Antagonistic effects of atipamezole, flumazenil and 4-aminopyridine on anesthesia and stress-related neurohormonal and metabolic changes induced by medetomidine, midazolam and ketamine in cats

（ネコにおけるメデトミジン、ミダゾラムおよびケタミン併用麻酔と
ストレス関連性神経内分泌と代謝変化に対するアチパメゾール、
フルマゼニルおよび4-アミノピリジンの拮抗効果）

The United Graduate School of Veterinary Science

Yamaguchi University

Naotami UEOKA

Mrach 2015

Table of Contents

General introduction

The selective α_2-adrenoceptor agonist, medetomidine (MED) is mainly used as a sedative, analgesic and muscle relaxant agent. However, it induces adverse cardiovascular effects such as hypertension and bradycardia in cats [1-3]. Midazolam (MID) is a water-soluble benzodiazepine that is used as an anxiolytic in human medicine [4, 5]. MID alone has not been used as a sedative agent for cats, because it induces ataxia, restlessness, and abnormal behaviours that make cats more difficult to approach and restrain, and does not induce profound sedation in cats [6]. A combination of MED with MID has been reported to enhance the sedative and analgesic actions of the individual drug in rats [7] and pigs [8], and to produce deep sedation in dogs [9]. This combination has also been reported to greatly reduce the anesthetic induction dose of sodium thiopental and propofol in dogs [10]. On the other hand, ketamine (KET) is widely used in feline practice as a dissociative anesthetic agent. KET induces anesthesia rapidly and causes minimal depression of the respiratory and cardiovascular systems, and has a wide margin of safety [11]. A wide range of KET doses are used for different purposes in feline practice. MED–MID combination may be also used as a premedication prior to KET anesthesia in cats. The combination of MID or MED with KET has been successfully used in cats [12-15]. However, to the best of our knowledge, there are no reports on MED–MID or MED–MID–KET combinations in cats. Our preliminary studies indicated that a combination of MED–MID–KET produced good anesthesia with excellent muscle relaxation and analgesia in cats.

Antagonism may be required when the anesthetized animals show profound depression of vital signs, adverse effects of the given agents, and/or delayed recovery from anesthesia. A selective α_2-adrenoceptor antagonist, atipamezole (ATI) is used as an antagonist for sedation or bradycardia induced by MED in cats [16-18]. Flumazenil (FLU), a potent and specific benzodiazepine antagonist, antagonizes behavioral and neurological effects of benzodiazepines such as muscle

relaxation and sedation [19, 20]. The effect of intravenous administration of variable-dose FLU following a fixed-dose of KET and MID has been studied in healthy cats [21]. 4-Aminopyridine (4AP) reverses non-depolarizing neuromuscular and sympathetic ganglion blockade mainly due to the enhanced release of acetylcholine in cats [22] and dogs [23], and partially antagonizes the anesthesia produced by KET or barbiturates in cats [24, 25]. In cats, however, there is little information available on the antagonistic effects of ATI, FLU and 4AP alone or in various combinations against the anesthesia induced by a combination of MED, MID and KET.

Stress consists of the biological responses of an animal in an attempt to cope with a disruption or threat to homeostasis. Stressors such as anxiety, excitement, pain, anesthesia, and other factors are well known to induce neurohormonal and metabolic changes in animals that are characterized by increases in blood levels of cortisol, catecholamines, glucose, and nonesterified fatty acids (NEFA) and a decrease in blood insulin levels. The α_2-adrenoceptor-mediated actions are closely coordinated with these events. Med has been reported to suppress catecholamine release, insulin release and lipolysis, and induce hyperglycemia, which are reversed by ATI, in dogs and cats [26-28]. Also, KET has been reported to increase plasma catecholamine and cortisol concentrations in dogs [29]. However, there are no data available on the neurohormonal and metabolic effects of MED–MID–KET anesthesia in cats. In addition, it is important to examine stress-related neurohormonal and metabolic responses of ATI, FLU, 4AP, and various combinations for appropriate use of antagonistic agents against MED–MID–KET anesthesia in cats.

In chapter 1, the antagonistic effects of ATI, FLU and 4AP alone and their combinations after anesthesia produced by a fixed dose of MED, MID and KET injected intramuscularly were evaluated in cats.

In chapter 2, the antagonistic effects of ATI, FLU and 4AP, both alone and in various combinations on key stress-related neurohormonal and metabolic changes after anesthesia with MED–MID–KET were investigated in healthy cats.

Chapter 1

Antagonistic effects of atipamezole, flumazenil and 4-aminopyridine against anesthesia with medetomidine, midazolam and ketamine combination in cats

Introduction

The selective α_2-adrenoceptor agonist, medetomidine (MED) is mainly used as a sedative, analgesic and muscle relaxant agent. However, it induces adverse cardiovascular effects such as hypertension and bradycardia in cats [1-3]. Midazolam (MID) is a water-soluble benzodiazepine that is used as an anxiolytic in human medicine [4,5]. MID alone has not been used as a sedative agent for cats, because it induces ataxia, restlessness, and abnormal behaviours that make cats more difficult to approach and restrain, and does not induce profound sedation in cats [6]. A combination of MED with MID has been reported to enhance the sedative and analgesic actions of the individual drug in rats [7] and pigs [8], and to produce deep sedation in dogs [9]. This combination has also been reported to greatly reduce the anaesthetic induction dose of sodium thiopental and propofol in dogs [10]. On the other hand, ketamine (KET) is widely used in feline practice as a dissociative anaesthetic agent. KET induces anesthesia rapidly and causes minimal depression of the respiratory and cardiovascular systems, and has a wide margin of safety [11]. A wide range of KET doses are used for different purposes in feline practice. MED–MID combination may be also used as a premedication prior to KET anesthesia in cats. The combination of MID or MED with KET has been successfully used in cats [12-15]. However, to the best of our knowledge, there are no reports on MED–MID or MED–MID–KET combinations in cats. Our preliminary studies indicated that a combination of MED–MID–KET produced good anesthesia with excellent muscle relaxation and analgesia in cats.

Antagonism may be required when the anaesthetized animals show profound depression of vital signs, adverse effects of the given agents, and/or delayed recovery from anesthesia. A selective α_2-adrenoceptor antagonist, atipamezole (ATI) is used as an antagonist for sedation or bradycardia induced by MED in cats [16-18]. Flumazenil (FLU), a potent and specific benzodiazepine antagonist, antagonizes behavioral and neurological effects of benzodiazepines

such as muscle relaxation and sedation [19,20]. The effect of intravenous administration of variable-dose FLU following a fixed-dose of KET and MID has been studied in healthy cats [21]. 4-Aminopyridine (4AP) reverses nondepolarizing neuromuscular and sympathetic ganglion blockade mainly due to the enhanced release of acetylcholine in cats [22] and dogs [23], and partially antagonizes the anesthesia produced by KET or barbiturates in cats [24, 25]. In cats, however, there is little information available on the antagonistic effects of ATI, FLU and 4AP alone or in various combinations against the anesthesia induced by a combination of MED, MID and KET.

The purpose of this study was to evaluate the antagonistic effects of intravenously administered ATI, FLU and 4AP alone and their combinations after anesthesia produced by a fixed dose of MED, MID and KET injected intramuscularly in cats.

Materials and methods

Animals and designs

Our experimental protocols were approved by the Animal Research Committee of Tottori University. Eight healthy intact, adult mixed-breed cats (5 females and 3 males) ranging in body weight from 3.0 to 3.9 kg were used. The cats were housed individually and fed dry food and water *ad libitum*. Routine hematological and plasma biochemical tests were performed before the study commenced. All values were within the normal physiological range. Food was withheld for 12 h before the experiment. One h before the experiment, the animals were placed in the experimental room controlled at 25 °C by air conditioning. Eight cats received eight different treatments at the rate of one treatment per week in a randomized crossover study design.

Each cat was given the mixture of 0.05 mg/kg MED (medetomidine HCl, 1 mg/ml, Domitor, Meiji Seika Kaisha Ltd., Tokyo, Japan) and 0.5 mg/kg MID (midazolam, 5 mg/ml, Dormicum, Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan) followed 10 min later by 10 mg/kg KET (ketamine HCl, 50 mg/ml, Ketalar, Sankyo Co., Ltd., Tokyo, Japan) intramuscularly. MED and MID were mixed in the same syringe immediately before injection. MED–MID and KET were injected into the semimembranousus muscle. The MED–MID administration caused rapid sedation and no painful response to the injection; lateral recumbency with excellent muscle relaxation was achieved within 10 min in all of cats. KET induced anesthesia smoothly within 5 to 10 min, without signs of pain in response to intramuscular injection or a hypertonic or cataleptic state. In this study, general anesthesia was defined as without behavioral responses to analgesic and other stimuli under complete lateral recumbency without movements described below.

Twenty min after KET injection, the cats were given either physiological saline solution (PSS) at a dose of 0.1 ml/kg (control), 0.2 mg/kg ATI (atipamezole HCl, 5 mg/ml, Antisedan, Meiji

Seika Kaisha Ltd., Tokyo, Japan), 0.1 mg/kg FLU (flumazenil, 0.1 mg/ml, Anexate, Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan), 0.5 mg/kg 4AP (4-aminopyridine, Wako Pure Chemical Industries, Ltd., Tokyo, Japan), ATI–FLU, FLU–4AP, ATI–4AP or ATI–FLU–4AP intravenously. 4AP was dissolved in physiological saline solution at a concentration of 2.5 mg/ml. The potential antagonists were mixed in the same syringe immediately before injection, and injected into the cephalic vein.

Measurements

Elapsed times to recovery of the palpebral reflex, pedal reflex and tail clamp reflex were recorded after the injection of antagonists. Pedal and tail clamp reflexes were defined as the reflex withdrawal to clamping of interdigital web of the paw of a limb and the tail during three seconds using Kocher's forceps. Recovery times to head movement, sternal recumbency, standing and walking were also recorded. The degree of antagonism for anesthesia was assessed using a slight modification of previously published scoring methods [26] as follows. (1) Posture score: 0 = Normal; 1 = ataxia, but able to walk; 2 = completely prone, unable to walk; 3 = lateral recumbency, but able to move the tail or paw; and 4 = complete lateral recumbency without movement. (2) Analgesic scores: a reflex withdrawal to clamping of the tail, the skin of body surface at the paramedian abdomen and the interdigital web of paw of all four limbs during three seconds using Kocher's forceps. 0 = Normal response; 1 = reduced response; 2 = faint response; and 3 = no reflex. (3) Jaw tone score. 0 = Normal resistance to open the mouth; 1 = the jaw can be opened, but there is still some resistance; 2 = little resistance to open the mouth and obvious muscle relaxation; and 3 = no resistance. (4) Auricular score, in response to a clapping sound behind the auricula. 0 = Normal response; 1 = dull response, but able to move body or head; 2 = no body movement; and 3 = completely no reflex at all. Total score was calculated as the sum of

four scores, including (1) posture, (2) analgesia, (3) jaw tone, and (4) auricular scores. Rectal temperature was measured prior to injection of MED–MID (pre-value), immediately before injection of potential antagonists (0 time), and 30, 60, 90, 120, 150, 180, 240, and 300 min after injection of antagonists. Heart rate, respiration rate and each of the above four scores were recorded prior to injection of MED–MID (pre-value), immediately before administration of antagonists (0 time), and 1, 5, 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, and 300 min after injection of antagonists. At each time-point a cat was placed on an observation table for scoring. Posture, analgesia, jaw tone, and auricular scores were recorded in that order. Heart rate was measured using a stethoscope. Respiration rate was measured by observations of movements of the thorax. Cats were observed for behavioral and visible side effects such as excitation, congestion of conjunctiva, rigidity of limbs, muscle tremors, piloerection and emesis for 300 min after injection of potential antagonists.

Statistical analysis

Data of rectal temperature, heart and respiration rates: one-way analysis of variance (ANOVA) for repeated measures was used to examine effect of time within each treatment group and one-way ANOVA for treatment effect at each time-point. When ANOVA was significant, the Tukey test was used for multiple comparisons of the means. Data of elapsed times to recovery from anesthesia were also analyzed by one-way ANOVA for treatment effect, and Tukey's multiple comparison test was used to identify differences between means. For comparisons between treatment groups in the score data, the nonparametric, Mann-Whitney test was used. The significance level of all tests was set at $P<0.05$.

Results

Recovery time from anesthesia

Mean elapsed times to recovering the eyelid, pedal and tail clamp reflex after injection of potential antagonists were significantly shortened in the antagonists-injected groups when compared with the PSS-injected control (Table 1). Recovery times to head-up motion, prone position, standing and walking, were not significantly different among the control, FLU and 4AP groups. These times in the groups given ATI were significantly shortened in comparison with the control or non-ATI groups. Mean elapsed times to head-up motion or prone position in ATI–FLU, ATI–4AP and ATI–FLU–4AP groups were significantly shortened compared to those in the ATI group. Mean elapsed times to either head-up motion or prone position in ATI–FLU–4AP group were significantly shorter than those in the ATI–FLU and ATI–4AP groups. Recovery to prone position was most rapid in the ATI–FLU–4AP group. However, there were no significant differences in recovery times to standing and walking among the ATI–FLU, ATI–4AP and ATI–FLU–4AP groups (Table 2).

