreased insulin, and decreased peripheral use of glucose associated with inhalant anesthesia [11,46]. Me induces dose-dependent hyperglycemia with the inhibition of insulin release in healthy cats [25]. In the present study, during anesthesia, pre-operative glucose concentrations were higher in the Me + Af and MM + Af groups than in the Af groups, demonstrating that Me premedication facilitates hyperglycemia during isoflurane anesthesia in cats. This enhancement of hyperglycemia by Me may be mainly due to the Me-

induced inhibition of insulin release via α_2-adrenoceptors on pancreatic ß-cells [16]. Hyperglycemia may limit the use of Me and MM in combination with Af in cats with metabolic and neurohormonal problems, such as diabetes mellitus, ketosis, and glycosuria.

Changes in NEFA concentrations are important metabolic indicators of the stress response since NEFA is affected by hormones like cortisol and catecholamines [11]. Lipolytic activity is stimulated by cortisol and catecholamines [11]. In the present study, NEFA concentrations in the Af IV and Af IM groups tended to decrease pre- and/or post-operatively and increase during the recovery phases in both castrated and ovariohysterectomized cats. These results may be attributed to the complicated effects of decreased catecholamine and increased cortisol during anesthesia and post-surgery. In un-anesthetized cats, Me is reported to reduce plasma NEFA concentration; however, Mi does not reduce plasma NEFA [25,26]. In the present study, pretreatments with Me or MM in combination with Af, especially compared with Af IV, greatly reduced pre- and/or postoperative NEFA concentrations during anesthesia. Lipolytic activity is suppressed by Me via α_2-adrenoceptors on adipose tissues, which also decreases cortisol and catecholamine [11,16]. Although NEFA concentration fluctuations may not be directly harmful to anesthetized cats, a decrease in plasma NEFA concentration induced by pretreatments of Me or MM combined with Af may be a clinically significant metabolic index of inhibition of sympathetic-adrenal activation in isoflurane-anesthetized cats undergoing surgery.

In conclusions, the present study revealed that, in both castrated and ovariohysterectomized cats, the addition of Me or MM to Af improved the quality of recovery from isoflurane anesthesia and surgery. Both plasma adrenaline and noradrenaline concentrations decreased during isoflurane anesthesia in cats premediated with Af with and without Me, except for MM + Af IM. Treatment with MM + Af prevented an excessive decrease in catecholamine concentrations during anesthesia and surgery. Combinations of Me or MM with Af suppressed an excessive postoperative increase in plasma cortisol in ovariohysterectomized cats. The addition of Me or MM to Af facilitated

hyperglycemia during isoflurane anesthesia in cats. Pretreatments with Me or MM in combination with Af greatly reduced pre- and/or postoperative NEFA concentrations. We conclude that pretreatments with Me or MM in combination with Af are useful for the prevention of stress-related hormonal and metabolic responses, other than hyperglycemia, during isoflurane anesthesia and surgery in feline veterinary practice.

Chapter 3

Effects of atipamezole and flumazenil on stress-related hormonal and metabolic responses in cats anesthetized with medetomidine, midazolam, ketamine, and isoflurane undergoing ovariohysterectomy or castration

Introduction

In feline veterinary medicine, the alpha(α)$_2$-adrenoceptor agonist medetomidine is an effective analgesic and muscle relaxant. However, it induces undesirable effects, such as bradyarrhythmia, hyperglycemia, and emesis in cats [10,26,36]. A combination of medetomidine with midazolam and ketamine (MMK) produces good anesthesia in cats, with fewer adverse effects or better analgesia potentiation [9,39,43,48]. Antagonism may be required when anesthetized animals show a profound depression of vital signs, adverse effects, and/or delayed recovery from anesthesia. Atipamezole (Ati) and flumazenil (Flu) either completely or partially antagonize the effects of MMK in cats [13,20,23,42,47,48,50]. After surgery and when combined with MMK, these antagonists can accelerate awakening from anesthesia and are useful in anesthesia-associated emergency and critical care. In cats, Ati, both alone and in combination with Flu administered intravenously (IV), effectively antagonizes the anesthetic and neurohormonal effects induced by MMK [47,48]. Compared to Ati alone, Ati in combination with Flu leads to a similar recovery time from MMK anesthesia but improves the quality of recovery by reducing excitation and hyperaesthesia [17]. The antagonistic effects of Ati and Ati–Flu also differ depending on the route and timing of administration in MMK-anesthetized normal and castrated cats [9,13].

Stressors such as anxiety, excitement, pain, anesthesia, and surgery induce neurohormonal and metabolic changes in animals; these changes are characterized by elevated blood cortisol levels, catecholamines, glucose, and non-esterified fatty acids (NEFA) [11]. Actions mediated by α_2-adrenoceptors are closely coordinated with these events [3,26]. MMK anesthesia suppresses the release of catecholamine and cortisol, suppresses lipolysis, and induces hyperglycemia in cats [47]. It is important to evaluate cats' stress-related hormonal and metabolic responses due to the post-operative administration of potential antagonists in clinical practice. In terms of the overall effect of these antagonists on general anesthesia and post-surgical recovery, the use of potential antagonists may be advantageous to recovery from anesthesia if the sympathoadrenal system is

adequately but not excessively activated. However, to the best of our knowledge, there are no published reports on the effects of Ati and Flu, both alone and in combination, on the stress responses of anesthetized cats undergoing surgery. Therefore, this study aimed to evaluate the effects of the intramuscular (IM) and IV administrations of Ati and Flu, both alone and in combination and at different administration times, on key stress-related neurohormonal and metabolic changes in MMK–isoflurane-anesthetized cats undergoing ovariohysterectomy and castration.

Materials and methods

Animals

One-hundred and eight client-owned mixed-breed cats (54 males, 54 females) were prospectively recruited at the Kamohara Animal Hospital for ovariohysterectomy or castration. They were clinically healthy and ranged in age from 6 months to 1 year. On average, males weighed 4.1 ± 0.7 kg (mean ± standard deviation [SD]), and females weighed 3.0 ± 0.4 kg. Each cat owner provided informed consent for data collection. Physical and routine hematological examinations before the study revealed that all values were within normal physiological ranges. All cats fasted for 12 h but had *ad libitum* access to water. Owners brought their cats to our hospital early in the morning on the day of surgery. After preparation for surgery and anesthesia, each cat rested in a darkened cage for 2–3 h before anesthesia. After complete recovery from anesthesia, all cats received water *ad libitum* and food.

Study protocol

The cats were randomly assigned to one of nine treatment groups (six cats/group) for both ovariohysterectomy and castration. Each cat was intramuscularly (IM) administered a mixture of 50-μg/kg medetomidine hydrochloride (Dorbene, Syva Laboratorios, S.A., Spain) and 0.5-mg/kg midazolam (Sandoz, Tokyo, Japan), followed by an IM administration of 5-mg/kg ketamine (Fujita Pharmaceutical, Tokyo, Japan) 10 min later. Medetomidine and midazolam were mixed in the same syringe immediately before injection. A further 10 min after the ketamine injection, anesthesia was induced with 4% isoflurane in oxygen at a total gas flow rate of 1.5 L/min using a face mask attached to the ADS 1000 veterinary anesthesia delivery system (Engler, Hialeah, FL). Then, a cuffed endotracheal tube was inserted. Cats were placed supine and maintained at a surgical depth of isoflurane anesthesia through a non-rebreathing system under controlled ventilation. During isoflurane anesthesia, the cats underwent castration or ovariohysterectomy

using standard methods. Pre-operatively, an analgesic (0.3-mg/kg meloxicam; Inflacam, Chanelle Pharmaceuticals Manufacturing Ltd, Ireland) was injected subcutaneously in all cats, followed by once daily for several days after surgery if necessary. Lactated Ringer's solution was infused IV at 10 mL/kg/h during anesthesia and surgery. The duration of isoflurane anesthesia was 30 and 60 min in castrated and ovariohysterectomized cats, respectively.

When the inhalation of isoflurane was halted, an antagonist was administered as follows, depending on the treatment group: 0.1-mL/kg physiological saline solution IM (control group), 50-μg/kg Flu (Anexate; Astellas Pharmaceutical, Tokyo, Japan) IM, 100-μg/kg Ati (atipamezole hydrochloride; Orion corporation, Pharmaceutical, Espoo, Finland) IM, 200-μg/kg Ati IM, 100-μg/kg Ati + 50-μg/kg Flu IM, 200-μg/kg Ati + 50-μg/kg Flu IM, 50-μg/kg Flu IV, 100-μg/kg Ati IV, or 100-μg/kg Ati + 50-μg/kg Flu IV; groups will be referred to as control, Flu IM, Ati100 IM, Ati200 IM, Ati100 + Flu IM, Ati200 + Flu IM, Flu IV, Ati100 IV, and Ati100 + Flu IV, respectively. Antagonists were mixed in the same syringe immediately before injection and injected into the cephalic vein. The endotracheal tube was extubated once a laryngeal reflex was visible. During the recovery process, the cats remained in separate cages in a room with an air temperature of 25 °C. General postoperative management and care were performed in all cats. More than 120 min after the administration of the potential antagonists, another analgesic, butorphanol (0.1–0.4 mg/kg; Vetorphale, Meiji Seika, Tokyo, Japan) was injected IM, to cats with signs of pain such as vocalization, anorexia and posture. There were no issues with surgery or anesthesia in any of the cats.

Anesthesia and intraoperative monitoring

An agent-specific precision vaporizer was used to administer isoflurane. Gas samples were drawn from the breathing circuit through a tube attached to an adapter positioned at the oral end of an endotracheal tube. During anesthesia, the expired end-tidal isoflurane (EtIso) and carbon

dioxide ($EtCO_2$) concentrations, arterial oxygen saturation of pulse oximetry (SpO_2), heart rate (HR), respiration rate (RR), rectal temperature (RT), and mean blood pressure (MBP) using the oscillometric method, were assessed either continuously or intermittently, with a multi-parameter monitor (BSM-5192; Nihon Kohden, Tokyo). During controlled ventilation, RR was adjusted to a range of 25–40-mmHg $EtCO_2$, and ranged from 8 to 15 breaths/min in all groups. SpO_2 was >98% in all cases. During anesthesia, EtIso concentration ranged from 0.40% ± 0.05% to 0.70% ± 0.23% (mean ± SD) in the castration groups and from 0.43% ± 0.05% to 0.70% ± 0.05% in the ovariohysterectomy groups. HR was between 97 ± 17 and 108 ± 9 beats/min in the castration groups and between 93 ± 25 and 116 ± 20 beats/min in the ovariohysterectomy groups. MBP was between 87 ± 13 and 121 ± 25 beats/min in the castration groups and between 90 ± 11 and 120 ± 14 mmHg in the ovariohysterectomy groups. RT was between 38.0 °C ± 0.7 °C pre-operatively and 36.9 °C ± 0.9 °C post-operatively in the castration groups and between 37.9 °C ± 0.6 °C pre-operatively and 35.9 °C ± 0.9 °C post-operatively in the ovariohysterectomy groups. There were no significant differences in EtIso, HR, MBP, RR, $EtCO_2$ and SpO_2 values across groups in both surgeries.

Behavioral recovery scoring

The overall quality of recovery from anesthesia was assessed using a previously published scoring method [47] as follows: score 1 = excellent; score 2 = good; score 3 = moderate; score 4 = poor; score 5 = extremely poor. The observer was blind to treatment. Time to extubation and head-up motion after administering these potential antagonists were also assessed in all groups.

Blood sample collection

Blood samples (2 mL) were collected from the jugular or cephalic vein on five occasions: before pretreatment (baseline), after completing the surgical procedure while under anesthesia

(post-operatively), and 10, 60, and 120 min after the administration of the potential antagonists (recovery phases after the discontinuation of anesthesia). Post-operative blood samples were collected 25 min after isoflurane anesthesia in the castration groups and 55 min after isoflurane anesthesia in the ovariohysterectomy groups.

Sample processing and analysis

Blood was mixed with ethylenediaminetetraacetic acid to prevent clotting. Samples were immediately centrifuged to separate the plasma, which was frozen at −76 °C until analysis. We assessed glucose, NEFA, cortisol, adrenaline, and noradrenaline levels according to previously published methods [25,47]. In brief, glucose and NEFA levels were determined by an enzyme assay and a spectrophotometer. A solid phase-antibody radioimmunoassay measured cortisol levels. Catecholamines were extracted on activated alumina and measured using high-performance liquid chromatography and an electrochemical detector.

Statistical analysis

All data were analyzed using Prism 7.0 (GraphPad, CA). All data are presented as mean ± SD. All data (other than score data) were tested for normality using the Shapiro-Wilk test. A repeated-measures one-way analysis of variance (ANOVA) was used to examine differences across variables within each group. *Post hoc* Dunnett's multiple comparisons tests were used to identify differences from baseline within each group. One-way ANOVA and *post hoc* Tukey's multiple comparisons tests were used to determine differences across groups. In all tests, the significance level was set at $P < 0.05$. Score data were analyzed using the Wilcoxon–Mann–Whitney test for treatment comparisons; $P < 0.00556$ was considered significant using a Bonferroni correction.

Results

Adrenaline

In all groups of castrated cats, the concentration of adrenaline either decreased or tended to decrease post-operatively compared with baseline levels (Figure 14). In the control group, adrenaline was lower 10 min post-saline injection than at baseline and after that increased gradually at 120 min compared to post-operation. In the groups that received Ati and/or Flu, adrenaline concentrations 10 min post-antagonist injection did not differ from baseline values. In combined groups with Ati100 or Ati200, adrenaline was higher at 60 min compared to post-operation. There were no differences in adrenaline between the groups at any time.

In all groups of ovariohysterectomized cats, adrenaline either decreased or tended to decrease post-operatively compared with baseline levels (Figure 14). In the control group, adrenaline was lower 10 min post-saline injection than at baseline but higher at 60 min than post-operation. In groups that received Ati and/or Flu, adrenaline concentrations 10 min after antagonist administration were not different from baseline values. In the Ati100 IV group, adrenaline was higher 10 min after antagonist administration than post-operation, and higher than that of the control group. There were no other differences between the groups at any time.