Anesthetic and analgesic scores

In the PSS-injected control group, a profound anesthesia was observed for approximately 90 min after MED–MID–KET injection. Thereafter, the cats recovered gradually, but ataxia continued until 300 min after PSS administration. In postural score, there were no significant differences between the control and non-ATI injected groups. Cats that received ATI alone had significantly lower postural scores at 45 to 240 min after injection when compared with PSS or non-ATI injected groups. Similarly, the cats receiving ATI–FLU, ATI–4AP and ATI–FLU–4AP had significantly lower postural scores at 1 to 240 min after injection of antagonists when

compared with the control or non-ATI injected groups. There were no significant differences in postural score between the groups combined with ATI.

The differences among the groups in analgesic, jaw tone, and auricular scores were similar to those in postural score. In each component score, there was no significant difference between the control and FLU group. Analgesic and jaw tone scores in the 4AP group were significantly lower than those in the control group at 60 to 90 min after injection of 4AP. The cats in the FLU–4AP group had significantly lower analgesic, jaw tone, and auricular scores at 30 to 150 min when compared with controls. Each component score in the groups combined with ATI was significantly lower than that in the control cats at 1 to 180 min. The cats in ATI–FLU–4AP group had the lowest component score after injection of antagonists (Figure 1).

The results for total score are shown in Figure 2. In total score, there was no significant difference between the control and FLU groups. Total score in both 4AP and FLU–4AP groups was slightly and significantly lower than that in the control. The cats in the ATI group had a significantly lower total score at 15 to 240 min when compared with PSS or non-ATI injected groups. The cats in both ATI–FLU and ATI–4AP groups had significantly lower total scores at 1 to 180 min when compared with PSS or non-ATI injected groups. The cats in the ATI–FLU–4AP group had the lowest total score at 1 to 60 min after antagonist injection when compared with the other groups. There were no significant differences in total score between the groups combined with ATI from 75 to 300 min after injection of potential antagonists.

Rectal temperature, heart and respiratory rates

The results of rectal temperature, heart and respiration rates are shown in Figure 3. Rectal temperature decreased significantly or tended to decrease from pre-values until 240 min in the

control and non-ATI injected groups. Rectal temperature in ATI, ATI–FLU and ATI–4AP groups tended to decrease until 60 min, but recovered to pre-values at 180 min after administration of antagonists. Recovery time from the decreased rectal temperature was fastest in the ATI–FLU–4AP group than the other groups.

Heart rates in the control and non-ATI injected groups were significantly reduced from pre-values. Heart rates in the ATI combined groups were significantly higher than those in control and non-ATI injected groups at 1 to 300 min after injection of antagonists. However, the cats in the ATI–FLU–4AP group showed tachycardia over 180 beats/min in the mean value at 1 to 120 min after injection. For example, heart rates (beats/min; mean±SD) increased significantly from 142±15 of pre-value to 197±37 at 1 min, 218±28 at 5 min, 213±17 at 90 min, and 187±34 at 120 min after ATI–FLU–4AP injection.

Respiratory rates in the control, FLU and FLU–4AP groups decreased significantly or tended to decrease from pre-values until 300 min after injection. Respiratory rates in the ATI, ATI–FLU and ATI–4AP groups were significantly higher than those in the control and non-ATI injected groups at 1 to 150 min after injection of potential antagonists. There were no significant differences in respiratory rates among the ATI, ATI–FLU and ATI–4AP groups. Respiration rates in the ATI–FLU–4AP group tended to be higher than those in the other groups at 5 to 150 min after antagonist injection, and showed tachypnea at 90 min after injection. (Figure 3)

Behavioral side effects

Excitement, vocalizing and aversion to body touch were observed in some of cats received ATI, 4AP, ATI–FLU or ATI–4AP, and in most of the cats that received ATI–FLU–4AP. Congestion of the conjunctiva was observed in some of cats receiving ATI, ATI–4AP or

ATI–FLU–4AP. Rigidity of limbs was observed in some cats that received FLU and 4AP, and in most after ATI, ATI–FLU, ATI–4AP or ATI–FLU–4AP. Emesis was observed in the control and non-ATI injected groups during recovery. Muscle tremors were observed in many cats that received ATI–4AP and ATI–FLU–4AP (Table 3).

Table 1. Recovery times to eyelid, pedal and tail clamp reflexes after administrations of atipamezole (ATI), flumazenil (FLU), and 4-aminopyridine (4AP) alone or their combinations in cats anesthetized with medetomidine-midazolam-ketamine

	Elapsed time (min) to		
Groups	Eyelid reflex	Pedal reflex	Tail clamp reflex
Control	58±34	83±28	98±31
ATI	1±0 [a]	2±1 [a]	5±10 [a]
FLU	6±10 [a]	62±15 [a,b]	64±22 [a,b]
4AP	12±17 [a]	32±24 [a,b,c]	51±29 [a,b]
ATI–FLU	1±0 [a]	1±0 [a,c,d]	1±0 [a,c,d]
FLU–4AP	3±5 [a]	31±24 [a,b,c,e]	49±22 [a,b,e]
ATI–4AP	1±0 [a]	1±0 [a,c,d,f]	2±1 [a,c,d,f]
ATI–FLU–4AP	1±0 [a]	1±0 [a,c,d,f]	1±0 [a,c,d,f]

Each value represents mean±S.D. of eight cats; a=significantly different from Control (P<0.05); b=significantly different from ATI (P<0.05); c=significantly different from FLU (P<0.05); d=significantly different from 4AP (P<0.05); e=significantly different from ATI–FLU (P<0.05); f=significantly different from FLU–4AP (P<0.05); g=significantly different from ATI–4AP (P<0.05).

Table 2. Recovery times to head-up motion, prone position, standing and walking after administrations of atipamezole (ATI), flumazenil (FLU), and 4-aminopyridine (4AP) alone or their combinations in cats anesthetized with medetomidine-midazolam-ketamine

Groups	Elapsed time (min) to		
	Head-up	Prone position	Standing and walking
Control	189±47	197±50	225±48
ATI	33±21 [a]	44±21 [a]	76.3±14 [a]
FLU	194±52 [b]	209±52 [b]	236±60 [b]
4AP	143±35 [b,c]	155±38 [b,c]	186±33 [b,c]
ATI–FLU	8±17 [a,b,c,d]	31±24 [a,c,d]	76±26 [a,c,d]
FLU–4AP	139±31 [a,b,c,e]	145±32 [a,b,e]	183±44 [b,e]
ATI–4AP	15±16 [a,c,d,f]	21±21 [a,b,c,d,f]	68±20 [a,c,d,f]
ATI–FLU–4AP	2±1 [a,b,c,d,f,g]	8±6 [a,b,c,d,e,f]	58±17 [a,b,c,d,f]

Each value represents mean±S.D. of eight cats; a=significantly different from Control (P<0.05); b=significantly different from ATI (P<0.05); c=significantly different from FLU (P<0.05); d=significantly different from 4AP (P<0.05); e=significantly different from ATI–FLU (P<0.05); f=significantly different from FLU–4AP (P<0.05); g=significantly different from ATI–4AP (P<0.05).

Table 3. Behavioral side effects after administrations of atipamezole (ATI), flumazenil (FLU), and 4-aminopyridine (4AP) alone or their combinations in cats anesthetized with medetomidine-midazolam-ketamine

Groups	Excitement symptoms (Initiation; duration)	Congestion of conjunctiva (Initiation; duration)	Rigidity of four limbs (Initiation; duration)	Emesis (Initiation)	Muscle tremors (Initiation; duration)
Control	0/8*	0/8	0/8	3/8 (148, 158, 189)	0/8
ATI	2/8 (68±11; 83±32) †	1/8 (90; 30)	4/8 (53±19; 41±26)	0/8	0/8
FLU	0/8	0/8	1/8 (75; 15)	3/8 (187, 212, 214)	0/8
4AP	1/8 (120; 30)	0/8	2/8 (135±21; 25±7)	2/8 (144, 202)	0/8
ATI–FLU	3/8 (110±17; 80±35)	0/8	4/8 (34±14; 40±41)	0/8	0/8
FLU–4AP	0/8	0/8	0/8	1/8 (140)	0/8
ATI–4AP	3/8 (60±26; 62±15)	2/8 (68±32; 23±11)	3/8 (37±28; 33±24)	0/8	4/8 (71±38; 49±43)
ATI–FLU–4AP	7/8 (74±36; 35±27)	3/8 (70±9; 25±17)	6/8 (7±4; 62±69)	0/8	5/8 (52±34; 27±20)

* positive / examined cats.

† Time (mean±SD, min) after injection of potential antagonists in positive cats.

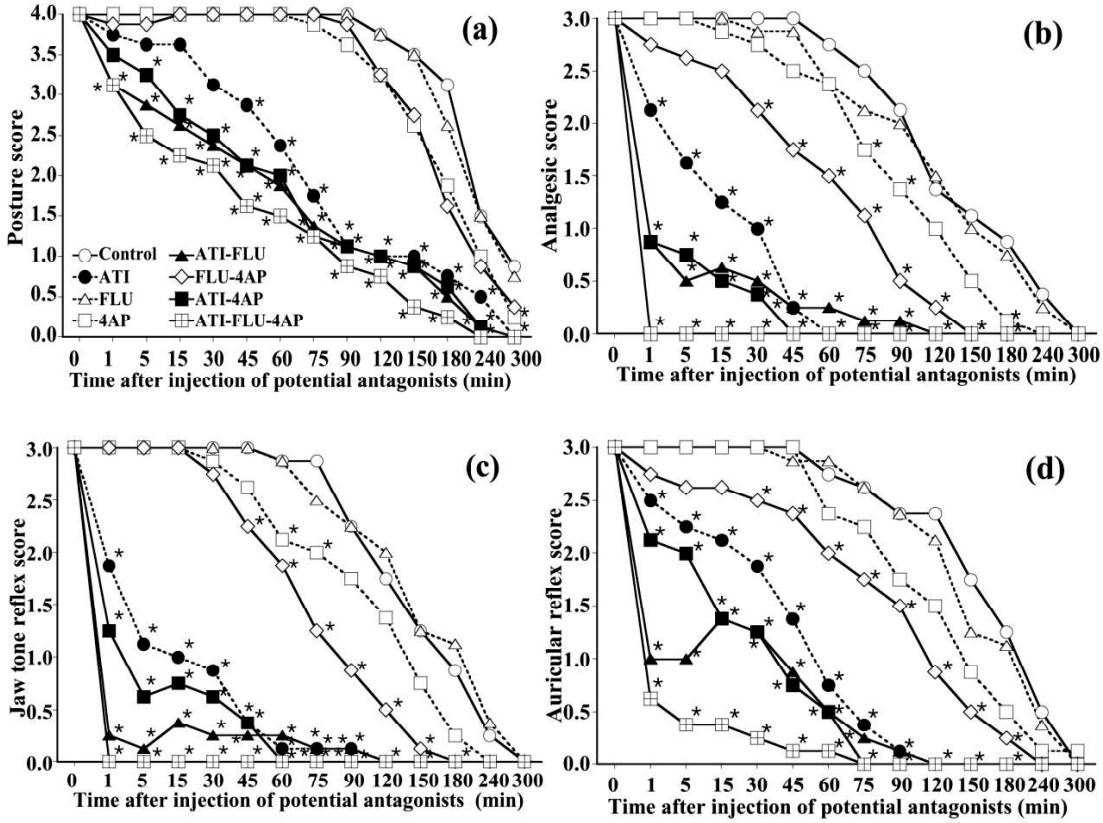


Figure 1. Antagonistic effects of atipamezole (ATI), flumazenil (FLU) and 4-aminopyridine (4AP), as assessed by scoring posture (a), analgesia (b), jaw tone reflex (c) and auricular reflex (d), in cats anesthetized with medetomidine-midazolam-ketamine. Each point indicates the mean value of eight cats. * Significantly different from the control group (P<0.05).