Noradrenaline

In all groups of castrated cats, noradrenaline decreased or tended to decrease post-operatively compared with baseline levels (Figure 15). In the control group, noradrenaline was lower 10 min after saline injection than at baseline, whereas in groups injected with potential antagonists, noradrenaline did not differ from baseline. There were no differences in noradrenaline between the groups at any time.

In all groups of ovariohysterectomized cats, noradrenaline decreased or tended to decrease post-operatively compared with baseline (Figure 15). In the control group, noradrenaline was

lower 10 min after saline injection than at baseline. In the Flu IV group, noradrenaline was lower 10 min after injection than at baseline and higher at 120 min than post-operation. In the Ati100 IM and Ati200 IM groups, noradrenaline was higher 60 min after Ati injection than post-operation; similar changes were observed in the Ati100 + Flu IV groups. In the Ati100 IV group, noradrenaline was higher 10 min after injection compared to post-operation, and higher than in both the control and Flu IV groups. There were no other differences in noradrenaline between the groups at any time.

Cortisol

In all groups of castrated cats, cortisol decreased or tended to decrease at post-operation and 10 min after injection compared with baseline levels (Figure 16). Cortisol was higher 60 and/or 120 min after injection than post-operation in control, Flu IM, Ati200 IM, Ati200 + Flu IM, and Ati100 + Flu IV groups; however, there were no differences in cortisol between the groups at any time.

In all groups of ovariohysterectomized cats, cortisol decreased or tended to decrease post-operation compared to baseline (Figure 16). Cortisol was lower or tended to be lower 10 min after injection compared to baseline levels in all groups, except for the Ati100 IV group, in which cortisol was higher at this time point compared to post-operation. In all groups, cortisol was higher or tended to be higher 60 and/or 120 min after injection compared with the baseline and/or post-operation in all groups. At 10 min after injection, cortisol was higher the Ati100 IV group than all other groups, including the control. There were no other differences in cortisol concentrations between the groups at any time.

Glucose

In all groups of castrated cats, glucose concentration increased post-operation and 10 min after injection compared with baseline levels (Figure 17). In the control group, glucose returned to baseline levels 120 min after saline injection, whereas in all other groups, it was lower 60 min after injection than post-operation. There were no differences in glucose concentrations between the groups at any time.

In ovariohysterectomized cats, glucose was higher post-operation and 10 min after injection than the baseline in all groups other than the Ati100 IV and Ati100 + Flu IV groups. It did not differ 10 min after injection (Figure 17). Elevated glucose concentrations returned to nearly baseline values 60 and 120 min after injection in all groups. There were no differences in glucose concentrations between the groups at any time.

NEFA

In all castrated and ovariohysterectomized cats, NEFA concentration decreased or tended to decrease post-operation compared with baseline levels (Figure 18). NEFA concentration was lower 10 min after discontinuing anesthesia, although sometimes insignificantly, in all groups other than the Ati100 IV group, in which it returned to baseline levels after 10 min. In all groups, NEFA was greater 60 and/or 120 min after injection compared to post-operation.

Recovery time and behavioral recovery scores

In both castrated and ovariohysterectomized cats, recovery times to extubation and head-up were rapid in the groups listed in decreasing order as follows: Ati100 + Flu IV, Ati100 IV, Ati200 + Flu IM, Ati100 + Flu IM, Ati200 IM, Ati100 IM, Flu IV, Flu IM, and the control groups (Table 5). However, there were no differences in the recovery times of the Ati100 IM and Ati200 IM groups, the Ati100 + Flu IM and Ati200 + Flu IM groups, and the Ati100 IV and Ati100 + Flu IV groups.

Behavioral scores were lower in the Ati100 IV and Ati100 + Flu IV groups than in the Ati100 IM and Ati200 IM groups in castrated cats (Table 6). A similar pattern was observed in ovariohysterectomized cats, but these differences were not statistically significant.

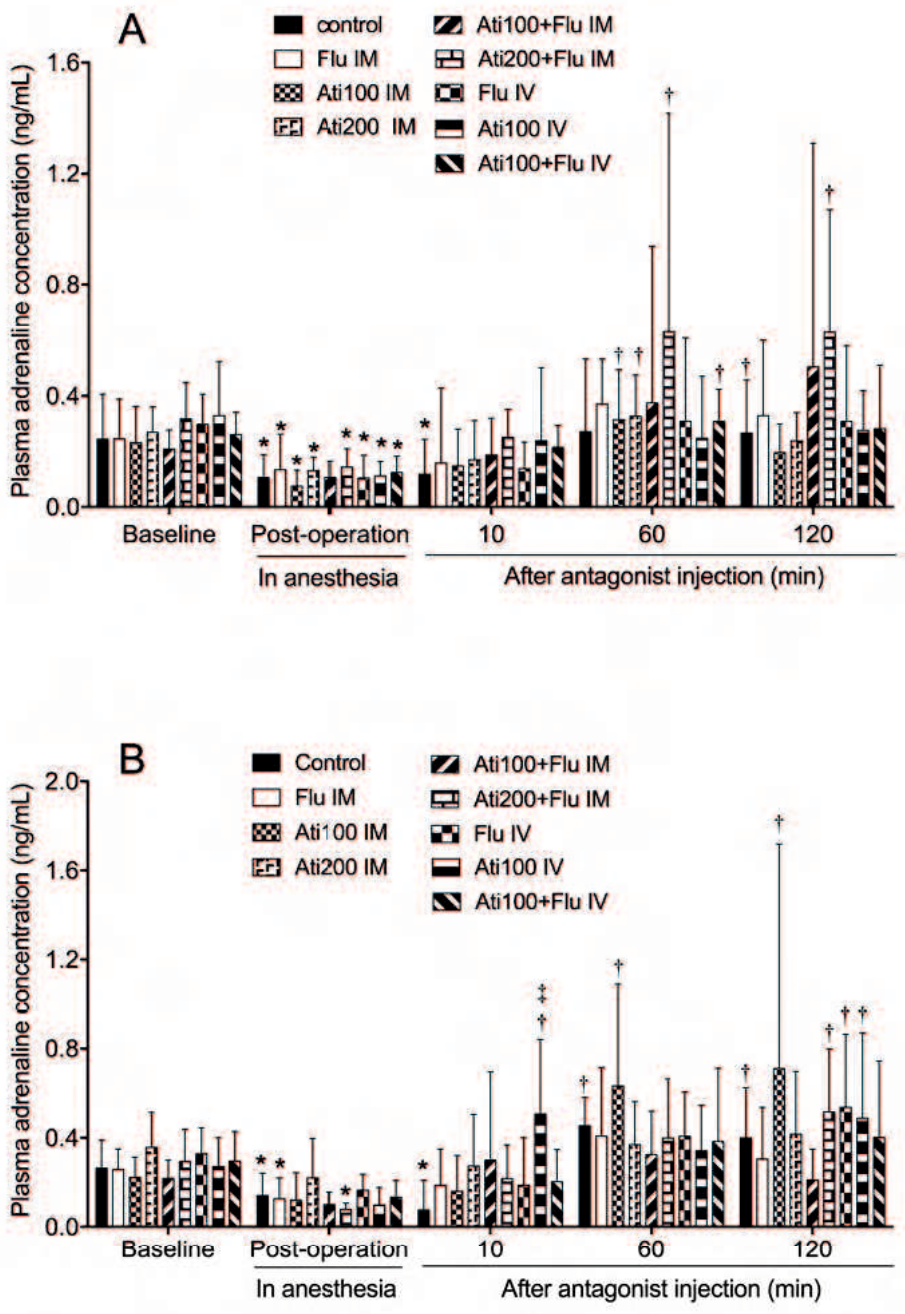


Figure 14. Effects of atipamezole (Ati) and flumazenil (Flu), both alone and in combination, on plasma adrenaline concentrations (ng/mL) in isoflurane-anesthetized cats premedicated with medetomidine (50 µg/kg), midazolam (0.5 mg/kg), and ketamine (5 mg/kg) undergoing castration (A) or ovariohysterectomy (B). Each vertical bar indicates the mean and SD of six cats. IM = intramuscularly; IV = intravenously; * significantly different from baseline; † significantly different from post-operation; ‡ significantly different from control; the significance level is $P < 0.05$.

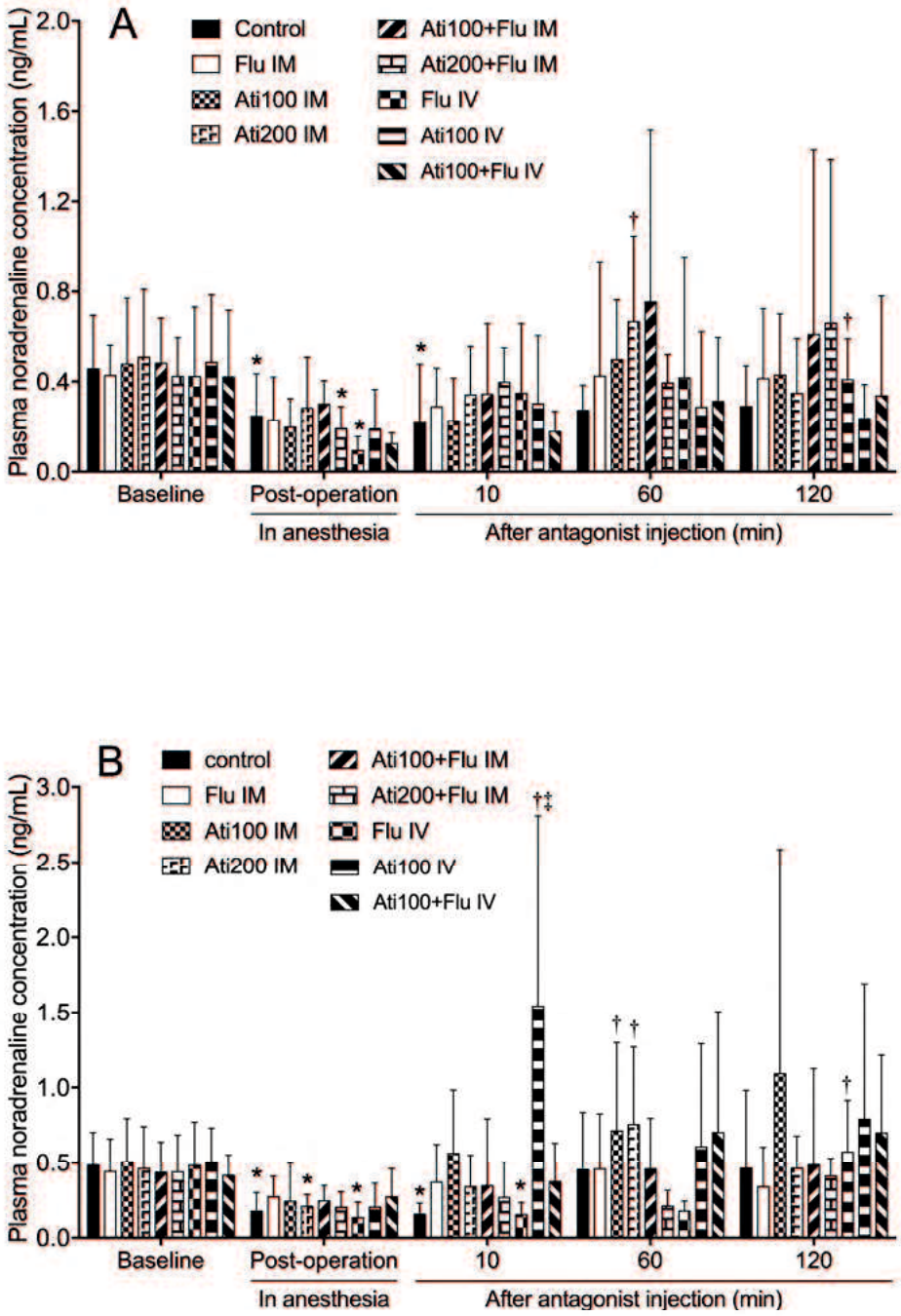


Figure 15. Effects of atipamezole (Ati) and flumazenil (Flu), both alone and in combination, on plasma noradrenaline concentrations (ng/mL) in isoflurane-anesthetized cats premedicated with medetomidine (50 μg/kg), midazolam (0.5 mg/kg), and ketamine (5 mg/kg) undergoing castration (A) or ovariohysterectomy (B). Each vertical bar indicates the mean and SD of six cats. IM = intramuscularly; IV = intravenously; * significantly different from baseline; † significantly different from post-operation; ‡ significantly different from control or Flu IV; the significance level is $P < 0.05$.

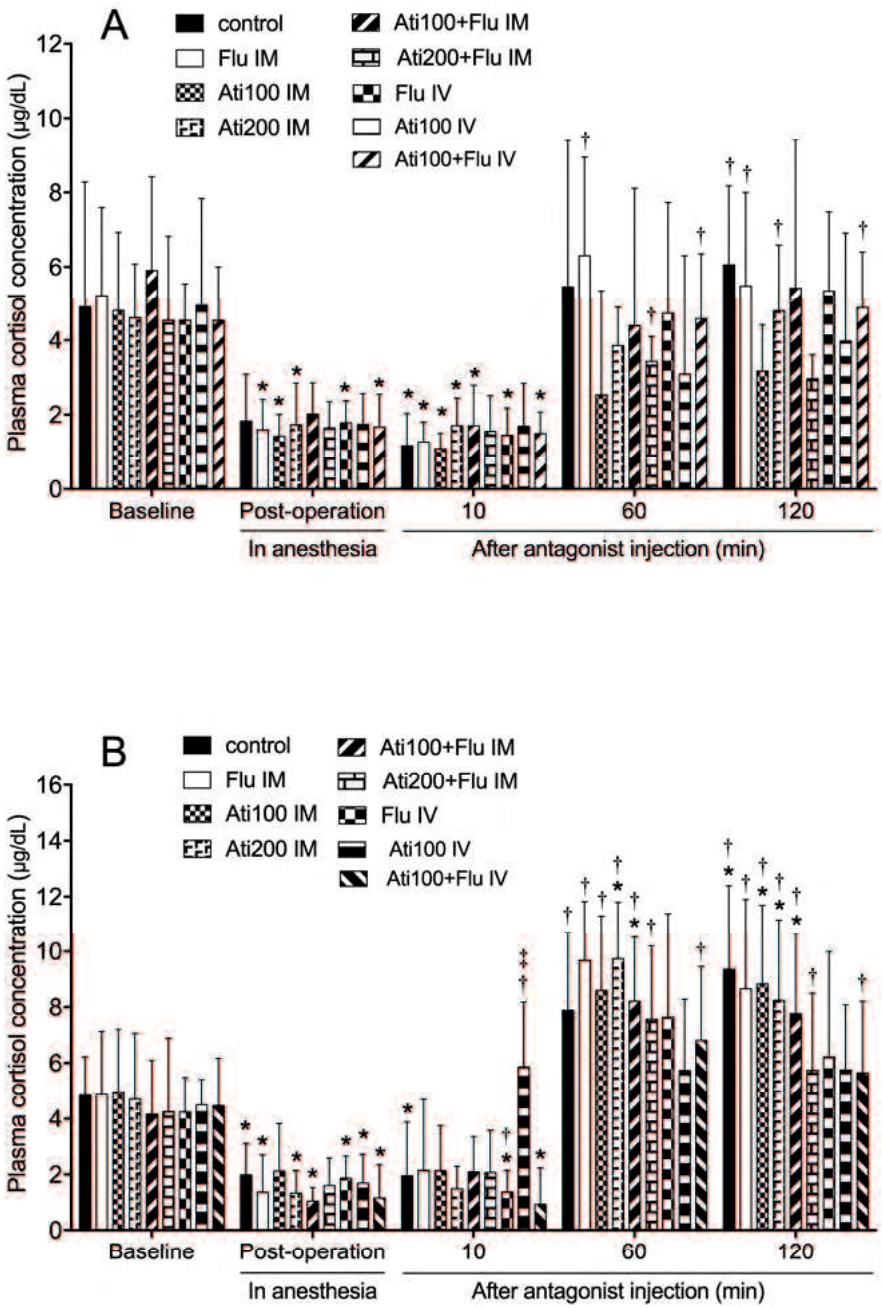
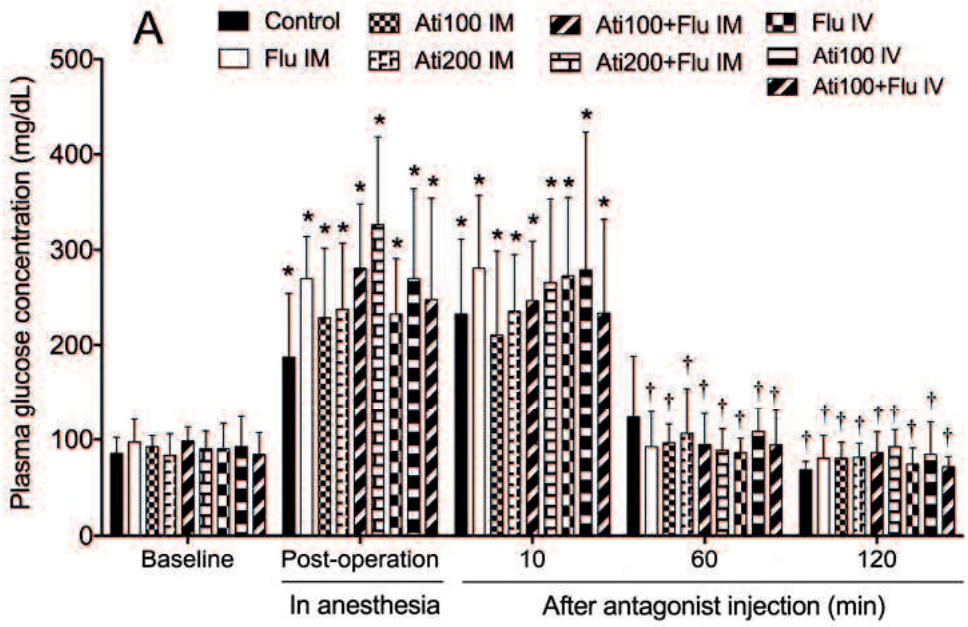


Figure 16. Effects of atipamezole (Ati) and flumazenil (Flu), both alone and in combination, on plasma cortisol concentrations (μg/dL) in isoflurane-anesthetized cats premedicated with medetomidine (50 μg/kg), midazolam (0.5 mg/kg), and ketamine (5 mg/kg) undergoing castration (A) or ovariohysterectomy (B). Each vertical bar indicates the mean and SD of six cats. IM = intramuscularly; IV = intravenously; * significantly different from baseline; † significantly different from post-operation; ‡ significantly different from control, Flu IM, Ati100 IM, Ati200 IM, Ati100+Flu IM, Ati200+Flu IM, Flu IV or Ati100+Flu IV; the significance level is $P < 0.05$.



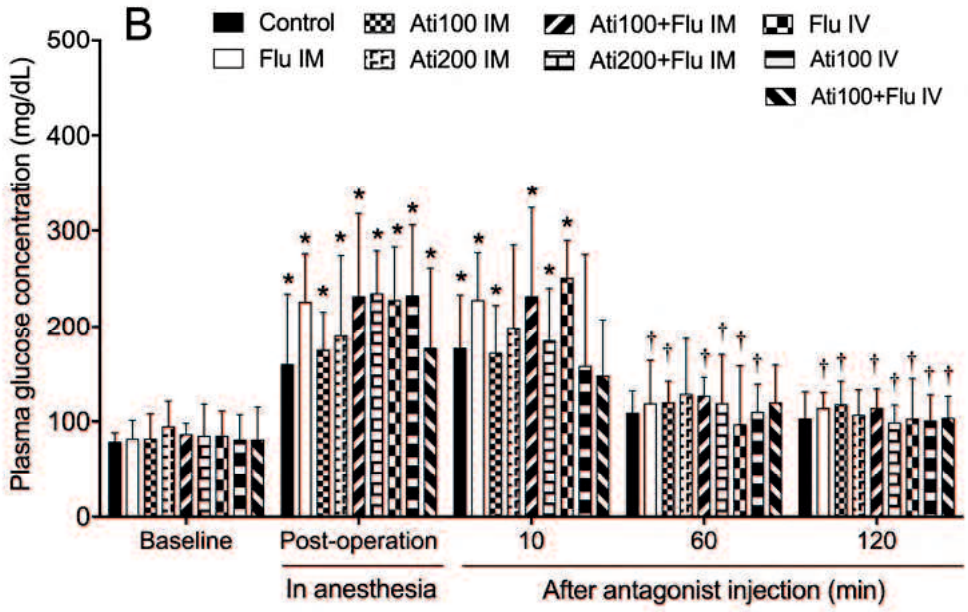


Figure 17. Effects of atipamezole (Ati) and flumazenil (Flu), both alone and in combination, on plasma glucose concentrations (mg/dL) in isoflurane-anesthetized cats premedicated with medetomidine (50 µg/kg), midazolam (0.5 mg/kg), and ketamine (5 mg/kg) undergoing castration (A) or ovariohysterectomy (B). Each vertical bar indicates the mean and SD of six cats. IM = intramuscularly; IV = intravenously; * significantly different from baseline; † significantly different from post-operation; the significance level is $P < 0.05$.

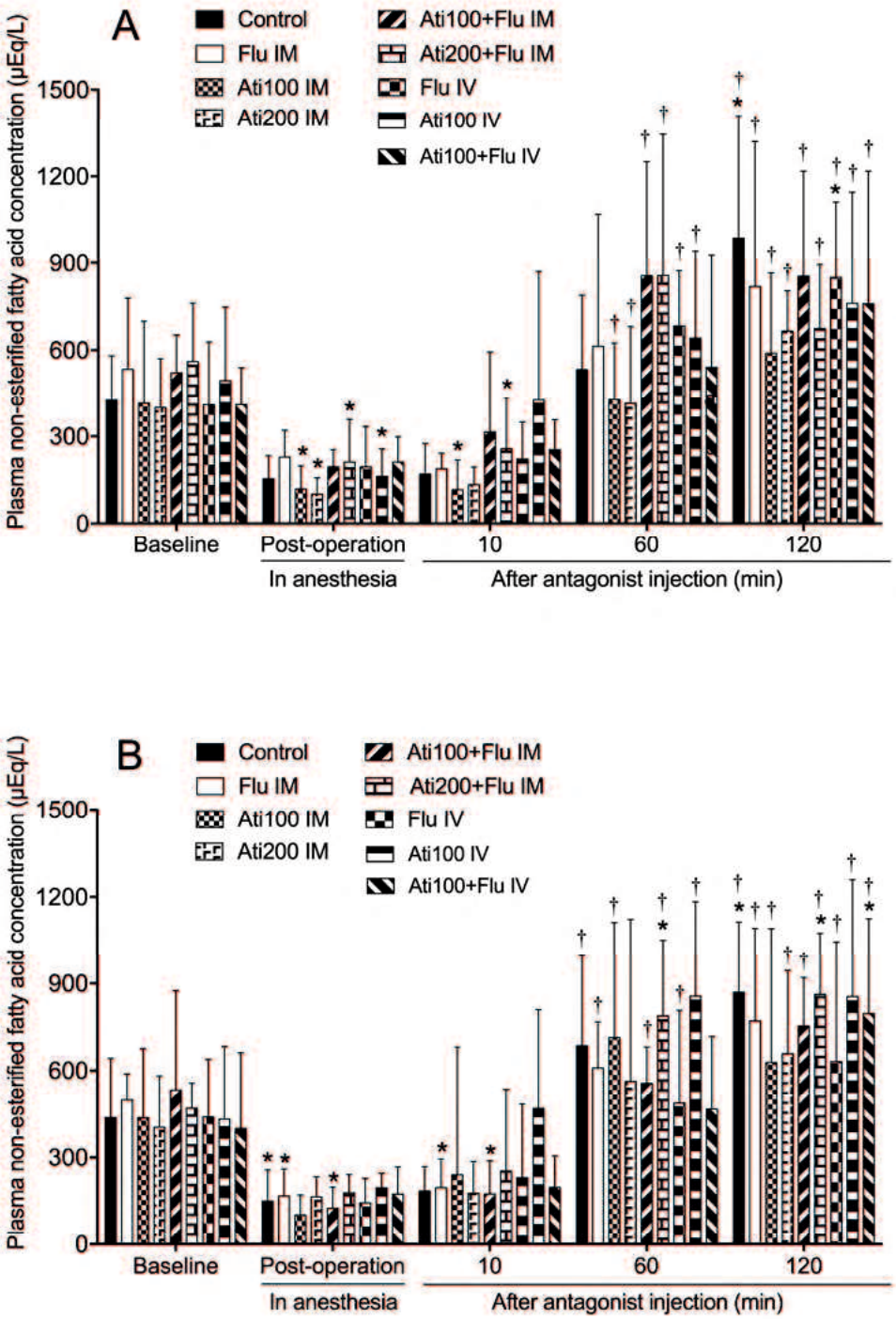


Figure 18. Effects of atipamezole (Ati) and flumazenil (Flu), both alone and in combination, on plasma non-esterified fatty acid concentrations (μEq/L) in isoflurane-anesthetized cats premedicated with medetomidine (50 μg/kg), midazolam (0.5 mg/kg), and ketamine (5 mg/kg) undergoing castration (A) or ovariohysterectomy (B). Each vertical bar indicates the mean and SD of six cats. IM = intramuscularly; IV = intravenously; * significantly different from baseline; † significantly different from post-operation; the significance level is $P < 0.05$.

Table 5. Recovery time after the administration of atipamezole (Ati) and flumazenil (Flu), both alone and in combination, in isoflurane-anesthetized cats premedicated with medetomidine (50 μg/kg), midazolam (0.5 mg/kg), and ketamine (5 mg/kg) undergoing ovariohysterectomy or castration.

Group	Time to extubation (min)		Time to head-up (min)	
	Castration	Ovariohysterectomy	Castration	Ovariohysterectomy
Control	26.0 ± 4.7	31.0 ± 7.3	69.7 ± 8.7	65.2 ± 9.6
Flu IM[a]	14.7 ± 1.9*	17.0 ± 2.4*	51.9 ± 6.9*	49.8 ± 9.5*
Ati100 IM	8.7 ± 0.5*†	8.7 ± 0.8*†	18.2 ± 1.5*†	19.7 ± 1.0*†
Ati200 IM	7.3 ± 1.0*†	9.0 ± 1.4*†	15.5 ± 1.6*†	18.8 ± 0.8*†
Ati100 + Flu IM	5.7 ± 1.0*†	6.2 ± 1.2*†	17.3 ± 2.3*†	16.5 ± 3.3*†
Ati200 + Flu IM	4.7 ± 0.8*†§	5.5 ± 1.1*†	14.5 ± 2.9*†	14.5 ± 2.8*†
Flu IV[b]	8.3 ± 2.5*†	9.7 ± 1.8*†	34.7 ± 4.0*†§‡‖¶	38.8 ± 3.7*†§‡‖‡
Ati100 IV	4.0 ± 0.9*†§#	3.8 ± 1.5*†#	8.8 ± 2.5*†§‖#	8.0 ± 2.0*†§‡#
Ati100 + Flu IV	2.8 ± 0.8*†§‡#	2.8 ± 0.4*†§‡#	7.2 ± 1.7*†§‡‖#	7.2 ± 2.1*†§‡#

Values represent mean ± SD of six cats; [a] IM — intramuscularly; [b] IV — intravenously; * significantly different from control; † significantly different from Flu IM; § significantly different from Ati100 IM; ‡ significantly different from Ati200 IM; ‖ significantly different from Ati100 + Flu IM; ¶ significantly different from Ati200 + Flu IM; # significantly different from Flu IV; the significance level is $P < 0.05$.

Table 6. Behavioral recovery scores after the administration of atipamezole (Ati) and flumazenil (Flu), both alone and in combination, in isoflurane-anesthetized cats premedicated with medetomidine (50 μg/kg), midazolam (0.5 mg/kg), and ketamine (5 mg/kg) undergoing ovariohysterectomy or castration.