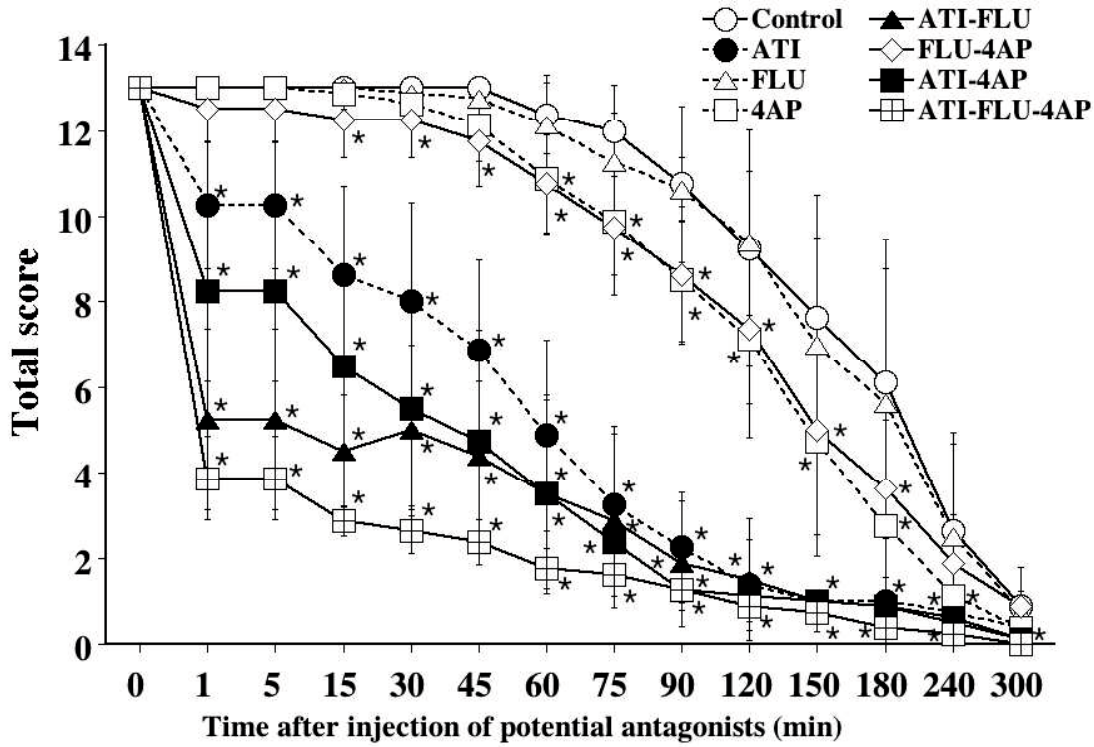


Figure 2. Antagonistic effect of atipamezole (ATI), flumazenil (FLU) and 4-aminopyridine (4AP), as assessed by total score calculated as the sum of four component scores, in cats anesthetized with medetomidine-midazolam-ketamine. Each point indicates the mean value of eight cats. * Significantly different from the control group (P<0.05).

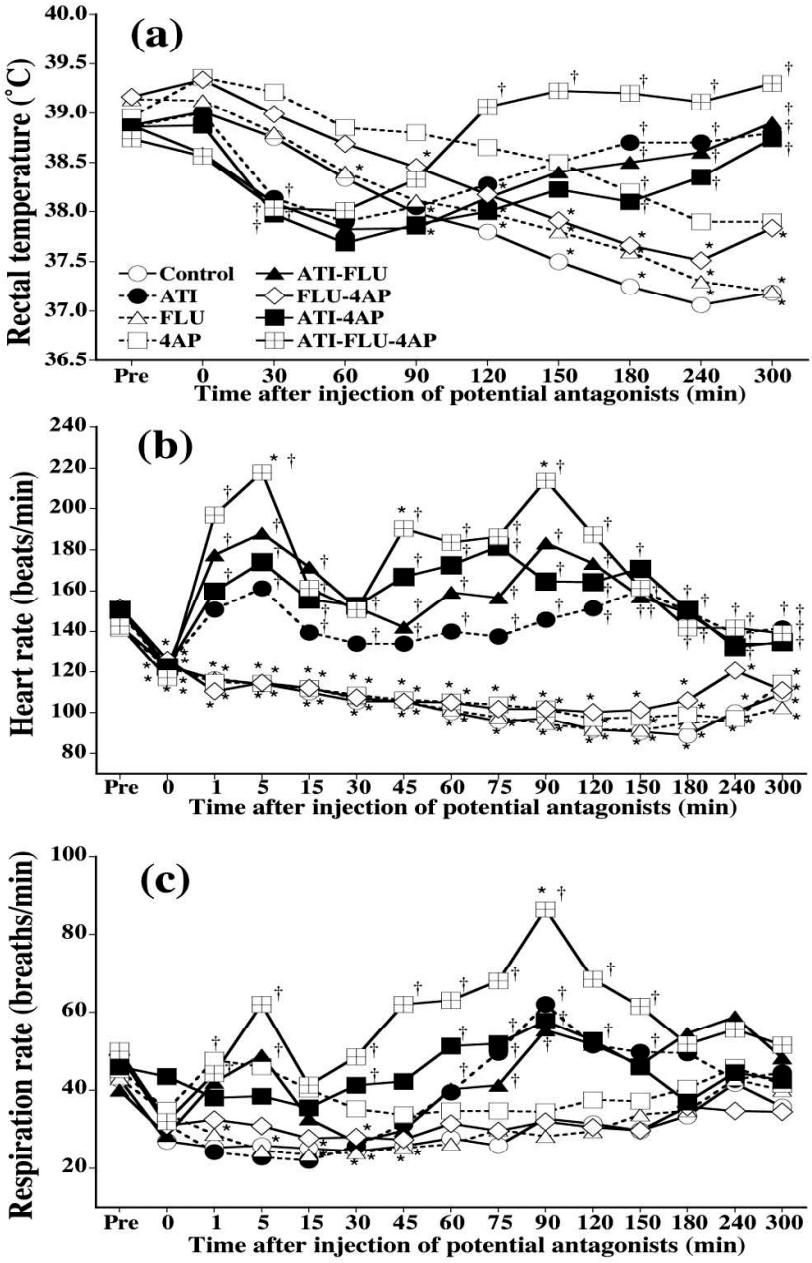


Figure 3. Changes in rectal temperature (a), heart rate (b) and respiration rate (c) after administration of atipamezole (ATI), flumazenil (FLU) and 4-aminopyridine (4AP) in cats anesthetized with medetomidine-midazolam-ketamine. Each point indicates the mean value of eight cats. * Significantly different from pre-value (P<0.05). † Significantly different from the control group (P<0.05).

Discussion

The present study showed that MED–MID–KET at the doses used produced good anesthesia with excellent muscle relaxation and analgesia in cats. The clinically recommended dose of MED as a sedative-analgesic in cats is reported to be 0.01 to 0.04 mg/kg intravenously and 0.04 to 0.08 mg/kg intramuscularly [26]. Also, it is well known that the wide ranges in KET doses (2 to 4 mg/kg intravenously and 10 to 30 mg/kg intramuscularly) are used for different purposes in feline practice [11]. A previous study reported that intravenous administration of 0.05 and 0.5 mg/kg MID after 3 mg/kg KET had beneficial effects on behavioural responses in cats [13]. It caused a greater proportion of cats to assume a laterally recumbent position with head down compared with KET alone. In addition, doses of MID of 0.5 mg/kg or above reduced muscle rigidity observed in cats which received KET alone, and greatly diminished a nociceptive response to the tail or paw clamp in cats [13]. Based on the previous findings described above, we determined intramuscular doses of 0.05 mg/kg MED, 0.5 mg/kg MID and 10 mg/kg KET for this study. Therefore, this fixed-dose of MED–MID–KET produced general anesthesia with excellent muscle relaxation and analgesia for approximately 90 min in cats.

In this study, the ATI dose of 0.2 mg/kg was selected for the reversal of 0.05 mg/kg MED, because the effective reversal dose of ATI in cats has been found to be two to four times that of MED [17, 18, 27]. A FLU dose of 0.1 mg/kg was determined based on a previous report that, assuming a agonist-antagonist ratio of 13:1, a FLU dose of 0.04 mg/kg or above would be enough for complete reversal of 0.5 mg/kg MID, and that an intravenous administration of 0.1 mg/kg FLU shortened the period of initial recovery stages following 0.5 mg/kg MID and 3 mg/kg KET in cats [21]. 4AP dose of 0.5 mg/kg was selected bases on reports it partially antagonized the effects of KET or pentobarbital anesthesia in cats [24, 25].

The present study demonstrated that either FLU or 4AP alone did not markedly antagonize MED–MID–KET anesthesia. On the other hand, ATI alone, ATI–FLU, ATI–4AP and ATI–FLU–4AP significantly hastened the recovery from anesthesia induced by MED–MID–KET and a combination of ATI–FLU–4AP was most effective. It is therefore concluded that ATI alone and combinations with ATI are much more useful for antagonizing MED–MID–KET anesthesia in cats. However, the present study indicated that the quality of recovery was smoother in ATI alone or ATI–FLU than after both ATI–4AP and ATI–FLU–4AP, because the rigidity of limbs, muscle tremors and excitement that were observed in most cats received ATI–FLU–4AP, and muscle tremors were observed in many cats after ATI–4AP during recovery process.

The present study revealed that combinations with ATI were effective in antagonizing the hypothermia, bradycardia and hypopnea induced by MED–MID–KET anesthesia. Reversal of bradycardia is mainly due to ATI [18]. On the other hand, Savola [16] reported that the anti-cholinergic drug, atropine was not effective in antagonizing MED-induced bradycardia in anesthetized rats. However, Short et al [28] and Ko et al [29] have reported in dogs that atropine and/or glycopyrrolate were more effective in preventing MED-induced bradycardia, but induced hypertension and pulsus alternans. In addition, Bergstrom [30] and Alibhai et al [31] have reported that an anti-cholinergic drug enhances hypertension produced by MED in dogs, although there are no published data showing such findings in cats. Therefore, if undesirable events occurred on cats when an anti-cholinergic drug was given as a premedicant to MED administration, the administration of ATI would be recommended for the reversal of these effects.

MED induces second-degree atrioventricular block and vomiting [28]. In our study, both cardiac arrhythmia assessed by auscultation and vomiting occurred more frequently in the control and non-ATI injected groups than the other groups. Therefore, combinations with ATI can prevent these side effects. In the present study, excitement was not observed in the FLU group, but

occurred frequently in the groups combined with ATI and most frequently in the ATI–FLU–4AP group. Ilkiw [6] reported that MID alone induced abnormal behaviours or excitement-like symptoms such as ataxia and restlessness in cats. In our study, MID might play a minor role in the observed excitement because these symptoms occurred frequently in the ATI–FLU and ATI–FLU–4AP groups in which the effects of MID were antagonized by FLU. Although a combination of ATI, FLU and 4AP was most effective in antagonizing the anesthesia induced by MED–MID–KET in our study, it induced limb rigidity and muscle tremors during the recovery phase. These results indicate that the use of a mixture of ATI, FLU and 4AP as antagonists for MED–MID–KET anesthesia is not always suitable for a smooth recovery.

In the present study, ATI, ATI–FLU, ATI–4AP and ATI–FLU–4AP combinations were effective in antagonizing the anesthesia and adverse effects induced by MED–MID–KET in cats. ATI alone effectively reversed the anesthesia, hypothermia, bradycardia and hypopnea produced by MED–MID–KET, with minimal adverse effects. However, the ATI–FLU combination may have some disadvantages because FLU is required at high dose in cats and is expensive. The ATI–4AP combination may be practical because 4AP is cheaper than FLU and this combination effectively hastens the recovery from anesthesia. However, the dose of 4AP should be carefully chosen because of its toxicity [12]. A combination of ATI–FLU–4AP is most effective in antagonizing the MED–MID–KET anesthesia, but tachypnea, excitement symptoms and muscle tremors occur frequently. Therefore, ATI alone can be used to shorten the effects of MED–MID–KET in cats. The combination of ATI, FLU and 4AP may be used after overdosage of MED–MID–KET.

In conclusion, ATI alone and combinations with ATI are much more effective for antagonizing the anesthesia and side effects induced by MED–MID–KET. ATI alone can be used

as a safe and effective agent for antagonizing the MED–MID–KET anesthesia in cats. The use of ATI–FLU–4AP may be not always produce smooth recovery from anesthesia.

Chapter 2

Effects in cats of atipamezole, flumazenil and 4-aminopyridine on stress-related neurohormonal and metabolic responses induced by medetomidine, midazolam and ketamine

Introduction

The α_2-adrenoceptor agonist medetomidine (MED) is mainly used for sedation, analgesia and muscle relaxation in veterinary medicine. However, it induces undesirable effects such as hyperglycemia, hypoinsulinemia, emesis, diuresis and bradyarrhythmias in dogs and cats [32-34]. Our previous study demonstrated that a combination of MED, the benzodiazepine agonist midazolam (MID) and the dissociative anesthetic agent ketamine (KET) produced good anesthesia in cats, with excellent muscle relaxation and analgesia [35]. Antagonism may be required when the anesthetized animals show a profound depression of vital signs, adverse effects and/or delayed recovery from anesthesia. Atipamezole (ATI), flumazenil (FLU) and 4-aminopyridine (4AP) completely or partially antagonise the effects of MED, MID and KET, respectively, in cats[16-18, 21,24]. These antagonists are beneficial in utilizing MED–MID–KET anesthesia in a clinic and are useful in the support of emergency and critical care associated with anesthesia. We previously evaluated the antagonistic effects of intravenously administered ATI, FLU and 4AP (alone and in various combinations) for anesthesia, produced by a fixed dose of MED–MID–KET injected intramuscularly in cats [35]. The combination of ATI–FLU–4AP was the most effective in antagonizing the anesthesia induced by MED–MID–KET; however, it was unsuitable for a smooth recovery from anesthesia because the triple antagonist regimen was associated with clinical manifestations such as tachycardia, tachypnea, excitement and muscle tremors [35]. Whether these symptoms constitute stressful events associated with the use of the ATI–FLU–4AP combination remains unclear. Stress-related hormonal responses associated with this combination may provide useful information.