Group	Recovery score	
	Castration	Ovariohysterectomy
Control	2 (2–3)	2 (2–3)
Flu IM[a]	3 (1–4)	2 (2–3)
Ati100 IM	3 (3–4)	3 (3–4)
Ati200 IM	4 (3–4)	4 (3–4)
Ati100 + Flu IM	2 (1–3)	3 (2–3)
Ati200 + Flu IM	3 (2–3)	3 (2–3)
Flu IV[b]	3 (2–3)	2 (2–3)
Ati100 IV	2 (1–2) *†	2 (1–3)
Ati100 + Flu IV	2 (1–2) *†	2 (2–3)

Values represent the median (range) of six cats; [a] IM — intramuscularly; [b] IV — intravenously; * significantly different from Ati100 IM; † significantly different from Ati200 IM; the significance level is $P < 0.00556$ by Mann-Whitney U test and Bonferroni correction.

Discussion

The present study demonstrated that MMK-isoflurane anesthesia reduced plasma concentrations of adrenaline, noradrenaline, cortisol, and NEFA. It also increased glucose concentrations in both castrated and ovariohysterectomized cats. This study's neurohormonal and metabolic changes are similar to those noted in previous studies of MMK-anesthetized cats that did not undergo surgery [47]; the only difference noted here is that cortisol concentration decreased with MMK-isoflurane anesthesia. The present findings indicate that premedication with MMK can be useful for perioperatively suppressing sympathetic-adrenal activity, other than hyperglycemic effects, in isoflurane-anesthetized cats.

The rationale for using fixed dosing of Ati and Flu as antagonists for MMK has been outlined in our earlier study [47,48]. In the present study, the antagonists' intravenous and intramuscular routes of administration were selected based on the immediate onset of action that is most often used during emergency anesthetic situations and on slower action with accelerated awakening time, respectively. The present study revealed that in both castrated and ovariohysterectomized cats, recovery times from anesthesia were rapid in the following order; Ati 100 + Flu IV ≤ Ati 100 IV < Ati 200 + Flu IM ≤ Ati 100 + Flu IM < Ati 200 IM ≤ Ati 100 IM < Flu IV < Flu IM < control. These findings indicate that, compared to IM administration, the IV delivery of Ati alone and in combination with Flu induces more rapid recovery from anesthesia, regardless of the timing of antagonist administration (beyond 50 min after medetomidine administration in castrated cats and 80 min in ovariohysterectomized cats). On the other hand, the present study revealed that castrated cats had a better quality of recovery from anesthesia after the IV administration of Ati alone and combined with Flu than with the IM administration of Ati alone. However, even with IM administration, the addition of Flu to Ati compared with Ati alone improved the quality of recovery. These results agree with a previous finding that the use of a

combination of Flu and Ati, in comparison to Ati alone, improves the quality of recovery by reducing excitation and hyperaesthesia [13].

In ovariohysterectomized cats, the IV administration of Flu alone did not affect changes to noradrenaline concentrations in the non-medicated control, demonstrating Flu itself does not have a large influence on reversing the inhibition of catecholamine release caused by MMK-isoflurane anesthesia. This result is consistent with previous findings of the effect of intravenous Flu against MMK anesthesia in cats that are not undergoing surgery [47]. Conversely, the IV administration of Ati rapidly and greatly reversed the decrease in adrenaline and noradrenaline induced by MMK-isoflurane anesthesia in ovariohysterectomized cats. Still, this effect was not significant in castrated cats, suggesting that the effect of Ati on catecholamine release depends on the timing of antagonist administration and the degree of surgical injury. IM administrations of Ati alone and Ati–Flu also accelerated recovery from the decreased adrenaline and noradrenaline concentrations induced by MMK-isoflurane anesthesia. Noradrenaline levels in groups that received Ati alone were greater 10 and/or 60 min post-injection than those who received Ati–Flu combinations. The increase may be due to the effect of un-antagonized residual midazolam since IM administration of midazolam alone increases plasma adrenaline and noradrenaline concentrations in cats [26]. Therefore, IM administration of Ati effectively reverses the inhibition of catecholamine release induced by MMK-isoflurane anesthesia in ovariohysterectomized cats.

In the present study, MMK-isoflurane anesthesia decreased plasma cortisol concentrations, indicating that it inhibits adrenocortical activity in both castrated and ovariohysterectomized cats. This may be mainly due to medetomidine since premedication with medetomidine is reported to reduce or delay the increase in plasma cortisol concentrations induced by ovariohysterectomy in dogs [6,29]. In castrated cats, both the IM and IV administrations of Flu alone, Ati alone, and combinations of Ati–Flu did not affect the changes in cortisol release observed in control. On the other hand, in ovariohysterectomized cats, Ati IV reversed the decreased cortisol concentration

induced by MMK-isoflurane anesthesia 10 min post-injection, demonstrating that intravenous Ati rapidly increases adrenocortical activity after anesthesia and surgery; this effect was not observed in groups that received Ati injections IM. These results suggest that, similar to the effect on catecholamine release, the effect of Ati IV on the release of cortisol depends on the timing and route of administration as well as the degree of surgical injury. However, compared to Ati alone, Ati–Flu combinations IV did not produce a rapid increase in cortisol concentration in the present study. Although the precise reason for this pattern is unknown, the Ati–Flu combination may help prevent excessive adrenocortical activity after administration in cats anesthetized with MMK-isoflurane.

In the current study, moderate hyperglycemia was induced by MMK-isoflurane anesthesia in both castrated and ovariohysterectomized cats. This hyperglycemia may be responsible for several effects: medetomidine-induced inhibition of insulin release mediated by α_2-adrenoceptors [26], surgical injuries, decreased insulin and decreased peripheral insulin use of glucose associated with inhalant anesthesia [11]. In the present study, Ati IV, both alone and in combination with Flu, reduced post-operative hyperglycemia in ovariohysterectomized cats more quickly. This result suggested that the effect of Ati is due to the suppression of medetomidine-induced hyperglycemia through the blockade of α_2-adrenoceptors. Other than this result, the IM and IV administrations of Ati, both alone and combined with Flu, did not affect the changes in post-operative blood glucose levels observed in the control group castrated and ovariohysterectomized cats. This may be due to the timing of antagonist administration (50 min after medetomidine administration in castrated cats and at 80 min in ovariohysterectomized cats) as well as the post-operative surgical injuries.

Changes in NEFA concentrations are clinically significant metabolic indicators of the stress response since NEFA is affected by hormones like cortisol and catecholamines [11]. Lipolytic activity is stimulated by cortisol and catecholamines [11] and inhibited by medetomidine [25]. In the present study, NEFA concentrations were reduced by MMK-isoflurane anesthesia in both

castrated and ovariohysterectomized cats, which may be attributable to the decreased catecholamine and cortisol levels and the inhibited lipolysis by medetomidine. The present study showed that Ati IV hastened recovery from post-operatively decreased NEFA concentrations. This may be related to the antagonism for medetomidine-induced inhibition of lipolysis, and increased catecholamine and cortisol levels, as previously mentioned. Other than this, however, the IM administration of Ati alone and in combination with Flu did not affect the post-operative changes in NEFA concentration after MMK-isoflurane anesthesia in both castrated and ovariohysterectomized cats. This result indicated that the IM route of administration does not largely influence post-operative lipolysis.

In conclusion, MMK-isoflurane anesthesia induced the inhibition of catecholamine and cortisol release, the inhibition of lipolysis, and hyperglycemia in castrated and ovariohysterectomized cats. Compared with intramuscular administration, the intravenous administration of Ati alone and in combination with Flu induces more rapid recovery from anesthesia, regardless of the timing of administration. Compared to Ati alone, the addition of Flu improved the quality of recovery. The IV administration of Ati alone effectively reverses the post-operative neurohormonal and metabolic effects induced by MMK-isoflurane anesthesia in cats undergoing ovariohysterectomy. The IM administration of Ati–Flu combinations can induce rapid recovery from anesthesia without largely altering the stress-related neurohormonal and metabolic changes induced by MMK-isoflurane anesthesia and surgery. This study is the first to demonstrate that differences in the route and timing of the administration of Ati and Flu as antagonists for MMK-isoflurane anesthesia alter post-operative stress-related hormonal and metabolic responses.

Chapter 4

Reversal effects of atipamezole, flumazenil, and 4-aminopyridine on bradycardia and increases in blood pressures induced by medetomidine, midazolam, and ketamine in isoflurane-anesthetized cats

Introduction

A combination of the α_2-adrenoceptor agonist, medetomidine (Me), the benzodiazepine agonist, midazolam (Mi), and the dissociative anesthetic agent, ketamine (Ke) produces good anesthesia in cats, with excellent muscle relaxation and analgaesia [9,39,43,48]. Atipamezole (Ati), flumazenil (Flu), and 4-aminopyridine (Ap) completely or partially antagonize the effects of Me, Mi, and Ke, respectively in cats [9,20,21,23]. These antagonists are useful when using Me + Mi + Ke (MMK) anesthesia in the clinic and are also useful during anesthetic emergencies or critical cases in which reversal is warranted.

Our previous studies evaluated the antagonistic effects of Ati, Flu, and Ap given intravenously, both alone and in combination on anesthesia and hormonal and metabolic changes produced by a fixed dose of MMK in cats [47,48]. In those studies, Ati, both alone and in combination, was found to be effective for antagonizing the anesthetic, neurohormonal, and metabolic effects induced by MMK, without prompting excessive stress responses in cats. The use of Ap and Flu was not recommended in the antagonism of the hormonal and metabolic effects induced by MMK, even if they were effective in accelerating recovery signs from anesthesia [47,48]. However, the use of Flu combined with Ati in comparison with Ati alone was reported to reduce excitation and hyperesthesia during the recovery process of antagonizing MMK anesthesia in cats [13]. Although the triple combination (Ati, Flu, and Ap) was the most effective in terms of accelerating recovery from anesthesia induced by MMK, it was unsuitable for smooth antagonism, because of adverse reactions such as tachycardia, tachypnoea, excitement, muscle tremors, and excessive stress-related hormonal responses [47,48]. However, when evaluating the clinical usefulness of the triple antagonist regimen it would appear insufficient to conclude only on aspects of behavioral and hormonal effects. In previous studies, it has been reported that AT reversed the increase in blood pressure induced by dexmedetomidine but induced short-acting hypotensive effects, and did not noticeably improve dexmedetomidine-induced bradycardia in isoflurane-

anesthetized cats [33,54]. Therefore, it is also important to examine the effect of the reversals of arterial blood pressure to ensure the appropriate use of these antagonists during an anesthetic emergency when using MMK. The purpose of this study was to investigate the effects of Ati, Flu, and Ap, both alone and in combination, on arterial blood pressure and heart rate in healthy cats administered MMK under isoflurane and oxygen anesthesia.

Materials and methods

Animals

Twelve healthy adult mixed-breed cats (5 males and 7 females), weighing 3.4 ± 0.5 kg (mean ± SD) and aged 3.8 ± 1.2 years, were used. The cats were housed in the laboratory for at least one month before study initiation and were fed a standard diet of commercial dry food. All cats were fasted for 12 hours and were not medicated before anesthesia. Anesthesia was carried out in a room with a temperature controlled at 24–25 °C. After anesthesia, the cats were allowed to wake up. Routine hematological examination carried out before the study found all test values to be within the normal physiological ranges. The experimental protocol was approved by the Animal Research Committee of Tottori University, Tottori, Japan.

Experimental protocol

The 12 cats were assigned to four of eight treatment groups in a randomized design, with at least two weeks between treatments for each cat. The number of cats in each treatment group was six. Anesthesia was induced with 4% isoflurane (Forane, Dinabott, Osaka, Japan) in oxygen at a total gas flow rate of 1.5 L/min using a face mask attached to an anesthetic circle system (Beaver 20, Kimura MED., Tokyo, Japan). After induction of anesthesia, a cuffed endotracheal tube (inside diameter, 3.0–4.0 mm; outside diameter, 4.2–5.6 mm; and length, 16–18 cm) was inserted, and the cuff was inflated. The cats were placed in lateral recumbency and kept at end-tidal concentration of 2% isoflurane (equivalent to 1.25 minimal alveolar concentration) under controlled ventilation. A polyethylene 23-gage catheter was inserted into either the right or left femoral artery for arterial pressure monitoring and blood gas sampling. Electrocardiogram electrodes were placed at the extremities. These preparations lasted approximately 20 minutes after induction of anesthesia. Subsequently, arterial blood sampling was performed immediately after catheterization, and arterial carbon dioxide partial pressure ($PaCO_2$) and oxygen partial

pressure were measured with a blood gas analyser (Blood Gas System 278, Ciba Corning Diagnostic Corp., Tokyo, Japan) at 37 °C. During the controlled ventilation, the $PaCO_2$ was maintained between 30 and 35 mmHg by adjustment of respiratory frequency (10 to 15/min) at a constant tidal volume (15 mL/kg) using a ventilator (Model B2, Igarashi Ika Kogyo, Tokyo, Japan). If the $PaCO_2$ was not within the required limits, the ventilation was changed, and, after 5 minutes, the measurement of $PaCO_2$ was repeated. Arterial oxygen partial pressure ranged from 568 to 621 mmHg during the controlled ventilation in all cats. After ventilation-assisted isoflurane anesthesia had been maintained for 30 minutes, circulation variables were measured, and the values obtained were recorded as the baseline values. Subsequently, the cats were administered intramuscularly a mixture of 0.05 mg/kg of Me (medetomidine hydrochloride, 1 mg/mL, Domitor, Meiji Seika Kaisha, Tokyo, Japan) and 0.5 mg/kg of Mi (midazolam hydrochloride, 5 mg/mL, Dormicum, Astellas Pharma, Tokyo, Japan), followed by 10 mg/kg of Ke (ketamine hydrochloride, 50 mg/mL, Ketalar, Daiichisannkyo Kaisha, Tokyo, Japan) intramuscularly 10 minutes later. Me and Mi were mixed in the same syringe immediately before injection. Twenty minutes after Ke injection, the cats were administered an intravenous dose of either physiological saline solution at 0.1 mL/kg (control), 0.2 mg/kg Ati (atipamezole hydrochloride, 5 mg/kg, Antisedan, Meiji Seika Kaisha, Tokyo, Japan), 0.1 mg/kg Flu (Flumazenil, 0.1 mg/mL, Anexate, Yamanouchi Pharmaceutical, Tokyo, Japan), 0.5 mg/kg Ap (4-aminopyridine, Wako Pure Chemical Industries, Tokyo, Japan), or one of all four possible combinations (Ati + Flu, Flu + Ap, Ati +Ap, or Ati + Flu + Ap). Ap was dissolved in saline solution at a concentration of 2.5 mg/mL. The potential antagonists were mixed as relevant in the same syringe immediately before injection, and injected directly into the jugular vein using a disposable syringe with the 23-gauge needle. The jugular vein was easy identified by dilation due to temporarily pressing the well-trimmed neck in preparation. The success of the needle insertion into the vein was verified by the blood inflow due to a light suction of the syringe immediately after the needle insertion. The

injection speed of drug solution was approximately 0.2 mL per second. No problems occurred during the injection.