Stress consists of the biological responses of an animal in an attempt to cope with a disruption or threat to homeostasis [36]. Stressors such as anxiety, excitement, pain, anesthesia and other factors are known to induce neurohormonal and metabolic changes in animals [37]. These changes

are characterised by increases in blood levels of cortisol, catecholamines, glucose and non-esterified fatty acids (NEFA) and a decrease in blood insulin levels [37]. Actions mediated by α_2-adrenoceptors are closely coordinated with these events. In dogs and cats, MED has been reported to suppress catecholamine release, insulin release and lipolysis, and it has been reported to induce hyperglycemia; these changes have been found to be reversed by ATI [32, 33, 38]. In addition, KET has been reported to increase plasma catecholamine and cortisol levels in dogs [39]. However, no data are available on the neurohormonal and metabolic effects of MED–MID–KET anesthesia in cats. In addition, it is important to examine the stress-related neurohormonal and metabolic responses of ATI, FLU and 4AP to ensure the appropriate use of antagonistic agents during the usage of MED–MID–KET anesthesia in cats. The present study aimed to investigate the effects of ATI, FLU and 4AP, both alone and in various combinations, on key stress-related neurohormonal and metabolic variables in healthy cats anesthetized with MED–MID–KET.

Materials and methods

Animals

Seven healthy mixed-breed cats (two intact males, two intact females, two neutered males and one neutered female), with an age [mean ± standard deviation (SD)] of 4.0 ± 1.8 years and a weight of 4.2 ± 0.7 kg were used. The cats were housed in the laboratory for at least 1 month before study initiation and were fed standard commercial dry food. Routine hematological examination before the study revealed that all values were within normal physiological ranges. The experimental protocol was approved by the Animal Research Committee of Tottori University, Tottori, Japan.

Experimental protocol

The seven cats were consistently used in each of the eight groups according to a randomised design. There were at least 3 weeks between treatments for each cat. Cats were intramuscularly administered the mixture of 0.05 mg/kg of MED (medetomidine hydrochloride, 1 mg/ml Domitor; Meiji Seika Kaisha, Tokyo, Japan) and 0.5 mg/kg of MID (midazolam hydrochloride, 5 mg/ml Dormicum; Astellas Pharma, Tokyo, Japan), which was followed by intramuscular administration of 10 mg/kg of KET (ketamine hydrochloride, 50 mg/ml Ketalar; Daiichisannkyo Kaisha, Tokyo, Japan) 10 min later. MED and MID were mixed in the same syringe immediately before injection. Twenty minutes after KET injection, the cats were administered an intravenous dose of either 0.1 ml/kg physiological saline solution (control), 0.2 mg/kg ATI (atipamezole hydrochloride, 5 mg/ml Antisedan; Meiji Seika Kaisha, Tokyo, Japan), 0.1 mg/kg FLU (0.1 mg/ml Anexate; Yamanouchi Pharmaceutical, Tokyo, Japan), 0.5 mg/kg 4AP (Wako Pure Chemical Industries, Tokyo, Japan) or all possible combinations (ATI–FLU, FLU–4AP, ATI–4AP or ATI–FLU–4AP). 4AP was dissolved in the saline solution at a concentration of 2.5 mg/ml. The potential antagonists were

mixed in the same syringe immediately before injection and injected into the jugular vein. The cats were fasted for 12 h before the injection of each agent. Food and water were offered again 1 h after the last blood sampling of the day. During the sampling period, the cats were kept in a room with the air temperature set at 25°C.

Instrumentation and sample collection

On the day before the experiment, a 17-G central venous catheter (EXCV catheter kit; Tyco Healthcare Japan, Tokyo, Japan) was introduced into the jugular vein under general anesthesia. Before catheter placement, 6.6–8.8 mg/kg of propofol (Rapinovet, Scering-Plough Animal Health, Osaka, Japan) was intravenously administered until adequate anesthesia was induced. Anesthesia was maintained with a constant infusion of 0.22–0.44 mg/kg/min of propofol. The catheter was flushed with 1.5 ml of heparinised physiological saline solution, capped and fixed. The cats were then placed in individual cages to rest overnight. Blood samples (2 ml per sampling) were collected from the catheter prior to the injection of MED–MID (pre-test value), immediately before injection of the antagonist agents ATI/FLU/4AP (time 0) as well as at 0.5, 1, 2, 3, 4, 5, 6 and 24 h after injection of the antagonists. After each sampling, the cats were returned to their cages. Packed cell volume and the other routine hematological variables were monitored throughout the sampling period. After each experiment, the catheter was removed and the cats were allowed to recover.

Sample processing and analysis

Blood was mixed with ethylenediaminetetraacetic acid to prevent clotting. Samples were immediately centrifuged at 4°C; the plasma was then separated and frozen at –80°C until analysis

for levels of glucose, insulin, cortisol, epinephrine, norepinephrine and NEFA. Glucose, NEFA, insulin, cortisol and catecholamine levels were measured according to previously published methods [33, 41]. Glucose and NEFA levels were determined by an enzyme assay using commercially available kits (Glucose CII-test and NEFA C-test Wako, respectively, Wako Pure Chemical, Osaka, Japan) and measured by means of a spectrophotometer. Insulin levels were measured by double-antibody radioimmunoassay with a kit (I-AJ16, Eiken Chemical Company, Tokyo, Japan). Cortisol was measured by single-antibody radioimmunoassay using a kit (Gamma Coat Cortisol, Nihon Sheering, Chiba, Japan). Catecholamines were extracted on activated alumina and measured using high-performance liquid chromatography and an electrochemical detector (Coulochem II, ESA, Chelmsford, Massacheusetts, USA). As an internal standard, 3,4-dihydroxybenzylamine was used. The percentage recovery of authentic 3,4-dihydroxybenzylamine was 55%–70%. Intra- and inter-assay coefficients of variation and limits of detection and quantitation for assay of each variable are shown in Table 4.

Behavioural scoring of recovery

The overall quality of recovery from anesthesia was assessed using a modification of previously published scoring methods [40] as follows: Score 1 (excellent) = smooth, quiet, comfortable, no stiffness, no shivering and no hypersensitivity to touch; Score 2 (good) = mild rigidity of either or both of rigidity of the thoracic and pelvic limbs, mild sensitivity to touch and mild shivering with symptoms persisting transiently; Score 3 (moderate) = marked rigidity of all four limbs, shivering and hypersensitivity to touch with signs persisting < 30 min; Score 4 (poor) = more marked demonstration of events mentioned before, with these persisting > 30 min and Score 5 (extremely poor) = marked rigidity of all four limbs, marked hypersensitivity to touch and

possible aggression with signs persisting > 60 min. The observer scoring the above mentioned behaviours was blinded to each treatment.

Statistical analysis

All data obtained were analysed using Prism statistical software (Version 4, GraphPad Software, San Diego, California, USA). One-way analysis of variance for repeated measurements was used to examine the time effect on glucose, insulin, cortisol, epinephrine, norepinephrine and NEFA concentrations within each group. When a significant difference was found, the Tukey test was used for multiple comparisons of the means. The area under the curve (AUC) for 0–6 h was calculated for each biochemical variable. AUC was measured by calculating the sum of the trapezoids formed by the data points and the x-axis. Additionally, AUC for 0–2 h was calculated for insulin value. The AUC data were tested for normality with Shapiro-Wilk test. When the data were normally distributed, the t-test with the Bonferroni correction was used for paired comparisons between the groups. When the AUC data were not normally distributed, the Wilcoxon signed-rank test with the Bonferroni correction was used to compare the difference between the groups. In both tests, the significant level was P value of < 0.00625. The score data were analysed using the Wilcoxon signed-rank test for paired treatment comparisons, and the P value of < 0.00625 was considered significant by the Bonferroni correction. For other tests, P values of < 0.05 were considered statistically significant.

Results

NEFA

Plasma NEFA concentrations decreased significantly at 0–2 h compared with pre-test values in the control and FLU–4AP groups, but did not significantly decrease in the groups administered

ATI (Figure 4A). The AUC data from 0 to 6 h in the NEFA value were significantly higher in the ATI–4AP and ATI–FLU–4AP groups than the control and FLU groups, and in the ATI–FLU, ATI–4AP and ATI–FLU–4AP groups than the FLU–4AP group (Figure 4B).

Glucose

Compared with pre-test values, plasma glucose concentrations increased significantly at 0.5–2 h in the control group, at 0 h in the ATI and ATI–FLU groups, at 0–0.5 h in the ATI–4AP and ATI–FLU–4AP groups and at 0–3 h in the FLU, 4AP and FLU–4AP groups (Figure 5A). The AUC values from 0 to 6 h were significantly higher in the FLU, 4AP and FLU–4AP groups than the ATI and ATI–FLU–4AP groups (Figure 5B).

Insulin

Plasma insulin concentrations decreased significantly at 0 and 1 h compared with the baseline in the FLU group (Figure 6A). There were no significant differences in AUC values from 0 to 6 h between any of the treatment groups (Figure 6B). However, the AUC values from 0 to 2 h were significantly higher in the ATI–FLU and ATI–4AP groups than the control group, in the ATI–FLU, ATI–4AP and ATI–FLU–4AP groups than the FLU group, and in the ATI–4AP group than the 4AP group (Figure 6C).

Cortisol

In the ATI–FLU–4AP group, the cortisol concentrations increased significantly at 3–5 h compared with the baseline (Figure 7A). Significant increases in cortisol concentrations were also

observed at 3 and 6 h in the ATI–FLU group. The AUC values from 0 to 6 h were significantly higher in the ATI–FLU–4AP group than the FLU and FLU–4AP groups (Figure 7B).

Epinephrine

The epinephrine concentrations decreased significantly at 0–5 h compared with the baseline in the control and FLU–4AP groups, and at 0.5–6 h in the FLU and 4AP groups (Figure 8A). The epinephrine concentrations in the groups administered ATI did not decrease significantly at 0.5–6 h compared with the baseline. The AUC values from 0 to 6 h were significantly higher in the ATI–FLU–4AP group than in the control and non-ATI groups (Figure 8B).

Norepinephrine

The norepinephrine concentrations decreased significantly at 0.5–4 h compared with the baseline in the control, at 0–5 h in the 4AP and FLU–4AP groups and at 0–6 h in the FLU group (Figure 9A). The norepinephrine concentrations in the groups administered ATI did not decrease significantly at 0–24 h compared with the baseline. On the other hand, two cats in the ATI–FLU–4AP group showed large increases in epinephrine concentrations at 2–4 h compared with the baseline. For example, the concentrations in one cat increased from 1.48 ng/ml to 12.57 ng/ml at 3 h, whereas those in the other cat increased from 0.75 ng/ml to 7.89 ng/ml at 3 h. The AUC value from 0 to 6 h was significantly higher in the ATI–FLU–4AP group than the non-ATI groups, and in the ATI–4AP group than the FLU group (Figure 9B).

Recovery score

Behavioural quality during the recovery process from anesthesia in each group was similar to previously described results [35]. Recovery score data were significantly higher in the ATI–FLU–4AP group than in the non-ATI groups (Table 5).

Table 4. Intra- and inter-assay coefficients of variation and limits of detection and quantification for assay of each variable used in this study

Variable	Method of assay	Coefficients of variation		Limits of detection		Limits of quantification
		Intra-assay	Inter-assay	Lower	Upper	
Glucose	Enzyme	0.5–2.0%	1.0–2.5%	3.8 mg/dL	700 mg/dL	15 mg/dL
NEFA	Enzyme	1.2–3.0%	1.9–3.2%	0 μEq/L	2000 μEq/L	16 μEq/L
Insulin	Radioimmunoassay	2.1–6.8%	2.4–5.6%	1 μU/mL	370 μU/mL	1.1 μU/mL
Cortisol	Radioimmunoassay	3.5–5.0%	4.2–8.7%	0.23 μg/dL	60 μg/dL	0.30 μg/dL
Epinephrine	HPLC-ECD	0.5–2.0%		20 pg/mL		20 pg/mL
Norepinephrine	HPLC-ECD	0.2–1.5%		10 pg/mL		11 pg/mL

NEFA = non-esterified fatty acid.

HPLC-ECD = high-performance liquid chromatography and electrochemical detection.

Table 5. Behavioural recovery score after administration of antagonists, either alone or in combination, in cats anaesthetized with MED–MID–KET

Group	Recovery score
Control	1 (1–2)
ATI	2 (1–3)
FLU	1 (1–2)
4AP	1 (1–2)
ATI–FLU	2 (1–3)
ATI–4AP	2 (1–5)
FLU–4AP	1 (1–2)
ATI–FLU–4AP	4 (2–5) [a, b, c, d]

Values represent the median and range in a parenthesis of seven cats; a, significantly different from control; b, significantly different from FLU; c, significantly different from 4AP; and d, significantly different from FLU–4AP. The significant level is $P < 0.00625$.

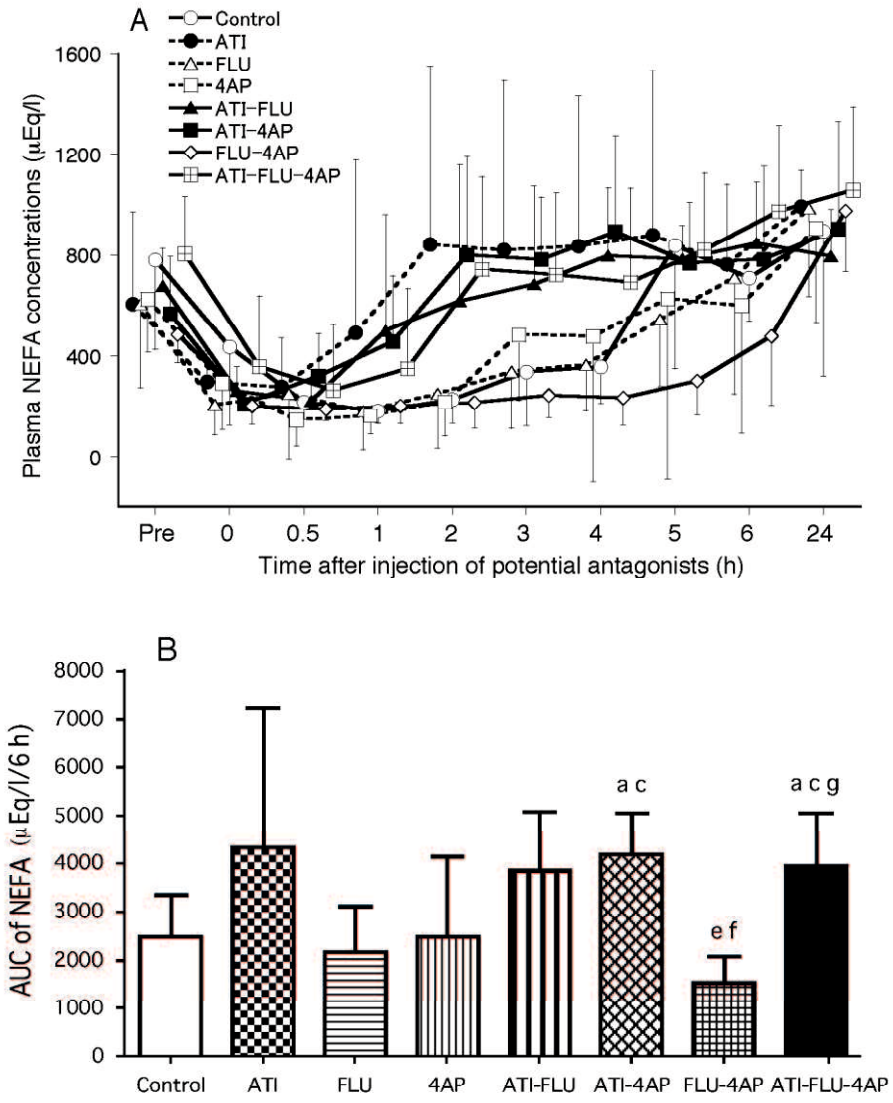


Figure 4 (A) Changes in plasma non-esterified fatty acid (NEFA) concentrations after administration of antagonists, either alone or in combination, in seven cats anesthetized with medetomidine, midazolam and ketamine (MED–MID–KET). Each point and vertical bars show the mean ± standard deviation (SD). (B) Area under the curve (AUC) data from 0 to 6 h for NEFA values after administration of antagonists. Each vertical bar indicates the mean and SD. **a**, significantly different from control; **b**, significantly different from atipamezole (ATI); **c**, significantly different from flumazenil (FLU); **d**, significantly different from 4-aminopyridine (4AP); **e**, significantly different from ATI–FLU; **f**, significantly different from ATI–4AP; **g**, significantly different from FLU–4AP. The significant level is $P < 0.00625$.

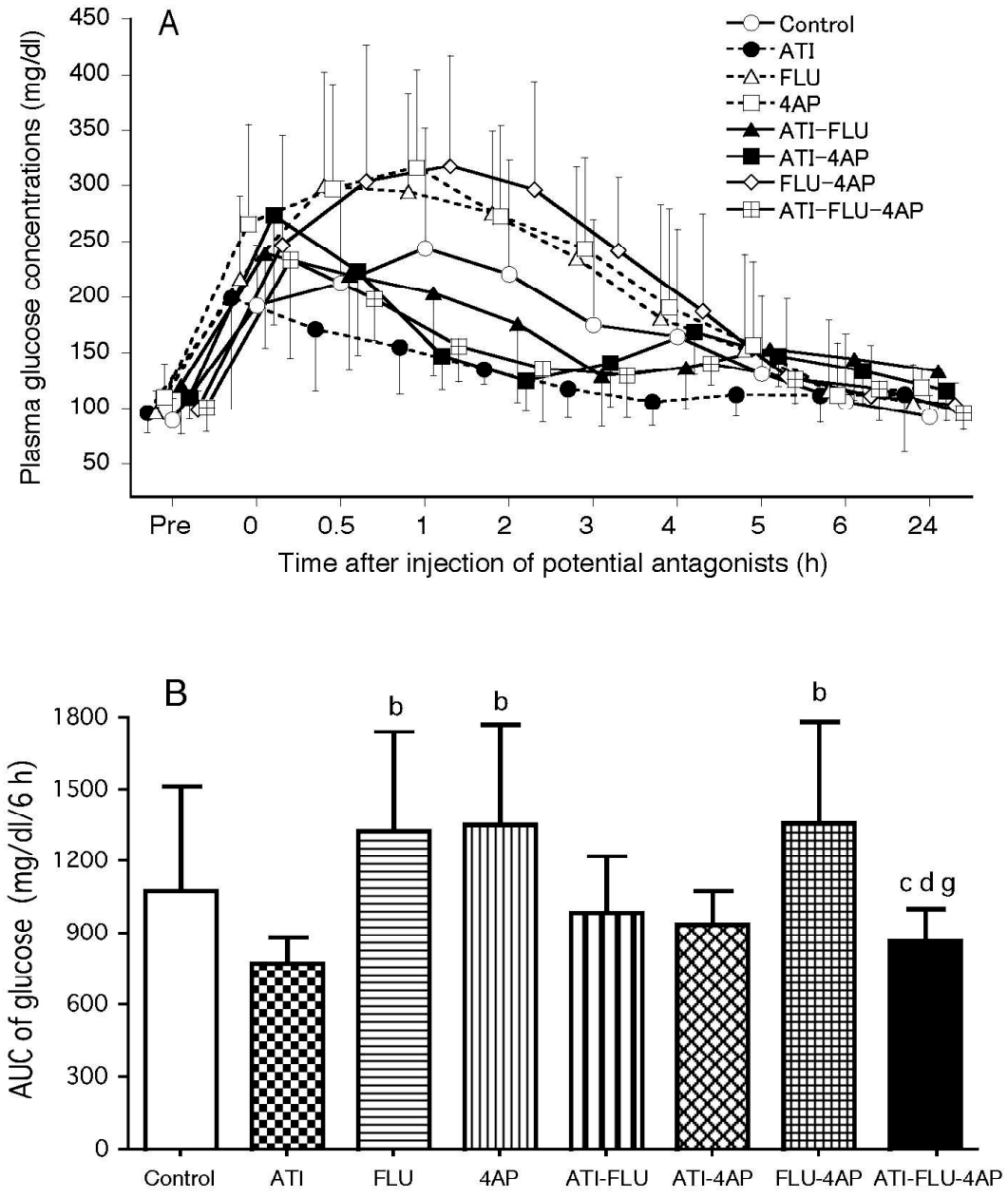
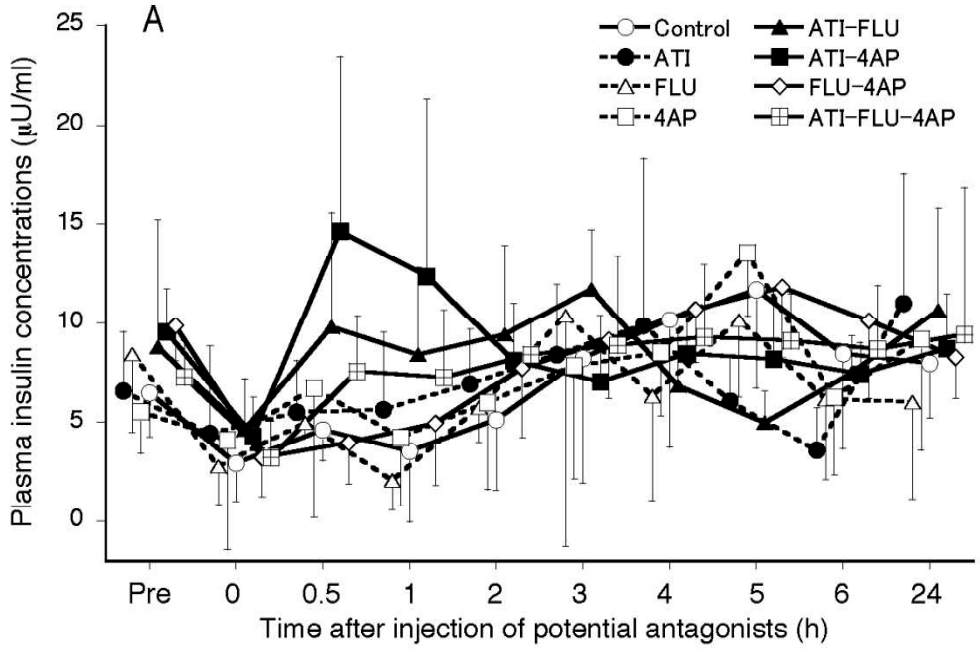
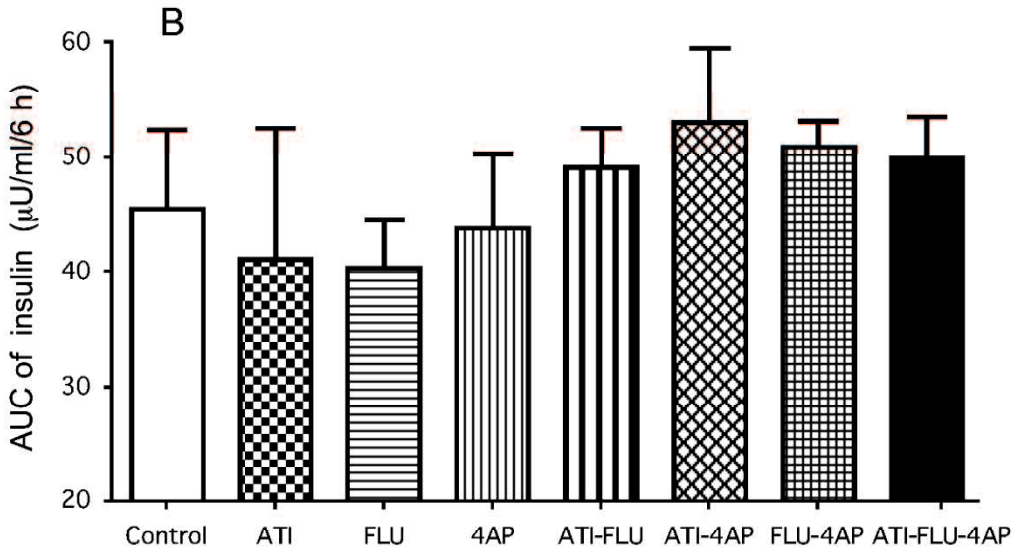


Figure 5 (A) Changes in plasma glucose concentrations after administration of antagonists, either alone or in combination, in seven cats anesthetized with MED–MID–KET. (B) AUC data from 0 to 6 h for glucose values after administration of antagonists. Plots, abbreviations and footnotes **a–g** are as described in Figure 4.


A
Control
ATI
FLU
4AP
ATI-FLU
ATI-4AP
FLU-4AP
ATI-FLU-4AP
Plasma insulin concentrations (μU/ml)
25
20
15
10
5
0
Pre
0
0.5
1
2
3
4
5
6
24
Time after injection of potential antagonists (h)


B
AUC of insulin (μU/ml/6 h)
60
50
40
30
20
Control
ATI
FLU
4AP
ATI-FLU
ATI-4AP
FLU-4AP
ATI-FLU-4AP

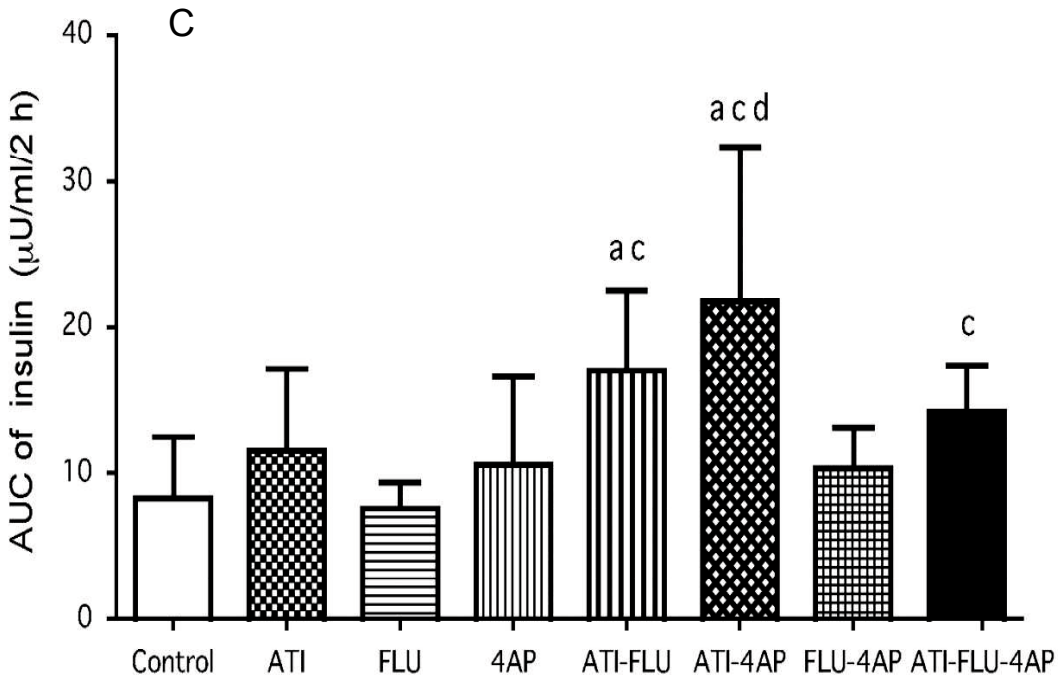


Figure 6 (A) Changes in plasma insulin concentrations after administration of antagonists, either alone or in combination, in seven cats anesthetized with MED–MID–KET. (B) AUC data from 0 to 6 h for insulin values after administration of antagonists. (C) AUC data from 0 to 2 h for insulin values after administration of antagonists. Plots, abbreviations and footnotes **a–g** are as described in Figure 4.