An agent specific precision vaporizer (Mark III, Acoma, Tokyo, Japan) was used to administer isoflurane. Gas samples were drawn from the breathing circuit through a tube attached to an adapter positioned between the Y-piece connection and the oral end of the endotracheal tube. The end-tidal anesthetic concentration was measured continuously using an infrared gas analyser (Capnomac, Datex, Helsinki, Finland) during anesthesia. Airway gas was continuously sampled at a rate of 200 mL/min, and the gas was not returned to the circuit. The volume of dead space from the tip of the endotracheal tube to the Y-piece connection (including the adapter for the gas sampling tube) was 6–8 mL. Arterial blood samples were drawn anaerobically into heparinized syringes. The volume of blood drawn for blood gas analysis was 0.3 mL per draw. No fluids were administered to the cats during the anesthesia. The rectal temperature was maintained between 36.7 and 38.5 °C throughout the experiment using a circulating water mat.

Circulation measurements

Systolic, diastolic, and mean arterial blood pressures (SAP, DAP, and MAP, respectively) were measured continuously using a pressure transducer placed at the heart level and a strip chart recorder (Minipolygraph RM-6100, Nihon Koden, Tokyo, Japan). A lead-II electrocardiogram was recorded continuously for heart rate (HR) and heart rhythm monitoring during anesthesia.

Statistical analysis

All data were analyzed using Prism statistical software (Version 4, GraphPad Software, San Diego, California, USA). The paired *t*-test was used to compare time data within each group. For intergroup comparisons, data were subjected to one-way analysis of variance. When significant F values were encountered, the Tukey's multiple comparison test was used to determine significant

differences between the treatment groups. For all tests, probability (P) values of < 0.05 were considered statistically significant.

Results

Arterial blood pressures

MAP increased significantly at 5 and 10 minutes following Me + Mi administration, and the increased MAP did not change significantly until 20 minutes after Ke administration in all groups (Table 7). In the control group, MAP gradually returned to near-baseline values during the 120 minutes after saline injection. In the Flu, Ap, and Flu + Ap groups, the MAP trend was similar to the control group. On the other hand, in the Ati, Ati + Flu, Ati +Ap, and Ati + Flu + Ap groups, administration of these potential antagonists resulted in a significant decrease in MAP as compared with the control group. The administration of Ati alone or in treatment combination resulted in severe hypotension for a short period of time (approximately 10 minutes). The lowest MAP value (mean ± SD) in Ati, Ati + Flu, Ati +Ap, and Ati + Flu + Ap group was 33 ± 11, 27 ± 4, 40 ± 13, and 30 ± 6 mmHg at 3 minutes after administration, respectively. There were no significant differences in the lowest MAP among the Ati, Ati + Flu, Ati + Ap, and Ati + Flu + Ap groups. After the lowest MAP was observed at 3 minutes after administration of the antagonists, the MAP gradually increased over the next 10 to 60 minutes and returned to baseline values. There were no significant differences in the change in MAP between the groups given Ati.

Similar results were obtained for SAP and DAP (Tables 8 and 9, respectively).

HR

In the control group, MMK administration significantly decreased HR relative to baseline up to 120 minutes after the saline injection (Table 10). In the other groups, administration of potential antagonists, both alone and in combination, did not significantly alter the bradycardia induced by MMK administration observed in the control group.

Table 7. Effects of atipamezole (Ati), flumazenil (Flu) and 4-aminopyridine (Ap), both alone and in combination, on mean arterial blood pressure (mmHg) after administration of medetomidine + midazolam + ketamine (Me + Mi + Ke) under isoflurane anesthesia in cats.

Time (min)	Control	Ati	Flu	Ap	Ati+Flu	Flu+Ap	Ati+Ap	Ati+Flu+Ap
Baseline	52 ± 7	55 ± 12	61 ± 15	59 ± 9	53 ± 8	61 ± 13	61 ± 17	59 ± 10
After Me+Mi								
5	108 ± 27 *	112 ± 26 *	100 ± 19 *	97 ± 13 *	92 ± 26 *	105 ± 37 *	107 ± 24 *	103 ± 24 *
10	114 ± 22 *	115 ± 27 *	104 ± 27 *	99 ± 12 *	94 ± 19 *	111 ± 33 *	117 ± 13 *	100 ± 23 *
After Ke								
10	107 ± 23 *	114 ± 36 *	105 ± 30 *	103 ± 16 *	94 ± 24 *	111 ± 25 *	120 ± 9 *	101 ± 22 *
20	99 ± 28 *	105 ± 29 *	102 ± 27 *	102 ± 19 *	89 ± 24 *	109 ± 24 *	112 ± 12 *	99 ± 21 *
After antagonists								
0.5	98 ± 28 *	72 ± 23	97 ± 28 *	84 ± 19	52 ± 11 †§	86 ± 31	63 ± 19	53 ± 17 †§
1	97 ± 29 *	42 ± 13 *†	99 ± 25 *‡	94 ± 24 *‡	33 ± 6 *†§¶	100 ± 25 *‡#	49 ± 16 *†§¶*f*	36 ± 8 *†§¶*f*
3	94 ± 30 *	33 ± 11 *†	98 ± 25 *‡	93 ± 23 *‡	27 ± 4 *†§¶	99 ± 24 *‡#	40 ± 13 *†§¶*f*	30 ± 6 *†§¶*f*
5	94 ± 31 *	35 ± 10 *†	97 ± 26 *‡	91 ± 21 *‡	30 ± 5 *†§¶	97 ± 24 *‡#	49 ± 11 *†§¶*f*	35 ± 9 *†§¶*f*
7	92 ± 32 *	42 ± 15 *†	96 ± 25 *	90 ± 19 *‡	34 ± 8 *†§¶	97 ± 23 *‡#	63 ± 17	48 ± 15 †§*f*
10	90 ± 32 *	51 ± 20	95 ± 23 *‡	87 ± 19 *	51 ± 19 §	96 ± 22 *‡#	73 ± 16	65 ± 20
15	90 ± 30 *	57 ± 19	92 ± 22	83 ± 19 *	59 ± 17	93 ± 20 *	75 ± 17	70 ± 11
30	85 ± 24 *	59 ± 14	84 ± 22 *	72 ± 13 *	53 ± 7 †§	87 ± 18 *#	70 ± 21	59 ± 9
45	76 ± 21	61 ± 13	77 ± 14 *	70 ± 12	56 ± 8	85 ± 17 *	71 ± 20	57 ± 11
60	74 ± 21 *	60 ± 12	70 ± 8	67 ± 10	55 ± 7	83 ± 17 *	69 ± 21	61 ± 11
75	67 ± 13 *	62 ± 15	69 ± 8	67 ± 11	56 ± 8	82 ± 19 *	67 ± 21	65 ± 12
90	65 ± 12 *	63 ± 17	66 ± 9	69 ± 12	58 ± 9	82 ± 16 *	68 ± 21	60 ± 11
105	64 ± 10 *	65 ± 23	63 ± 7	69 ± 13	57 ± 7	79 ± 18 *	68 ± 20	58 ± 14
120	64 ± 10 *	61 ± 17	63 ± 7	68 ± 12	57 ± 6	65 ± 13	66 ± 18	60 ± 12

Values are mean ± SD (n=6). * Significantly ($p < 0.05$) different from baseline value. † Significantly ($p < 0.05$) different from control group. ‡ Significantly ($p < 0.05$) different from Ati group. § Significantly ($p < 0.05$) different from Flu group. ¶ Significantly ($p < 0.05$) different from Ap group. # Significantly ($p < 0.05$) different from Ati+Flu group. *f* Significantly ($p < 0.05$) different from Flu+Ap group.

Table 8. Effects of atipamezole (Ati), flumazenil (Flu) and 4-aminopyridine (Ap), both alone and in combination, on systolic arterial blood pressure (mmHg) after administration of medetomidine + midazolam + ketamine (Me + Mi + Ke) under isoflurane anesthesia in cats.

Time (min)	Control	Ati	Flu	Ap	Ati+Flu	Flu+Ap	Ati+Ap	Ati+Flu+Ap
Baseline	84 ± 13	80 ± 13	85 ± 15	81 ± 11	81 ± 12	90 ± 17	86 ± 14	78 ± 9
After Me+Mi								
5	136 ± 37 *	133 ± 26 *	122 ± 21 *	117 ± 10 *	115 ± 25 *	129 ± 35 *	133 ± 26 *	112 ± 9 *
10	143 ± 32 *	138 ± 28 *	125 ± 31 *	120 ± 11 *	115 ± 19 *	134 ± 30 *	141 ± 21 *	111 ± 19 *
After Ke								
10	130 ± 26 *	136 ± 39 *	125 ± 33 *	123 ± 16 *	113 ± 22 *	135 ± 25 *	140 ± 14 *	113 ± 22 *
20	122 ± 30 *	126 ± 40 *	122 ± 30 *	122 ± 17 *	109 ± 23 *	131 ± 24 *	132 ± 13 *	113 ± 22 *
After antagonists								
0.5	120 ± 30 *	94 ± 24	116 ± 30 *	103 ± 18	72 ± 8	100 ± 31	83 ± 15	67 ± 15 †
1	120 ± 30 *	65 ± 16 *†	117 ± 29 *‡	114 ± 22 *‡	54 ± 4 *†§¶	123 ± 28 *#	72 ± 11 *†§¶*f*	56 ± 11 *†§¶*f*
3	118 ± 33 *	55 ± 15 *†	117 ± 29 *‡	113 ± 22 *‡	46 ± 4 *†§¶	121 ± 27 *‡#	62 ± 11 *†§¶*f*	47 ± 9 *†§¶*f*
5	117 ± 34 *	55 ± 14 *†	115 ± 29 *‡	112 ± 19 *‡	46 ± 5 *†§¶	120 ± 26 *‡#	72 ± 10 *†§¶*f*	53 ± 6 *†§¶*f*
7	116 ± 33 *	66 ± 20 †	113 ± 28 *‡	111 ± 18 *‡	55 ± 7 *†§¶	118 ± 25 *‡#	85 ± 10	68 ± 15 †§¶*f*
10	115 ± 34 *	76 ± 23	113 ± 27 *	108 ± 17 *	73 ± 17	119 ± 25 *	96 ± 11	84 ± 17
15	114 ± 32 *	85 ± 21	109 ± 26 *	104 ± 16 *	83 ± 13	117 ± 24 *	97 ± 15	90 ± 7
30	109 ± 27 *	82 ± 18	103 ± 24	96 ± 12 *	77 ± 7 *	110 ± 18 *	91 ± 19	78 ± 7
45	101 ± 24	84 ± 16	96 ± 17	92 ± 10	78 ± 6	106 ± 16	93 ± 17	76 ± 10
60	97 ± 23	83 ± 15	87 ± 7	89 ± 8	75 ± 6	107 ± 18	89 ± 18	77 ± 8
75	93 ± 19	82 ± 17	86 ± 6	88 ± 10	76 ± 6	105 ± 20	87 ± 18	84 ± 10
90	91 ± 18	84 ± 18	83 ± 5	70 ± 10	77 ± 6	105 ± 18	88 ± 17	79 ± 11
105	89 ± 17	86 ± 24	80 ± 5	89 ± 10	76 ± 5	102 ± 18	88 ± 18	75 ± 13 .
120	89 ± 16	83 ± 18	80 ± 6	88 ± 10	78 ± 6	99 ± 18	86 ± 15	79 ± 10

Values are mean ± SD (n=6). * Significantly ($p < 0.05$) different from baseline value. † Significantly ($p < 0.05$) different from control group. ‡ Significantly ($p < 0.05$) different from Ati group. § Significantly ($p < 0.05$) different from Flu group. ¶ Significantly ($p < 0.05$) different from Ap group. # Significantly ($p < 0.05$) different from Ati+Flu group. *f* Significantly ($p < 0.05$) different from Flu+Ap group.

Table 9. Effects of atipamezole (Ati), flumazenil (Flu) and 4-aminopyridine (Ap), both alone and in combination, on diastolic arterial blood pressure (mmHg) after administration of medetomidine + midazolam + ketamine (Me + Mi + Ke) under isoflurane anesthesia in cats.