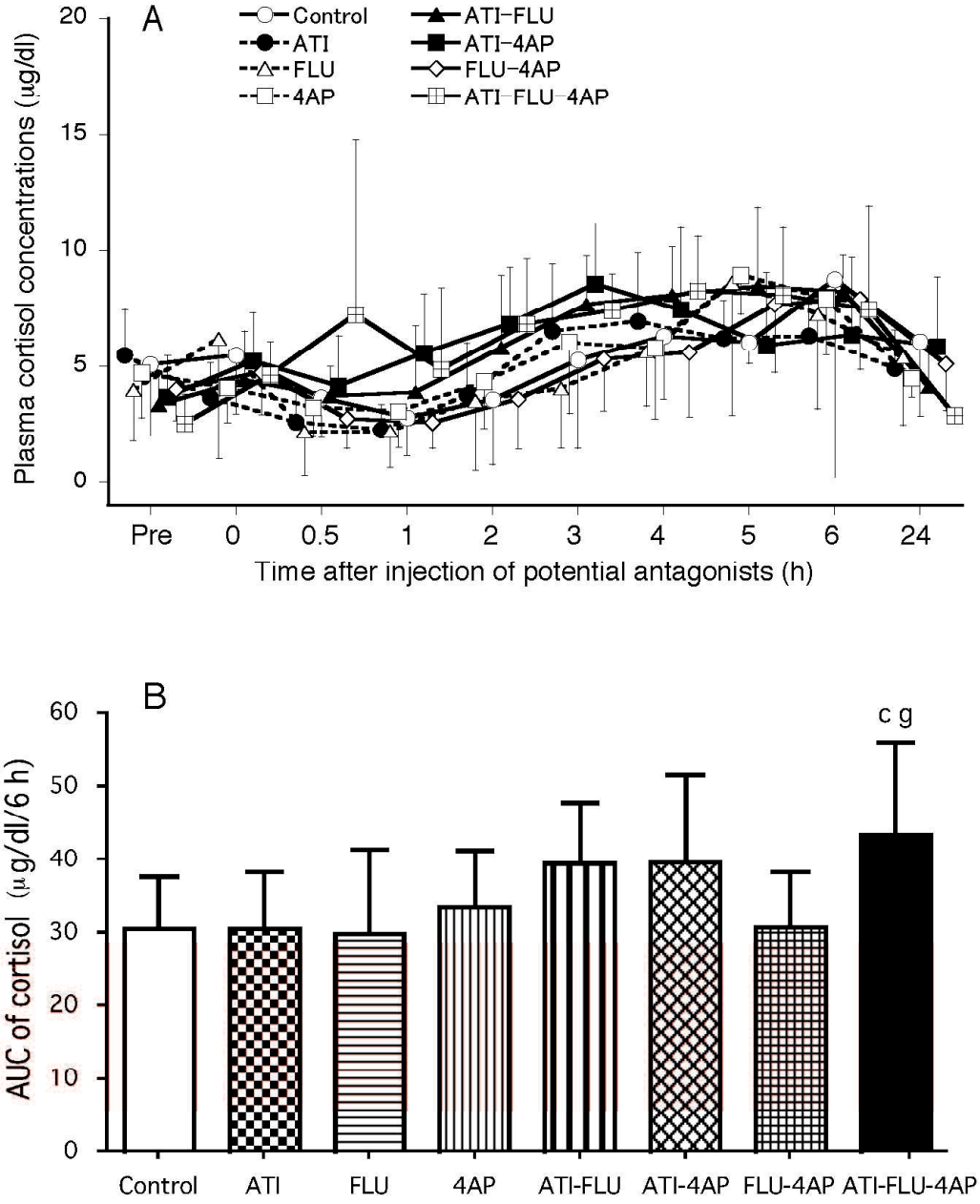


Figure 7 (A) Changes in plasma cortisol concentrations after administration of antagonists, either alone or in combination, in seven cats anesthetized with MED–MID–KET. (B) AUC data from 0 to 6 h for cortisol values after administration of antagonists. Plots, abbreviations and footnotes **a–g** are as described in Figure 4.

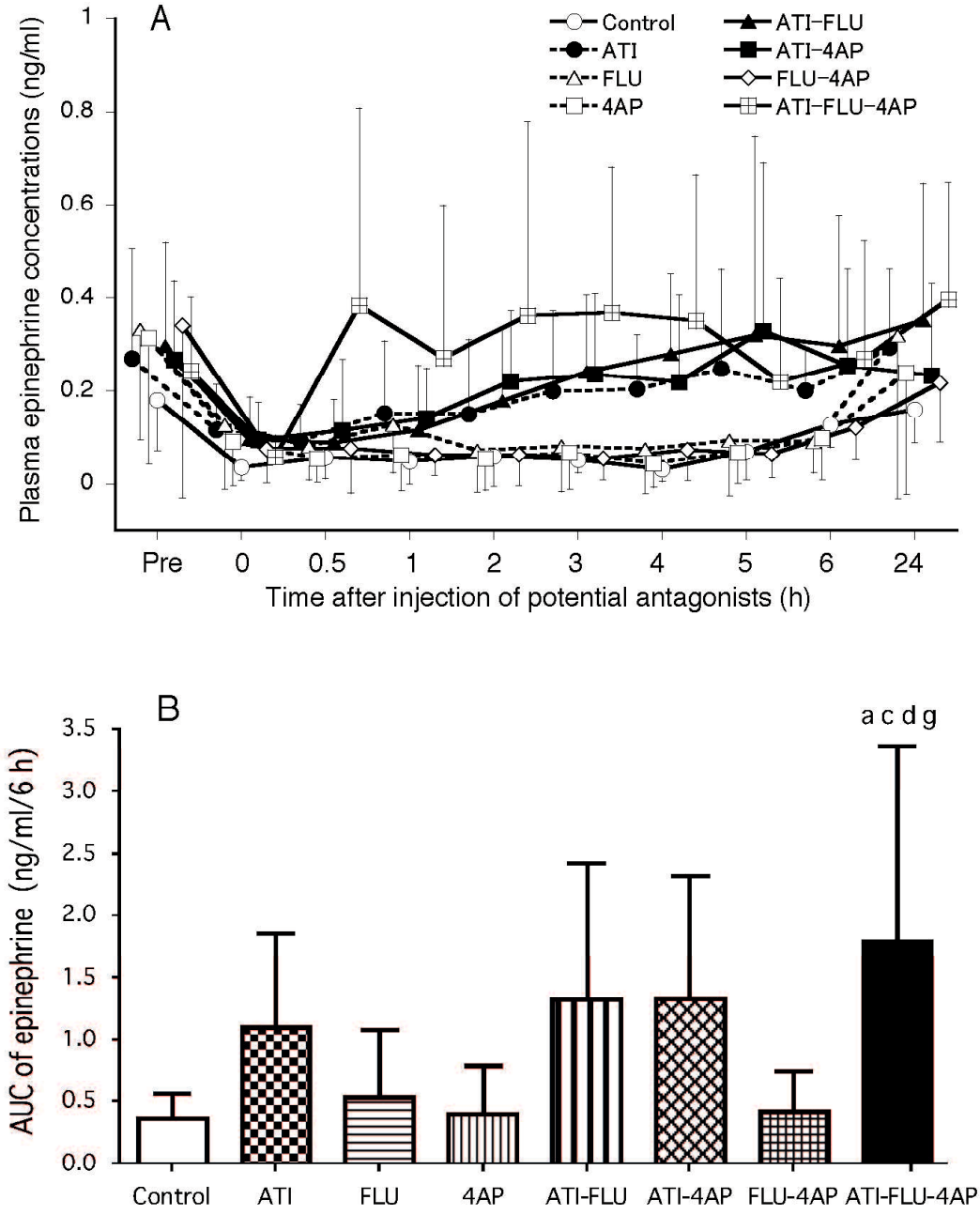


Figure 8 (A) Changes in plasma epinephrine concentrations after administration of antagonists, either alone or in combination, in seven cats anesthetized with MED–MID–KET. (B) AUC data from 0 to 6 h for epinephrine values after administration of antagonists. Plots, abbreviations and footnotes **a–g** are as described in Figure 4.

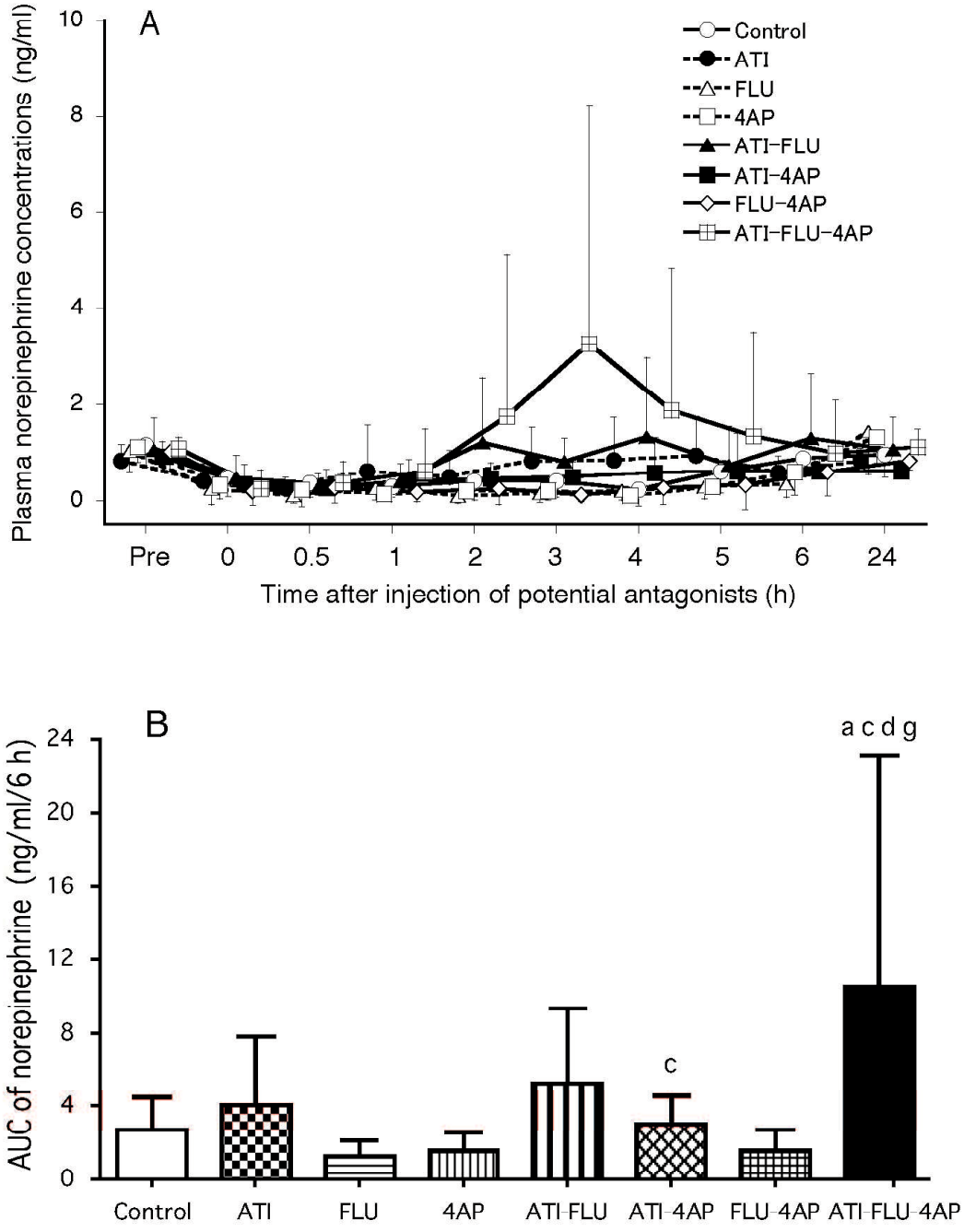


Figure 9 (A) Changes in plasma norepinephrine concentrations after administration of antagonists, either alone or in combination, in seven cats anesthetized with MED–MID–KET. (B) AUC data from 0 to 6 h for norepinephrine values after administration of antagonists. Plots, abbreviations and footnotes **a–g** are as described in Figure 4.