Time (min)	Control	Ati	Flu	Ap	Ati+Flu	Flu+Ap	Ati+Ap	Ati+Flu+Ap
Baseline	38 ± 7	46 ± 10	52 ± 16	47 ± 10	43 ± 6	50 ± 15	51 ± 13	46 ± 10
After Me+Mi								
5	93 ± 23 *	99 ± 24 *	89 ± 19 *	85 ± 12 *	79 ± 25 *	93 ± 37 *	94 ± 23 *	90 ± 22 *
10	100 ± 20 *	102 ± 26 *	91 ± 24 *	86 ± 13 *	82 ± 19 *	98 ± 31 *	104 ± 11 *	89 ± 23 *
After Ke								
10	94 ± 21 *	101 ± 35 *	92 ± 25 *	91 ± 16 *	83 ± 22 *	98 ± 26 *	106 ± 8 *	88 ± 20 *
20	87 ± 27 *	90 ± 36 *	90 ± 23 *	89 ± 18 *	78 ± 23 *	97 ± 24 *	101 ± 13 *	88 ± 20 *
After antagonists								
0.5	85 ± 28 *	60 ± 22	87 ± 24 *	74 ± 19	42 ± 8 †	78 ± 30 *	53 ± 20	43 ± 14 †§
1	84 ± 28 *	30 ± 11 *†	88 ± 22 *‡	82 ± 24 *‡	22 ± 4 *†§¶	89 ± 25 *‡#	36 ± 15 *†§¶*f*	25 ± 6 *†§¶*f*
3	82 ± 28 *	24 ± 7 *†	87 ± 23 *‡	79 ± 23 *‡	19 ± 4 *†§¶	88 ± 23 *‡#	28 ± 12 *†§¶*f*	20 ± 5 *†§¶*f*
5	81 ± 29 *	25 ± 8 *†	84 ± 23 *‡	78 ± 21 *‡	21 ± 5 *†§¶	86 ± 24 *‡#	38 ± 11 *†§¶	25 ± 8 *†§¶
7	80 ± 30 *	32 ± 13 *†	83 ± 22 *‡	78 ± 19 *‡	27 ± 7 *†§¶	85 ± 23 *‡#	51 ± 14	36 ± 15 †§¶*f*
10	79 ± 30 *	42 ± 18	83 ± 21 *‡	75 ± 19 *	40 ± 17 †§	84 ± 22 *‡#	60 ± 13	52 ± 20
15	79 ± 28 *	48 ± 19	82 ± 20 *	72 ± 18 *	49 ± 13	81 ± 19 *	60 ± 17	55 ± 12
30	73 ± 21 *	48 ± 15 †	74 ± 19 *‡	62 ± 13 *	42 ± 7 †§¶	76 ± 17 *‡#	58 ± 19	47 ± 9 †§¶*f*
45	64 ± 18 *	50 ± 14	68 ± 15	58 ± 12	44 ± 6	74 ± 15 *#	62 ± 18	45 ± 12 *f*
60	63 ± 21 *	50 ± 13	59 ± 9	56 ± 11	44 ± 6	73 ± 16 *#	58 ± 20	48 ± 11
75	56 ± 18 *	51 ± 16	58 ± 7	57 ± 12	45 ± 6	73 ± 17 *	57 ± 18	55 ± 14
90	54 ± 10 *	53 ± 17	57 ± 7	58 ± 13	47 ± 6	72 ± 17 *	58 ± 19	49 ± 13
105	53 ± 9 *	56 ± 22	54 ± 6	58 ± 13	46 ± 5	70 ± 17 *	58 ± 18	47 ± 14 .
120	52 ± 11 *	52 ± 16	55 ± 5	58 ± 14	48 ± 6	67 ± 19 *	61 ± 17	50 ± 12

Values are mean ± SD (n=6). * Significantly ($p < 0.05$) different from baseline value. † Significantly ($p < 0.05$) different from control group. ‡ Significantly ($p < 0.05$) different from Ati group. § Significantly ($p < 0.05$) different from Flu group. ¶ Significantly ($p < 0.05$) different from Ap group. # Significantly ($p < 0.05$) different from Ati+Flu group. *f* Significantly ($p < 0.05$) different from Flu+Ap group.

Table 10. Effects of atipamezole (Ati), flumazenil (Flu) and 4-aminopyridine (Ap), both alone and in combination, on heart rates (beats/min) after administration of medetomidine + midazolam + ketamine (Me + Mi + Ke) under isoflurane anesthesia in cats.

Time (min)	Control	Ati	Flu	Ap	Ati+Flu	Flu+Ap	Ati+Ap	Ati+Flu+Ap
Baseline	124 ± 21	131 ± 35	129 ± 30	126 ± 23	120 ± 16	122 ± 15	136 ± 11	118 ± 16
After Me+Mi								
5	92 ± 19 *	98 ± 35 *	105 ± 26 *	100 ± 22 *	94 ± 13 *	101 ± 30	99 ± 22 *	101 ± 34
10	87 ± 17 *	91 ± 20 *	91 ± 17 *	82 ± 15 *	90 ± 14 *	95 ± 17 *	87 ± 10 *	92 ± 16 *
After Ke								
10	102 ± 21 *	99 ± 22 *	91 ± 13 *	85 ± 9 *	97 ± 14 *	97 ± 6 *	92 ± 7 *	94 ± 14 *
20	104 ± 19 *	99 ± 21 *	92 ± 13 *	87 ± 9 *	96 ± 10 *	99 ± 6 *	94 ± 6 *	93 ± 13 *
After antagonists								
1	102 ± 22 *	103 ± 21 *	93 ± 12 *	82 ± 11 *	98 ± 12 *	94 ± 4 *	97 ± 5 *	87 ± 15 *
3	103 ± 20 *	106 ± 22 *	94 ± 14 *	80 ± 11 *	102 ± 12 *	95 ± 4 *	98 ± 10 *	91 ± 13 *
5	103 ± 20 *	106 ± 24 *	94 ± 14 *	80 ± 10 *	103 ± 13 *	95 ± 5 *	101 ± 9 *	95 ± 15 *
7	104 ± 19 *	107 ± 25 *	92 ± 13 *	79 ± 10 *	102 ± 10 *	95 ± 5 *	102 ± 7 *	96 ± 16 *
10	103 ± 19 *	110 ± 26 *	93 ± 12 *	81 ± 10 *	101 ± 8 *	96 ± 6 *	100 ± 7 *	94 ± 10 *
15	102 ± 20 *	110 ± 24 *	91 ± 12 *	81 ± 11 *	101 ± 9 *	97 ± 8 *	105 ± 6 *	94 ± 10 *
30	101 ± 15 *	110 ± 27 *	91 ± 12 *	85 ± 13 *	102 ± 8 *	98 ± 7 *	114 ± 8 *	99 ± 14 *
45	103 ± 11 *	106 ± 25 *	92 ± 15 *	87 ± 15 *	100 ± 9 *	98 ± 7 *	117 ± 10 *	100 ± 12 *
60	101 ± 18 *	107 ± 27 *	92 ± 14 *	88 ± 15 *	100 ± 9 *	98 ± 8 *	120 ± 9 *	96 ± 12 *
75	102 ± 17 *	105 ± 26 *	94 ± 16 *	89 ± 16 *	97 ± 10 *	98 ± 9 *	122 ± 10	94 ± 13 *
90	101 ± 17 *	104 ± 27 *	103 ± 35	89 ± 16 *	96 ± 11 *	98 ± 10 *	121 ± 12	94 ± 13 *
105	101 ± 18 *	109 ± 37 *	102 ± 35	88 ± 16 *	97 ± 12 *	99 ± 11 *	118 ± 12 *	96 ± 8 *
120	100 ± 20 *	108 ± 37	101 ± 37	88 ± 16 *	97 ± 10 *	97 ± 11 *	118 ± 11 *	93 ± 10 *

Values are mean ± SD (n=6). * Significantly ($p < 0.05$) different from baseline value.

Discussion

The rationale for fixed-dosing of Ati, Flu, and Ap as antagonists in MMK anesthesia has been outlined in our earlier study [47,48]. In the present study, an intravenous route of administration of the antagonists was chosen based on the immediate onset of action and the fact that this route is most often used during emergency anesthetic situations. From the results of this study, however, it is firstly proposed that the intravenous administration of antagonists containing Ati is not recommended due to the risk of causing hypotension in cats anesthetized with MMK and isoflurane.

The results of the present study revealed that MMK administration caused increases in SAP, DAP, and MAP in cats anesthetized with isoflurane, which was in agreement with a previous report showing that the diastolic blood pressure exhibited an increase during MMK anesthesia in cats [14]. MMK-induced increases in blood pressure under isoflurane anesthesia may mainly be due to the peripheral vasoconstricting action stimulated by Me. This is supported by a report that Me increased arterial pressure and systemic vascular resistance by severe vasoconstriction via α_2-adrenoceptors in isoflurane-anesthetized cats [19]. It has been also reported that during anesthesia with isoflurane in oxygen, arterial blood pressure can be maintained higher in cats premedicated with Me than in cats receiving the Mi + Ke combination [2].

The present results demonstrated that Ati alone or in combination with other drugs reversed the increase in blood pressure (SAP, DAP, and MAP) induced by MMK. In contrast, Flu, Ap, and Flu + Ap did not significantly change the increase in blood pressure induced by MMK. In addition, we found that there were no significant differences in the change in blood pressure among the Flu, Ap, and Flu + Ap treatment groups, or among the Ati, Ati + Flu, Ati + Ap, and Ati + Flu + Ap treatment groups. These results demonstrated that Ati was critical to reversing the arterial blood pressure elevation induced by MMK administration under isoflurane anesthesia in

cats. This effect may be due to the antagonism of Ati on the α_2-adrenoceptor-mediated vasoconstrictive action by medetomidine in anesthetized cats [19,42].

Meanwhile, in this study, intravenous administration of Ati alone or in treatment combination resulted in severe hypotension for a short period of time (approximately 10 minutes) under isoflurane anesthesia in cats that had received MMK. A similar, notably hypotensive effect of Ati (25 and 50 μg/kg) administered intramuscularly has been reported in isoflurane-anesthetized and ventilated cats that had received dexmedetomidine [54]. In that report, intramuscular administration of 50 μg/kg Ati resulted in hypotension (MAP < 60 mmHg) in 4 of 6 cats, and mean MAP decreased to approximately 60 mmHg and then increased to around 90 mmHg over the next 15 minutes, although it didn't actually specify when the peak hypotensive effect occurred [54]. On the other hand, in our study reported here, intravenous administration of 200 μg/kg Ati caused more severe hypotension (the lowest MAP value was 33 ± 11 mmHg; mean ± SD) than in that report. Thus, the intravenous administration of Ati appeared to cause a greater hypotension than its intramuscular administration, although there were differences in Ati dose between both studies. Therefore, the intravenous administration of Ati under isoflurane anesthesia in cats receiving MMK should be subject to close monitoring to enable identification of temporary but potentially rapidly developing hypotension. The present study suggests that the intravenous Ati should only be used in emergency situations and not on a routine basis. Furthermore, the present results suggest that the intravenous usage of Ati is discouraged, especially in critically ill animals. An intravenous Ati may be contraindicated in emergency situations because of impairing resuscitation efforts due to temporally severe hypotension, with the possible exception of cardiac arrest and cardiopulmonary resuscitation. Atropine was reported to prevent bradycardia and enhance the hypertension induced by dexmedetomidine in cats [35], and to maintain a higher blood pressure in cats anesthetized with MMK or Me + Ke [14]. In emergency situations it may be

necessary to also administer atropine if critical hypotension and bradycardia occur following reversal by Ati.

In the present study, MMK administration decreased the HR in cats anesthetized with isoflurane, which is in agreement with a previous report showing a decrease in HR during MMK anesthesia in cats [14,48]. This bradycardia induced by MMK may be due to the activation of α_2-adrenoceptors by Me, since Ati has been reported to efficiently antagonize the bradycardic action of Me in non-anesthetized cats [20]. However, the results in the present study indicated that Ati alone or in combination with other drugs did not reverse the decrease in HR induced by MMK under isoflurane anesthesia with controlled ventilation in cats. In fact, the present study showed that HR did not differ significantly between any of the treatments (Ati, Flu, Ap, and control). The finding of the inability of Ati to reverse bradycardia in the present study was in contrast to our previous report where combinations with Ati were effective in terms of antagonizing the bradycardia induced by MMK without isoflurane anesthesia in cats [48]. A previous study reported that Ati was ineffective in increasing the pulse rate in isoflurane-anesthetized cats that had received dexmedetomidine [54]. It has been also reported that in isoflurane-anesthetized cats receiving dexmedetomidine, intravenous administration of Ati reduced arterial blood pressure while resulting in only marginal increased HR [33]. Based on these previous reports, the ineffectiveness of Ati in reversing bradycardia induced by MMK in this study may reflect the fact that this experiment was performed under isoflurane anesthesia with controlled ventilation. In fact, it has been reported that dexmedetomidine produces initial bradycardia via the baroreflex and subsequently results in HR reduction mainly because of central sympathetic depression [53], and that isoflurane depresses the baroreflex control in HR and inhibit the HR baroreflex primarily by the sympathetic nervous system [31]. Therefore, the ineffectiveness of Ati in reversing bradycardia may be due primarily to depression of the baroreflex control in HR caused by isoflurane anesthesia.

In the present study, the hypotension caused by intravenous Ati may be due to rapid reversal of peripheral α_2-adrenoceptor-mediated vasoconstriction, while not affecting the central α_2-adrenoceptor-mediated bradycardia in cats anesthetized with isoflurane under controlled ventilation. If the bradycardia could be improved, the hypotension might be minimal, and the animal might be able to maintain cardiac output and thus blood pressure. A previous study has shown that intravenous Ati rapidly reversed the bradycardia produced by MMK in cats without isoflurane anesthesia [48]. Therefore, further studies are desired to investigate the effect of intravenous Ati on blood pressures after administration of MMK in cats without isoflurane anesthesia.

The only advantage of the use of Ap, especially triple combination with Ati and Flu, is the most accelerating recovery from MMK anesthesia [48]. However, this triple combination causes adverse reactions including tachycardia, tachypnoea, excitement, muscle tremors, and excessive stress-related hormonal responses [48]. Furthermore, in the present study, no advantage in using Ap as antagonist combinations was obtained for reversing the changes in blood pressure and HR caused by MMK in isoflurane-anesthetized cats. Therefore, based on these findings, the use of Ap as an antagonist for MMK anesthesia in cats is not recommended.

There are some limitations in this study. In our study design, the intravenous administration of antagonists for MMK was performed under controlled ventilation. As the controlled ventilation may have affected severe hypotension and the ineffectiveness of bradycardia improvement after Ati administration, it might be necessary to design the study under a spontaneous breathing. Although the present study was intended to investigate the changes of blood pressure and HR, it may be necessary to evaluate more detailed cardiovascular parameters such as systemic vascular resistance, stroke volume, and cardiac output. In this study, an intravenous route only was selected for administration of the antagonists in the expectation of an immediate effect in emergencies. As a result, severe hypotension occurred after intravenous administration of antagonists combined

with Ati. It may be interesting to perform a follow up study evaluating the intramuscular administration of the antagonist. In addition, the dose-dependent effect of antagonists, especially Ati, was not conducted in this study.