Discussion

The rationale for fixed-dosing of ATI, FLU and 4AP as antagonists for MED–MID–KET anesthesia has been outlined in our earlier study [35]. In the present study, for supportive management during emergencies immediately after the balanced anesthesia, we selected intravenous administration of antagonists because of the immediate effect (within 20 min after administration). Alternatively, intramuscular delivery, in comparison with intravenous administration of antagonists, may induce a lower stress response; conducting an intramuscular trial on the stress response in the future may be informative. A total sampling of 20 ml of blood over 24 h, as conducted in this study, may potentially affect the stress response. However, our previous study found that blood sampling using similar procedures did not significantly alter plasma concentrations of glucose, insulin, glucagon, cortisol, NEFAs, norepinephrine and epinephrine in non-medicated normal cats [41]. Therefore, it is conceivable that 2-ml blood sampling repeated 10 times at a 1-h interval over 24 h does not induce apparent effects on stress responses in healthy cats. In this study, the priority for determining the AUC time base of NEFA, glucose and catecholamines was based on the recovery time for changes that were recorded in the control group. The time period for the cortisol and insulin AUCs was determined to be the same as that for catecholamines and other metabolites as indicators of the stress hormonal response. The measurement at 24 h post-treatment was performed to reconfirm the return to pre-medication values.

The results of the present study revealed that MED–MID–KET anesthesia produced a moderate hyperglycaemia, primarily because of the effect of MED, which has previously been reported to induce a dose-dependent and marked hyperglycaemia in cats [33]. This hyperglycemic effect may limit the use of MED–MID–KET anesthesia in cats with metabolic and neurohormonal problems such as diabetes mellitus, ketosis and glycosuria. The present results demonstrated that ATI alone or in combination with other drugs reversed hyperglycemia induced by

MED–MID–KET. By contrast, FLU, 4AP and FLU–4AP did not decrease hyperglycemia; rather, surprisingly, FLU, 4AP and FLU–4AP enhanced MED–MID–KET–induced hyperglycemia. Although the precise mechanism is unknown, it has been reported that feline hyperglycemia induced by 40 μg/kg MED can be decreased by concomitant administration of 0.5 mg/kg MID [41]. The augmentation effect of FLU treatment on MED–MID–KET–induced hyperglycemia may be caused by the antagonism of the effect of MID suppressing the hyperglycemic action of MED. It was also found that 4AP treatment enhanced the hyperglycemic effect induced by MED–MID–KET. Although the precise mechanism of this effect is also unknown, treatments with FLU and/or 4AP are not clinically recommended for decreasing the hyperglycemia associated with MED–MID–KET anesthesia in cats. Therefore, ATI is potentially useful for treating hyperglycemic problems after MED–MID–KET anesthesia in cats.

In the present study, the plasma insulin concentration at 1 h following FLU treatment decreased significantly, suggesting that FLU at least partially influenced MED–MID–KET–induced hyperglycemia. Plasma insulin concentrations tended to be transiently decreased after MED–MID–KET anesthesia and reversed by ATI. The AUC from 0 to 2 h of plasma insulin concentration was higher in the groups combined with ATI than the non-ATI regimen groups. These results suggest that the reversal effects observed in MED–MID–KET-induced hyperglycemia were mainly due to the antagonistic effect of ATI for MED-induced inhibition of insulin secretion via α_2-adrenoceptors in the β cells of the pancreas. However, AUC from 0 to 6 h for plasma insulin concentration did not significantly differ between any of the treatments, indicating that ATI, FLU and 4AP, both alone and in various combinations do not have a long term influence on the plasma insulin concentration after MED–MID–KET anesthesia in cats.

The changes in NEFA concentrations are clinically significant because they are affected by hormones such as cortisol and catecholamines that are associated with the stress response [37]. In

the present study, plasma NEFA concentrations decreased after MED–MID–KET administration. This change was reversed by treatments with ATI, ATI–FLU and ATI–FLU–4AP. It has been reported that plasma NEFA concentrations are decreased by MED but that the addition of MID fails to have an effect on this in cats [33, 41]. However, ketamine (10 mg/kg) alone fails to alter NEFA concentrations in dogs [39]. Therefore, treatments including ATI are clinically effective in antagonizing the reduction in plasma NEFA induced by MED–MID–KET anesthesia in cats. The fluctuation of plasma NEFA concentration may not be directly harmful to the anesthetized cats; however, it is preferable that this NEFA change during and after anesthesia is as small as possible because it indirectly reflects the glucose homeostasis and metabolism in adipose tissue and the liver associated with sympathoadrenal activation or inhibition [42]. In this regard, treatments including ATI will be clinically significant as an index of feline metabolic stability.

We have previously shown that MED alone or an MED–MID combination does not affect the plasma cortisol concentration in cats [33, 41]. However, premedication with MED has been reported to reduce or delay the increase in plasma cortisol concentrations induced by ovariohysterectomy in dogs [43, 44]. In dogs, it has been reported that the MED–KET combination prevented this effect despite KET monotreatment increased plasma cortisol concentrations [39]. In the present study, MED–MID–KET anesthesia also failed to significantly affect plasma cortisol concentration in cats, similar to ATI, FLU, 4AP and FLU–4AP administration. However, cortisol release was stimulated by an intervention combination of ATI–FLU–4AP. The present study also reconfirmed that the recovery from anesthesia was extremely poor in the ATI–FLU–4AP group, as described in our previous study [35]. These results suggest that treatment with ATI–FLU–4AP is not clinically recommended because of the large increase in adrenocortical activity. Furthermore, plasma cortisol concentrations may be more excessively elevated after the antagonism by ATI–FLU–4AP when surgery such as ovariohysterectomy is added to MED–MID–KET anesthesia in feline practice.

It has been reported that MED greatly inhibits plasma epinephrine and norepinephrine concentrations in cats and dogs [32, 33]. In contrast, plasma epinephrine and norepinephrine concentrations have been found to be increased by MID in cats [41] and by KET in dogs [39]. The KET-induced increases in plasma concentrations of epinephrine and norepinephrine have been reported to be mitigated by MID [45] and even abolished by MED [39]. In addition, MID has been reported to decrease MED-induced inhibition of norepinephrine release in cats [41]. Ultimately, the present study revealed that the MED–MID–KET combination decreased plasma concentrations of both epinephrine and norepinephrine in cats, suggesting that these effects are mainly attributed to MED. The present study revealed that FLU, 4AP and FLU–4AP did not significantly alter MED–MID–KET–induced reduction in plasma concentrations of both epinephrine and norepinephrine. However, ATI alone, ATI–FLU, ATI–4AP and ATI–FLU–4AP reversed these effects. Furthermore, we found that ATI alone, ATI–FLU and ATI-4AP had similar effects but that the ATI–FLU–4AP combination caused large increases in both plasma epinephrine and norepinephrine concentrations during recovery from anesthesia. In particular, an increase in the norepinephrine concentration was prominent. It has been reported that 4AP can release norepinephrine from the sympathetic ganglion and perivascular nerve endings as well as being able to enhance the release of acethylcholine [22, 46]. These actions of 4AP may contribute to prominent elevation of norepinephrine after ATI–FLU–4AP administration. These results suggest that treatment with ATI–FLU–4AP is not clinically recommended because of a large increase in sympatho–adrenergic activity. Care must be taken in the further enhancement of sympatho–adrenergic activity when the triple antagonist regimen is used for antagonism to MED–MID–KET anesthesia in cats having cardiovascular problems such as tachycardia. In addition, FLU or 4AP alone is also not clinically recommended for reversing the inhibition of catecholamine release induced by MED–MID–KET in cats. On the other hand, as an overall effect

of the antagonists, the use of potential antagonists may be advantageous for recovery from anesthesia if the sympatho–adrenomedullary system is activated adequately but not excessively.

The results of this study revealed that ATI, both alone and in combination, is effective in antagonizing the neurohormonal and metabolic effects of MED–MID–KET in cats. The use of 4AP and FLU is not clinically recommended in the antagonism of the hormonal and metabolic effects induced by MED–MID–KET even if they are effective in hastening recovery from anesthesia [35]. It is also reported that the addition of FLU to ATI alone does not show a significant difference in the recovery time and total time of immobilization by MED–MID–KET anesthesia in cats, although excitation and hyperesthesia are not observed in the ATI–FLU combination [47]. ATI alone may give appropriate antagonism without large stress responses for the recovery from anesthesia. However, the triple combination (ATI–FLU–4AP) is not suitable for smooth antagonism because of large stress-related hormonal responses as well as poor recovery from anesthesia, including hypersensitivity and aggression.

General conclusion

In chapter 1, the present study showed that MED–MID–KET at the doses used produced good anesthesia with excellent muscle relaxation and analgesia in cats. It was demonstrated that either FLU or 4AP alone did not markedly antagonize MED–MID–KET anesthesia. On the other hand, ATI alone, ATI–FLU, ATI–4AP and ATI–FLU–4AP significantly hastened the recovery from anesthesia induced by MED–MID–KET and a combination of ATI–FLU–4AP was most effective. However, the present study indicated that the quality of recovery was smoother in ATI alone or ATI–FLU than after both ATI–4AP and ATI–FLU–4AP, because the rigidity of limbs, muscle tremors and excitement that were observed in most cats received ATI–FLU–4AP, and muscle tremors were observed in many cats after ATI–4AP during recovery process. The present study revealed that combinations with ATI were effective in antagonizing the hypothermia, bradycardia and hypopnea induced by MED–MID–KET anesthesia. Reversal of bradycardia is mainly due to ATI. Cardiac arrhythmia assessed by auscultation and vomiting occurred more frequently in the control and non-ATI injected groups than the other groups. Therefore, combinations with ATI can prevent these side effects. Excitement was not observed in the FLU group, but occurred frequently in the groups combined with ATI and most frequently in the ATI–FLU–4AP group. Although a combination of ATI, FLU and 4AP was most effective in antagonizing the anesthesia induced by MED–MID–KET in this study, it induced limb rigidity and muscle tremors during the recovery phase. ATI alone and combinations with ATI are much more effective for antagonizing the anesthesia and side effects induced by MED–MID–KET. ATI alone can be used as a safe and effective agent for antagonizing the MED–MID–KET anesthesia in cats. The use of ATI–FLU–4AP may be not always produce smooth recovery from anesthesia.

In chapter 2, the effects of ATI, FLU and 4AP, both alone and in various combinations on key stress-related neurohormonal and metabolic variables against anesthetized with MED–MID–KET

was investigated in healthy cats. The results of this study revealed that MED–MID–KET anesthesia produced a moderate hyperglycemia. The present results demonstrated that ATI alone or in combination with other drugs reversed hyperglycemia induced by MED–MID–KET. On the other hand, FLU, 4AP and FLU–4AP enhanced MED–MID–KET induced hyperglycemia. The augmentation effect of FLU treatment on MED–MID–KET –induced hyperglycemia may be caused by the antagonism of the effect of MID suppressing the hyperglycemic action of MED. It was also found that 4AP treatment enhanced the hyperglycemic effect induced by MED–MID–KET. Although the precise mechanism of this effect is also unknown, treatments with FLU and/or 4AP are not clinically recommended for decreasing the hyperglycemia associated with MED–MID–KET anesthesia in cats. The plasma insulin concentration at 1 h following FLU treatment decreased significantly, suggesting that FLU at least partially influenced MED–MID–KET induced hyperglycemia. Plasma insulin concentrations tended to be transiently decreased after MED–MID–KET anesthesia and reversed by ATI. Treatments including ATI are clinically effective in antagonizing the reduction in plasma NEFA induced by MED–MID–KET anesthesia in cats. MED–MID–KET anesthesia failed to significantly affect plasma cortisol concentration in cats, similar to ATI, FLU–4AP and FLU–4AP administration. However, cortisol release was stimulated by an intervention combination of ATI–FLU–4AP. The present study reconfirmed that the recovery from anesthesia was extremely poor in the ATI–FLU–4AP group. These results suggest that treatment with ATI–FLU–4AP is not clinically recommended because of the large increase in adrenocortical activity. The present study revealed that the MED–MID–KET combination decreased plasma concentrations of both epinephrine and norepinephrine in cats, suggesting that these effects are mainly attributed to MED. The present study revealed that FLU, 4AP and FLU–4AP did not significantly alter MED–MID–KET induced reduction in plasma concentrations of both epinephrine and norepinephrine. However, ATI alone and in combination reversed these effects. Furthermore, we found that ATI alone, ATI–FLU and

ATI–4AP had similar effects but that the ATI–FLU–4AP combination caused large increases in both plasma epinephrine and norepinephrine concentrations during recovery from anesthesia. These results suggest that treatment with ATI–FLU–4AP is not clinically recommended because of a large increase in sympatho–adrenergic activity. FLU or 4AP alone is also not clinically recommended for reversing the inhibition of catecholamine release induced by MED–MID–KET in cats.

In conclusion, the results revealed that ATI, both alone and in combination, is effective in antagonizing the neurohormonal and metabolic effects of MED–MID–KET in cats. The use of 4AP and FLU is not clinically recommended in the antagonism of the hormonal and metabolic effects induced by MED–MID–KET even if they are effective in hastening recovery signs from anesthesia. ATI alone may give appropriate antagonism without large stress responses for the recovery from anesthesia. However, the triple combination is not suitable for smooth antagonism because of large stress-related hormonal responses as well as poor recovery from anesthesia, including hypersensitivity and aggression.