In conclusion, MMK administration resulted in increased blood pressure and bradycardia in cats anesthetized with isoflurane under controlled ventilation. Administration of potential antagonists alone or in all possible combinations did not significantly alter the bradycardia. Flu, Ap alone, and Flu + Ap did not significantly alter the changes in blood pressures induced by MMK. Meanwhile, Ati alone or in combination reversed the increase in blood pressures induced by MMK, but transiently caused notable decreases in blood pressure relative to baseline. These results indicate that the intravenous use of Ati alone or combinations with Ati is effective for antagonizing hypertension induced by MMK in cats anesthetized with isoflurane but should be monitored closely due to the risk of temporary hypotension. The present results also suggest that the intravenous Ati should only be used in emergency situations and not on a routine basis.

General conclusion

In chapter 1, the present study showed that isoflurane anesthesia itself inhibited decreased adrenaline and noradrenaline concentrations but increased cortisol concentrations and hyperglycemia in castrated and ovariohysterectomized cats. Pretreatment with Me alone and in combination reduced cortisol release during isoflurane anesthesia and in the early-recovery phase as well as the improvement of the quality of recovery. No remarkable differences in sympathetic-adrenal and metabolic responses were observed between Me-treated groups, except that MM treatment prevented an excessive decrease in catecholamine concentrations during isoflurane anesthesia. This study demonstrated newly that pretreatments with Me alone and in combination are useful for the prevention of stress responses induced by isoflurane anesthesia and surgery in feline practice.

In chapter 2, the present study revealed that, in both castrated and ovariohysterectomized cats, the addition of Me or MM to Af improved the quality of recovery from isoflurane anesthesia and surgery. Both plasma adrenaline and noradrenaline concentrations decreased during isoflurane anesthesia in cats premediated with Af with and without Me, except for MM + Af IM. Treatment with MM + Af prevented an excessive decrease in catecholamine concentrations during anesthesia and surgery. Combinations of Me or MM with Af suppressed an excessive postoperative increase in plasma cortisol in ovariohysterectomized cats. The addition of Me or MM to Af facilitated hyperglycemia during isoflurane anesthesia in cats. Pretreatments with Me or MM in combination with Af greatly reduced pre- and/or postoperative NEFA concentrations. Therefore, it was concluded that pretreatments with Me or MM in combination with Af are useful for the prevention of stress-related hormonal and metabolic responses, other than hyperglycemia, during isoflurane anesthesia and surgery in feline veterinary practice.

In chapter 3, this study revealed that MMK-isoflurane anesthesia induced the inhibition of catecholamine and cortisol release, the inhibition of lipolysis, and hyperglycemia in castrated and ovariohysterectomized cats. Compared with intramuscular administration, the intravenous administration of Ati alone and in combination with Flu induces more rapid recovery from anesthesia, regardless of the timing of administration. Compared to Ati alone, the addition of Flu improved the quality of recovery. The IV administration of Ati alone effectively reverses the post-operative neurohormonal and metabolic effects induced by MMK-isoflurane anesthesia in cats undergoing ovariohysterectomy. The IM administration of Ati–Flu combinations can induce rapid recovery from anesthesia without largely altering the stress-related neurohormonal and metabolic changes induced by MMK-isoflurane anesthesia and surgery. This study is the first to demonstrate that differences in the route and timing of the administration of Ati and Flu as antagonists for MMK-isoflurane anesthesia alter post-operative stress-related hormonal and metabolic responses.

In chapter 4, the present study revealed that MMK administration resulted in increased blood pressure and bradycardia in cats anesthetized with isoflurane under controlled ventilation. Administration of potential antagonists alone or in all possible combinations did not significantly alter the bradycardia. Flu, Ap alone, and Flu + Ap did not significantly alter the changes in blood pressures induced by MMK. Meanwhile, Ati alone or in combination reversed the increase in blood pressures induced by MMK, but transiently caused notable decreases in blood pressure relative to baseline. These results indicated that the intravenous use of Ati alone or combinations with Ati is effective for antagonizing hypertension induced by MMK in cats anesthetized with isoflurane but should be monitored closely due to the risk of temporary hypotension. The present results also suggested that the intravenous Ati should only be used in emergency situations and not on a routine basis.

In conclusion, this study revealed that pretreatments with Me alone and in combination with Mi, Ke or Af are useful for the prevention of stress-related hormonal and metabolic responses,

other than hyperglycemia, during isoflurane anesthesia and surgery in feline veterinary practice. As to the effects of potential antagonists, IV administration of Ati alone effectively reverses the post-operative neurohormonal and metabolic effects induced by MMK-isoflurane anesthesia and surgery in cats, but IM administration of Ati–Flu combination can induce rapid recovery from anesthesia without largely altering the neurohormonal and metabolic changes. Therefore, this study demonstrated that differences in the route and timing of the administration of Ati and Flu alter post-operative stress-related hormonal and metabolic responses induced by MMK-isoflurane anesthesia and surgery. In addition, this study revealed that the IV use of Ati alone and in combinations is effective for antagonizing hypertension induced by MMK in cats anesthetized with isoflurane but should be monitored closely due to the risk of temporary hypotension.

Abstract

The α_2-adrenoceptor agonist medetomidine (Me) is mainly used for sedation and analgesia in veterinary medicine. However, it induces undesirable effects like hyperglycemia, hypoinsulinemia, emesis and bradyarrhythmias in cats. A combination of Me with midazolam (Mi) and/or ketamine (Ke) produces good anesthesia in cats, with a reduction of adverse effects or the potentiation of analgesia. Alfaxalone (Af) is a newly developed anesthetic agent that is useful for the sedation, induction, and maintenance of anesthesia in cats. However, during the recovery period, this agent produces more adverse events than propofol, including ataxia and muscular tremors. The quality of recovery from anesthesia with Af may be improved with the use of other sedative and/or inhalant anesthetic agents. Stressors such as anxiety, excitement, pain and anesthesia are known to induce neurohormonal and metabolic changes. These changes are characterized by increases in blood levels of cortisol, catecholamines, glucose and non-esterified fatty acids (NEFA) and a decrease in blood insulin levels. Actions mediated by α_2-adrenoceptors are closely coordinated with these events. However, there are no published reports on the effects of pretreatment with Me alone and in combinations on stress responses in isoflurane-anesthetized cats undergoing surgery. On the other hand, antagonism may be required when anesthetized animals show a profound depression of vital signs, adverse effects, and/or delayed recovery from anesthesia. Atipamezole (Ati), flumazenil (Flu) and 4-aminopyridine (Ap) either completely or partially antagonize the effects of Me + Mi + Ke (MMK) in cats. In terms of the overall effect of these antagonists on general anesthesia and post-surgical recovery, the use of potential antagonists may be advantageous to recovery from anesthesia if the sympathoadrenal system is adequately but not excessively activated. However, there are no published reports on the effects of Ati and Flu, both alone and in combinations, on the stress responses of anesthetized cats undergoing surgery. It is also important to examine the effect of the reversals of arterial blood pressure to ensure the

appropriate use of these antagonists during an anesthetic emergency when using MMK in cats. Therefore, the present study aimed to investigate the effects of pretreatments with Me, Mi, Ke and Af alone and in combinations on stress-related neurohormonal, metabolic and cardiovascular responses in isoflurane-anesthetized cats undergoing surgery, and their antagonistic effects by potential antagonists.

In chapter 1, the effects of pretreatment with Me, Mi and Ke alone and in combinations on stress-related neurohormonal and metabolic responses were evaluated in isoflurane-anesthetized cats undergoing ovariohysterectomy and castration. One hundred and twelve, client-owned healthy mixed-breed cats were recruited prospectively. In both surgeries, the cats were divided into 7 groups (8 cats per group): non-treatment (control), Me (50 μg/kg), Mi (0.5 mg/kg), Ke (5 mg/kg), Me + Mi, Me + Ke and Me + Mi + Ke administered intramuscularly. After pretreatments, we maintained anesthesia with isoflurane and oxygen. Venous blood was taken before pretreatment, pre- and post-operatively during anesthesia and at early- and complete-recovery. Both plasma adrenaline and noradrenaline decreased during anesthesia in all groups. Plasma cortisol increased during anesthesia and at early-recovery in non-Me-treated groups, whereas it decreased in Me-treated groups in both surgeries. Plasma insulin and NEFA decreased and glucose increased during anesthesia in all groups, but hyperglycemia and decrease in NEFA were greater in Me-treated groups. It is concluded that in isoflurane-anesthetized cats undergoing surgeries, premedication with Me alone and in combinations is useful for reducing perioperative stress-related increase in cortisol and catecholamines except for hyperglycemia.

In chapter 2, the effects of pretreatment with Af alone and in combinations with Me and Mi, on stress-related neurohormonal and metabolic responses were investigated in isoflurane-anesthetized cats undergoing ovariohysterectomy or castration. This study was designed to assess stress responses during clinically important perioperative stages in feline veterinary practice. Seventy-two client-owned, healthy, mixed-breed cats were prospectively recruited. In both

surgeries, the cats were divided into six groups (six cats per group): 1) Af intravenously (IV); 2) Af intramuscularly (IM); 3) Me IM + Af IV; 4) Me IM + Af IM; 5) Me + Mi IM + Af IV; and 6) Me + Mi IM + Af IM. Af, Me, and Mi dosages were 5 mg/kg, 50 μg/kg, and 0.5 mg/kg, respectively. After pretreatment, cats were maintained under anesthesia with isoflurane and oxygen. Venous blood was taken before pretreatment, pre- and post-operatively during anesthesia, and during early and complete recovery. Both plasma adrenaline and noradrenaline decreased during isoflurane anesthesia in cats premediated with Af alone and in combination with Me, except for the Me + Mi (MM) + Af IM group. Treatment with MM + Af prevented an excessive decrease in catecholamine during anesthesia and surgery. Combinations of Me or MM with Af suppressed an excessive increase in postoperative plasma cortisol in ovariohysterectomized cats. The addition of Me or MM to Af facilitated hyperglycemia during isoflurane anesthesia. Pretreatments with Me or MM in combination with Af, compared to Af IV, greatly reduced pre- and/or postoperative non-esterified fatty acids concentrations. The addition of Me or MM to Af improved the quality of recovery from anesthesia. These results indicate that pretreatments with Me or MM combined with Af are useful for preventing stress-related hormonal and metabolic responses, other than hyperglycemia, during isoflurane anesthesia and surgery, and for improving recovery quality in feline veterinary practice.

In chapter 3, the post-operative effects of Ati and Flu on stress-related neurohormonal and metabolic responses were evaluated in isoflurane-anesthetized cats premedicated with MMK and undergoing ovariohysterectomy or castration. Client-owned mixed-breed cats (n = 108) were treated with 50-μg/kg Me and 0.5-mg/kg Mi, followed by 5-mg/kg Ke (MMK) intramuscularly (IM), and maintained under isoflurane anesthesia. The cats were divided into nine groups (six cats/group): control (saline IM), 50-μg/kg Flu IM or intravenously (IV), 100-μg/kg Ati IM or IV, 200-μg/kg Ati IM, 100-μg/kg Ati + 50-μg/kg Flu IM or IV, and 200-μg/kg Ati + 50-μg/kg Flu IM. Five blood samples were taken: before pretreatment, post-operatively during anesthesia, and 10,

60, and 120 min after antagonist administration. MMK-isoflurane anesthesia decreased adrenaline, noradrenaline, cortisol, and NEFA levels. It also caused hyperglycemia. Compared to the controls, Ati IV rapidly reversed the decreased catecholamine and cortisol concentrations in ovariohysterectomized cats. Ati IV and/or Ati + Flu IV tended to induce rapid recovery from hyperglycemia and reverse the NEFA decreases. Compared to IM, the IV administration of Ati, alone and in combination with Flu, induced a more rapid recovery from anesthesia. Compared to Ati alone, the Ati–Flu combination improved the quality of recovery. Thus, Ati IV is effective for rapidly reversing stress-related neurohormonal and metabolic effects in MMK-isoflurane anesthetized cats. Ati–Flu IM aids in rapid recovery without altering post-operative neurohormonal and metabolic changes.

In chapter 4, the effects of a fixed dose of Ati, Flu, and Ap, both alone and in combinations, on changes in arterial blood pressure and heart rate induced by MMK were investigated under isoflurane anesthesia with controlled ventilation in healthy cats. Healthy adult mixed-breed cats were used for eight investigation groups (6 cats per group), with ≥ 2 weeks between interventions. Cats were anesthetized with end-tidal isoflurane concentration of 2% under controlled ventilation. A catheter was inserted into the right or left femoral artery for arterial pressure monitoring and blood gas sampling, and electrocardiogram electrodes were placed. Upon completed preparations, cats were administered a mixture of Me (50 μg/kg) and Mi (0.5 mg/kg), followed 10 minutes later by Ke (10 mg/kg) intramuscularly. Twenty minutes after Ke injection, the cats received intravenous injection with either a physiological saline solution at 0.1 mL/kg (control), or one of seven variations of experimental drugs, alone or in combination: Ati (0.2 mg/kg), Flu (0.1 mg/kg), Ap (0.5 mg/kg), Ati + Flu, Flu + Ap, Ati + Ap, and Ati + Flu + Ap. Arterial blood pressure and heart rate were continuously measured over 120 minutes after administration of potential antagonists. MMK induced an increase in blood pressure and bradycardia. Potential antagonists alone or in combination did not significantly alter the bradycardia. Flu, Ap alone, and Flu +Ap did

not significantly alter the changes in blood pressures induced by MMK. Meanwhile, administration of Ati alone or in combination reversed the increase in blood pressure induced by MMK but transiently caused excessive hypotension. These results revealed that Ati alone or in combination is effective for antagonizing hypertension induced by MMK; however, attention should be paid to temporary hypotension in cats anesthetized with isoflurane.

In conclusion, this study revealed that pretreatments with Me alone and in combinations with Mi, Ke or Af are useful for the prevention of stress-related hormonal and metabolic responses, other than hyperglycemia, during isoflurane anesthesia and surgery in feline veterinary practice. As to the effects of potential antagonists, this study demonstrated that differences in the route and timing of the administration of Ati and Flu alter post-operative stress-related hormonal and metabolic responses induced by MMK-isoflurane anesthesia and surgery. Furthermore, this study revealed that the IV use of Ati alone and in combinations is effective for antagonizing hypertension induced by MMK in cats anesthetized with isoflurane but should be monitored closely due to the risk of temporary hypotension. This study provided new information about the effects of pretreatments with Me, Mi, Ke and Af alone and in combinations on stress-related hormonal, metabolic and cardiovascular responses in isoflurane anesthetized cats undergoing surgery, and on their antagonistic effects by potential antagonists in MMK–isoflurane anesthetized cats undergoing surgery.

Acknowledgements

I would like to thank my doctoral course supervisor Professor Dr. Yoshiaki Hikasa (Tottori University) for his guidance and support throughout my graduate student years, for the critical reviewing of the manuscript. 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. Takuya Mizuno (Yamaguchi University) for their guidance as assistant supervisors during my study. Special thanks to Professor Dr. Toshio Ohta (Tottori University), Professor Dr. Tomohiro Imagawa (Tottori University), and Professor Dr. Takashi Takeuchi (Tottori University) for the review of this doctoral thesis.

I am grateful to my wife, Dr. Toshiko Kamohara for a variety of support in performing the study and manuscript preparation with data collection. I also wish to thank Kumiko Maeda and Erika Chikami for their assistance with data collection.

References

1. Adams HA. Endocrine reactions following S-(+)-ketamine. Anaesthesist. 1997; 46: 30–37.
2. Akkerdaas LC, Mioch P, Sap R, Hellebrekers LJ. Cardiopulmonary effects of three different anaesthesia protocols in cats. Vet Q. 2001; 23: 182–186.
3. Ambrisko TD, Hikasa Y. Neurohormonal and metabolic effects of medetomidine compared with xylazine in beagle dogs. Can J Vet Res. 2002; 66: 42–49.
4. Ambrisko TD, Hikasa Y, Sato K. Influence of medetomidine on stress-related neurohormonal and metabolic effects caused by butorphanol, fentanyl, and ketamine administration in dogs. Am J Vet Res. 2005; 66: 406–412.
5. Armstrong T, Wagner MC, Cheema J, Pang DS. Assessing analgesia equivalence and appetite following alfaxalone- or ketamine-based injectable anesthesia for feline castration as an example of enhanced recovery after surgery. J Feline Med Surg. 2018; 20: 73–82.
6. Benson GJ, Grubb TL, Neff-Davis C, Olson WA, Thurmon JC, Lindner DL, Tranquilli WJ, Vanio O. Perioperative stress response in the dog: effect of pre-emptive administration of medetomidine. Vet Surg. 2000; 29: 85–91.
7. Bouillon J, Duke T, Focken AP, Snead EC, Cosford KL. Effects of dexmedetomidine on glucose homeostasis in healthy cats. J Feline Med Surg. 2020; 22: 344–349.
8. Bruniges N, Taylor PM, Yates D. Injectable anaesthesia for adult cat and kitten castration: effects of medetomidine, dexmedetomidine and atipamezole on recovery. J Feline Med Surg. 2016; 18: 860–867.
9. Bruniges N, Yates D. Effects of atipamezole dosage and timing of administration on recovery time and quality in cats following injectable anaesthesia incorporating ketamine. J Feline Med Surg. 2020; 22: 589–597.
10. Cullen LK. Medetomidine sedation in dogs and cats: a review of its pharmacology, antagonism and dose. Br Vet J. 1996; 152: 519–535.

11. Desborough JP. The stress response to trauma and surgery. Br J Anaesth. 2000; 85: 109–117.

12. Deussing JM, Chen A. The corticotropin-releasing factor family: physiology of the stress response. Physiological Reviews Physiol Rev. 2018; 98: 2225–2286.

13.Ebner J, Wehr U, Baumgartner C, Erhardt W, Henke J. Partial antagonization of midazolam-medetomidine-ketamine in cats--atipamezole versus combined atipamezole and flumazenil. J Vet Med A Physiol Pathol Clin Med. 2007; 54: 518–521.

14. Ebner J, Wehr U, Busch R, Erhardt W, Henke J. A comparative clinical study of three different dosages of intramuscular midazolam-medetomidine-ketamine immobilization in cats. J Vet Med A Physiol Pathol Clin Med. 2007; 54: 418–423.

15. Eggers B, Tordiffe ASW, Lamberski N, Lawrenz A, Sliwa A, Wilson B, Meyer LCR. Evaluation of two doses of butorphanol-medetomidine-midazolam for the immobilization of wild versus captive black-footed cats (*felis nigripes*). J Zoo Wild Med. 2020; 51: 497–506.

16. Fagerholm V, Haaparanta M, Scheinin M. α2-Adrenoceptor regulation of blood glucose homeostasis. Basic Clin Pharmacol Toxicol. 2011; 108: 365–370.

17. Gates MC, Littlewood KE, Kongara K, Odom TF, Sawicki RK. Cross-sectional survey of anaesthesia and analgesia protocols used to perform routine canine and feline ovariohysterectomies. Vet Anaesth Analg. 2020; 47: 38–46.

18. Georgiyants MA, Vysotska OV, Seredenko NP, Chernii TV, Strashnenko HN, Haiduchyk PD. Study of the state of stress-implementing systems in abdominal delivery depending on anesthetic techniques. Wiad Lek. 2020; 73: 2378–2385.

19. Golden AL, Bright JM, Daniel GB, Fefee D, Schmidt D, Harvey RC. Cardiovascular effects of the alpha2-adrenergic receptor agonist medetomidine in clinically normal cats anesthetized with isoflurane. Am J Vet Res. 1998; 59: 509–513.

20. Granholm M, McKusick BC, Westerholm FC, Aspegrèn JC. Evaluation of the clinical efficacy and safety of dexmedetomidine or medetomidine in cats and their reversal with atipamezole. Vet Anaesth Analg. 2006; 33: 214–223.

21. Hatch RC, Booth NH, Kitzman JV, Wallner BM, Clark JD. Antagonism of ketamine anesthesia in cats by 4-aminopyridine and yohimbine. Am J Vet Res. 1983; 44: 417–423.

22. Hernández-Avalos I, Flores-Gasca E, Mota-Rojas D, Casas-Alvarado A, Miranda-Cortés AE, Domínguez-Oliva A. Neurobiology of anesthetic-surgical stress and induced behavioral changes in dogs and cats: a review. Vet World. 2021; 14: 393–404.

23. Ilkiw JE, Farver TB, Suter CM, McNeal D, Steffey EP. The effect of intravenous administration of variable-dose flumazenil after fixed-dose ketamine and midazolam in healthy cats. J Vet Pharmacol Ther. 2002; 25: 181–188.

24. Jaeger AT, Pypendop BH, Ahokoivu H, Honkavaara J. Cardiopulmonary effects of dexmedetomidine, with and without vatinoxan, in isoflurane-anesthetized cats. Vet Anaesth Analg. 2019; 46: 753–764.

25. Kanda T, Hikasa Y. Neurohormonal and metabolic effects of medetomidine compared with xylazine in healthy cats. Can J Vet Res. 2008; 72: 278–286.

26. Kanda T, Hikasa Y. Effects of medetomidine and midazolam alone or in combination on the metabolic and neurohormonal responses in healthy cats. Can J Vet Res. 2008; 72: 332–339.

27. Khalili-Mahani N, Martini CH, Olofsen E, Dahan A, Niesters M. Effect of subanaesthetic ketamine on plasma and saliva cortisol secretion. Br J Anaesth. 2015; 115: 68–75.

28. Khenissi L, Nikolayenkova-Topie O, Broussaud S, Touzot-Jourde G. Comparison of intramuscular alfaxalone and ketamine combined with dexmedetomidine and butorphanol for castration in cats. J Feline Med Surg. 2017; 19: 791–797.

29. Ko JC, Mandsager RE, Lange DN, Fox SM. Cardiorespiratory responses and plasma cortisol concentrations in dogs treated with medetomidine before undergoing ovariohysterectomy. J Am Vet Med Assoc. 2000; 217: 509–514.

30. Lagos-Carvajal A, Queiroz-Williams P, da Cunha A, Liu CC. Determination of midazolam dose for co-induction with alfaxalone in sedated cats. Vet Anaesth Analg. 2019; 46: 299–307.

31. Lee JS, Morrow D, Andresen MC, Chang KS. Isoflurane depresses baroreflex control of heart rate in decerebrate rats. Anesthesiology. 2002; 96: 1214–1222.

32. Mahdmina A, Evans A, Yates D, White KL. Comparison of the effects of buprenorphine and methadone in combination with medetomidine followed by intramuscular alfaxalone for anaesthesia of cats undergoing ovariohysterectomy. J Feline Med Surg. 2020; 22: 77–83.

33. Martin-Flores M, Sakai DM, Honkavaara J, Campoy L. Hemodynamic effects of low-dose atipamezole in isoflurane-anesthetized cats receiving an infusion of dexmedetomidine. J Feline Med Surg. 2018; 20: 571–577.

34. Mathis A, Pinelas R, Brodbelt DC, Alibhai HI, Comparison of quality of recovery from anaesthesia in cats induced with propofol or alfaxalone. Vet Anaesth Analg. 2012; 39: 282–290.

35. Monteiro ER, Campagnol D, Parrilha LR, Furlan LZ. Evaluation of cardiorespiratory effects of combinations of dexmedetomidine and atropine in cats. J Feline Med Surg. 2009; 11: 783–792.

36. Murahata Y, Hikasa Y. Comparison of the diuretic effects of medetomidine hydrochloride and xylazine hydrochloride in healthy cats. Am J Vet Res. 2012; 73: 1871–1880.

37. Nystrom MR, Odunayo A, Okafor CC. Assessment of hydromorphone and dexmedetomidine for emesis induction in cats. J Vet Emerg Crit Care. 2019; 29: 360–365.

38. Pocock G, Richards CD The action of volatile anaesthetics on stimulus-secretion coupling in bovine adrenal chromaffin cells. Br J Pharmacol. 1988; 95: 209–217.

39. Polson S, Taylor PM, Yates D. Analgesia after feline ovariohysterectomy under midazolam-medetomidine-ketamine anaesthesia with buprenorphine or butorphanol, and carprofen or meloxicam: a prospective, randomised clinical trial. J Feline Med Surg. 2012; 14: 553–559.

40. Raekallio MR, Virtanen M, Happonen I, Vainio OM. Adverse reactions of α_2-adrenoceptor agonists in cats reported in 2003-2013 in Finland. Vet Anaesth Analg. 2017; 44: 803–810.

41. Sano Y, Ito S, Yoneda M, Nagasawa K, Matsuura N, Yamada Y, Uchinaka A, Bando YK, Murohara T, Nagata K. Effects of various types of anesthesia on hemodynamics, cardiac function, and glucose and lipid metabolism in rats. Am J Physiol Heart Circ Physiol. 2016; 311: 1360–1366.

42. Savola JM. Cardiovascular action of medetomidine and their reversal by atipamezole. Acta Vet Scand. 1989; 85: 38–47.

43. Shah M, Yates D, Hunt J, Murrell J. Comparison between methadone and buprenorphine within the QUAD protocol for perioperative analgesia in cats undergoing ovariohysterectomy. J Feline Med Surg. 2019; 21: 723–731.

44. Sinclair MD. A review of the physiological effects of α_2-agonists related to the clinical use of medetomidine in small animal practice. Can Vet J. 2003; 44: 885–897.

45. Tamura J, Oyama N, Fukui S, Yamashita K. Comparison of the anesthetic effects between 5 mg/kg of alfaxalone and 10 mg/kg of propofol administered intravenously in cats. J Vet Med Sci. 2021; 83; 73–77.

46. Tanaka T, Nabatame H, Tanifuji Y. Insulin secretion and glucose utilization are impaired under general anesthesia with sevoflurane as well as isoflurane in a concentration-independent manner. J Anesth. 2005; 19: 277–281.

47. Ueoka N, Hikasa Y. Effects in cats of atipamezole, flumazenil and 4-aminopyridine on stress-related neurohormonal and metabolic responses induced by medetomidine, midazolam and ketamine. J Feline Med Surg. 2015; 17: 711–718.

48. Ueoka N, Hikasa Y. Antagonistic effects of atipamezole, flumazenil and 4-aminopyridine against anaesthesia with medetomidine, midazolam and ketamine combination in cats. J Feline Med Surg. 2008; 10: 47–54.

49. Umamaheswaran S, Dasari SK, Yang P, Lutgendorf SK, Sood AK. Stress, inflammation, and eicosanoids: an emerging perspective. Cancer Metastasis Rev. 2018; 37: 203–211.

50. Vähä-Vahe T. Clinical effectiveness of atipamezole as a medetomidine antagonist in cats. J Small Anim Pract. 1990; 31: 193–197.

51. Väisänen M, Raekallio M, Kuusela E, Huttunen P, Leppäluoto J, Kirves P, Vainio O. Evaluation of the perioperative stress response in dogs administered medetomidine or acepromazine as part of the preanesthetic medication. Am J Vet Res. 2002; 63: 969–975.

52. Warne LN, Beths T, Whittem T, Carter JE, Bauquier SH. A review of the pharmacology and clinical application of alfaxalone in cats. Vet J. 2015; 203: 141–148.

53. Xu H, Aibiki M, Seki K, Ogura S, Ogli K. Effects of dexmedetomidine, an alpha2-adrenoceptor agonist, on renal sympathetic nerve activity, blood pressure, heart rate and central venous pressure in urethane-anesthetized rabbits. J Auton Nerv Syst. 1998; 71: 48–54.

54. Zatroch KK, Sakai DM, Parry S, Campoy L, Martin-Flores M. Evaluation of atipamezole as a treatment for dexmedetomidine-induced cardiovascular depression in anesthetized cats. Am J Vet Res. 2019; 80: 455–460.