Abstract

The α_2-adrenoceptor agonist medetomidine (MED) is mainly used for sedation and analgesia in veterinary medicine. However, it induces undesirable effects such as hyperglycemia, hypoinsulinemia, emesis, diuresis and bradyarrhythmias in dogs and cats. The combination of MED, the benzodiazepine agonist midazolam (MID) and the dissociative anesthetic agent ketamine (KET) produce good anesthesia in cats with excellent muscle relaxation and analgesia. Antagonism may be required when the anesthetized animals show a profound depression of vital signs, adverse effects and/or delayed recovery from anesthesia. Atipamezole (ATI), flumazenil (FLU) and 4-aminopyridine (4AP) completely or partially antagonize the effects of MED, MID and KET, respectively, in cats. 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 is no report on stress-related neurohormonal and metabolic influences of antagonism against the anesthesia with MED–MID–KET in cats. The present study aimed to investigate the effects of ATI, FLU and 4AP, both alone and in various combinations, on anesthesia and key stress-related neurohormonal and metabolic changes induced by MED–MID–KET in healthy cats.

In chapter 1, the antagonistic effects of ATI, FLU and 4AP alone and their combinations after anesthesia produced by a fixed dose of MED, MID and KET injected intramuscularly were evaluated in cats. Eight cats received eight different treatments. Each cat was given the mixture of 0.05 mg/kg MED and 0.5 mg/kg MID followed 10 min later by 10 mg/kg KET intramuscularly. Twenty min after KET injection, the cats were given either physiological saline solution (PSS) at a dose of 0.1 ml/kg (control), 0.2 mg/kg ATI, 0.1 mg/kg FLU, 0.5 mg/kg 4AP, ATI–FLU,

FLU–4AP, ATI–4AP or ATI–FLU–4AP intravenously. Elapsed times to recovery of the palpebral reflex, pedal reflex and tail clamp reflex were recorded 13 times over 300 min period after the injection of antagonists. Recovery times to head movement, sternal recumbency, standing and walking were also recorded. The degree of antagonism for anesthesia was assessed using previously published scoring methods. Mean elapsed times to recovering the eyelid, pedal and tail clamp reflex after injection of potential antagonists were significantly shortened in the antagonists-injected groups compared with the PSS-injected control. The cats in the ATI group had a significantly lower total score compared with PSS or non-ATI injected groups. The cats in the ATI–FLU–4AP group had the lowest total score after antagonist injection compared with the other groups. Rectal temperature decreased significantly or tended to decrease from pre-values until 240 min in the control and non-ATI injected groups. Rectal temperature in ATI, ATI–FLU and ATI–4AP groups recovered to pre-values at 180 min after administration of antagonists. Heart rates and respiratory rates in the ATI combined groups were significantly higher than those in control and non-ATI injected groups after injection of antagonists. However, the cats in the ATI–FLU–4AP group showed tachycardia and tachypnea. Excitement, vocalizing, aversion to body touch and congestion of the conjunctiva were observed in some of cats received combinations with ATI and in most of the cats received ATI–FLU–4AP. In addition, muscle tremors were observed in many cats that received ATI–4AP and ATI–FLU–4AP. Emesis was observed in the control and non-ATI injected groups during recovery. These results demonstrated that either FLU or 4AP alone did not markedly antagonize MED–MID–KET anesthesia. ATI alone and combinations with ATI are much more effective for antagonizing the anesthesia and side effects induced by MED–MID–KET. ATI alone can be used as a safe and effective agent for antagonizing the MED–MID–KET anesthesia in cats. However, the use of ATI–FLU–4AP may be not always produce smooth recovery from anesthesia.

In chapter 2, the effects of ATI, FLU and 4AP, both alone and in various combinations on key stress-related neurohormonal and metabolic variables after anesthesia with MED–MID–KET were investigated in healthy cats. Seven cats received eight different treatments. Experimental groups and dosages of tested agents were same as those in chapter 1. Blood samples were collected 10 times during the 24-h test period from a central venous catheter introduced into the jugular vein. Plasma glucose, insulin, cortisol, epinephrine, norepinephrine and NEFA concentrations were measured. MED–MID–KET administration resulted in hyperglycemia and decreases in epinephrine, norepinephrine and NEFA concentrations. FLU or 4AP alone or FLU–4AP combination did not effectively antagonize the effects induced by MED–MID–KET but enhanced hyperglycemia. ATI alone was effective in antagonizing these effects. Compared with non-ATI regimens, combinations with ATI were more effective in antagonizing the effects induced by MED–MID–KET; however, ATI–FLU–4AP caused large increases in cortisol, epinephrine and norepinephrine concentrations. ATI, both alone and in combination, is effective in antagonizing the neurohormonal and metabolic effects of MED–MID–KET in cats. However, the ATI–FLU–4AP combination is not suitable because of large stress-related hormonal responses.

In conclusion, this study revealed that ATI, both alone and in combination, is effective in antagonizing the neurohormonal and metabolic effects of MED–MID–KET in cats. The use of 4AP and FLU is not clinically recommended in the antagonism of the hormonal and metabolic effects induced by MED–MID–KET. ATI alone may give appropriate antagonism without large stress responses for the recovery from anesthesia. However, the triple combination is not suitable for smooth antagonism because of large stress-related hormonal responses, as well as poor recovery from anesthesia, including hypersensitivity and aggression. This study provided new information on the antagonism for anesthesia with MED–MID–KET and its stress-related hormonal and metabolic changes in cats.

Acknowledgements

The author wishes to acknowledge Dr.Yoshiaki Hikasa (Tottori University) for his appropriate guidance as supervisor, critical review of the manuscript, and escorting the first steps as veterinary researcher. The author greatly appreciate Dr. Takashi Takeuchi (Tottori University) and Dr. Masaru Okuda (Yamaguchi University) for their technical advises and suggestions on this study. The authors wish to thank Dr. T. Ueoka of the Ueoka Animal Hospital for technical assistance.

References

1. Golden AL, Bright JM, Daniel GB, Fefee D, Schmidt D, Harvey RC (1998) Cardiovascular effects of the alpha2-adrenergic receptor agonist medetomidine in clinically normal cats anesthetized with isoflurane. American Journal of Veterinary Research 59, 509–513.
2. Lamont LA, Bulmer BJ, Grimm KA, Tranquilli WJ, Sisson DD (2001) Cardiopulmonary evaluation of the use of medetomidine hydrochloride in cats. American Journal of Veterinary Research 62, 1745–1749.
3. Selmi AL, Mendes GM, Lins BT, Figueiredo JP, Barbudo-Selmi GR (2005) Comparison of xylazine and medetomidine as premedicants for cats being anaesthetised with propofol-sevoflurane. Veterinary Record 157, 139–143.
4. Brown CR, Sanquist FH, Canup CA, Pedley TA (1979) Clinical, electroencephalographic and pharmacokinetic studies of a water-soluble benzodiazepine, midazolam maleate. Anesthesiology 50, 467–470.
5. Court MH, Greenbratt DJ (1992) Pharmacokinetics and preliminary observations of behavioral changes following administration of midazolam to dogs. Journal of Veterinary Pharmacology and Therapeutics 15, 343–350.
6. Ilkiw JE, Suter CM, Farver TB, McNeal D, Steffey EP (1996) The behaviour of healthy awake cats following intravenous and intramuscular administration of midazolam. Journal of Veterinary Pharmacology and Therapeutics 19, 205–216.
7. Salonen M, Reid K, Maze M (1992) Synergistics interaction between a$_{\square 2}$-adrenergic agonists and benzodiazepines in rats. Anesthesiology 76, 1004–1011.
8. Nishimura R, Kim H, Matsunaga S, Hayashi K, Tamura H, Sasaki N, Takeuchi A (1993) Sedative effect induced by a combination of medetomidine and midazolam in pigs. Journal of Veterinary Medical Science 55, 717–722.

9. Itamoto K, Hikasa Y, Sakonjyu I, Itoh H. Kakuta T, Takase K (2000) Anaesthetic and cardiopulmonary effects of balanced anesthesia with medetomidine-midazolam and butorphanol in dogs. Journal of Veterinary Medicine Series A 47, 411–420.
10. Kojima K, Nishimura R, Mutoh T, Hong SH, Mochizuki M, Sasaki N (2002) Effects of medetomidine-midazolam, acepromazine-butorphanol, and midazolam-butorphanol on induction dose of thiopental and propofol and on cardiopulmonary changes in dogs. American Journal of Veterinary Research 63, 1671–1679.
11. Flecknell PA (1994) Injectable anaesthetics. In: Hall, Taylor (eds), Anesthesia of the Cat. Bailliere Tindall: W.B. Saunders, pp. 129–156.
12. Verstegen J, Fargetton X, Zanker S, Donnay I, Ectors F (1991) Antagonistic activities of atipamezole, 4-aminopyridine and yohimbine against medetomidine/ketamine-induced anesthesia in cats. Veterinary Record 128, 57–60.
13. Ilkiw JE, Suter CM, McNeal D, Farver TB, Steffey EP (1996) The effect of intravenous administration of variable-dose midazolam after fixed-dose ketamine in healthy awake cats. Journal of Veterinary Pharmacolology and Therapeutics 19, 217–224.
14. Sparkes AH, Papasouliotis K, Viner J, Cripps PJ, Gruffydd-Jones TJ (1996) Assessment of orocaecal transit time in cats by the breath hydrogen method: the effects of sedation and a comparison of definitions. Research in Veterinary Science 60, 243–246.
15. Akkerdaas LC, Mioch P, Sap R, Hellebrekers LJ (2001) Cardiopulmonary effects of three different anesthesia protocols in cats. Veterinary Quarterly 23, 182–186.
16. Savola JM (1989) Cardiovascular action of medetomidine and their reversal by atipamezole. Acta Veterinaria Scandinavica 85, 38–47.
17. Vähä-Vahe T (1990) Clinical effectiveness of atipamezole as a medetomidine antagonist in cats. Journal of Small Animal Practice 31, 193–197.

18. Granholm M, McKusick BC, Westerholm FC, Aspegren JC (2006) Evaluation of the clinical efficacy and safety of dexmedetomidine or medetomidine in cats and their reversal with atipamezole. Veterinary Anesthesia and Analgesia 33, 214–223.
19. Brogden RN, Goa KL (1988) Flumazenil. A preliminary review of its benzodiazepine antagonist properties, intrinsic activity and therapeutic use. Drugs 35, 448–467.
20. Sumida T, Tagami M, Ide Y, Nagase M, Sekiyama H, Hanaoka K (1995) Intravenous midazolam suppresses noxiously evoked activity of spinal wide dynamic range neurons in cats. Anesthesia and Analgesia 80, 58–63.
21. Ilkiw JE, Farver TB, Suter CM, McNeal D, Steffey EP (2002) The effect of intravenous administration of variable-dose flumazenil after fixed-dose ketamine and midazolam in healthy cats. Journal of Veterinary Pharmacolology and Therapeutics 25, 181–188.
22. Durant NN, Lee C, Katz RL (1980) 4-Aminopyridine reversal of sympathetic ganglionic blockade in the anesthetized cat. Anesthesiology 52, 381–384.
23. Rupp SM, Shinohara Y, Fisher DM, Miller RD, Castagnoli NJr (1983) Pharmacokinetics and pharmacodynamics of 4-aminopyridine in anesthetized dogs. Journal of Pharmacology and Experimental Therapeutics 225, 351–354.
24. Hatch RC, Booth MH, Kitzman JV, Wallner BM, Clark JD (1983) Antagonism of ketamine anesthesia in cats by 4-aminopyridine and yohimbine. American Journal of Veterinary Research 44, 417–423.
25. Hatch RC, Kitzman JV, Clark JD, Zahner JM, Booth NH (1984) Reversal of pentobarbital anesthesia with 4-aminopyridine and yohimbine in cats pretreated with acepromazine and xylazine. American Journal of Veterinary Research 45, 2586–2590.
26. Sinclair MD (2003) A review of the physiological effects of α_2-agonists related to the clinical use of medetomidine in small animal practice. Canadian Veterinary Journal 44, 885–897.

27. Cullen LK (1996) Medetomidine sedation in dogs and cats: a review of its pharmacology, antagonism and dose. British Veterinary Journal 152, 519–535.
28. Short CE (1991) Effect of anticholinergic treatment on the cardiac and respiratory systems in dogs sedated with medetomidine. Veterinary Record 129, 310–313.
29. Ko JC, Fox SM, Mandsager RE (2001) Effects of preemptive atropine administration on incidence of medetomidine-induced bradycardia in dogs. Journal of American Veterinary Medical Association 218, 52–58.
30. Bergstom K (1988) Cardiovascular and pulmonary effects of a new sedative/analgesic (medetomidine) as a preanaesthetic drug in the dog. Acta Veterinaria Scandinavica 29, 109–116.
31. Alibhai HI, Clarke KW, Lee YH, Thompson J (1996) Cardiopulmonary effects of combinations of medetomidine hydrochloride and atropine sulphate in dogs. Veterinary Record 138, 11–13.
32. Ambrisko T, Hikasa Y (2002) Neurohormonal and metabolic effects of medetomidine compared with xylazine in beagle dogs. Canadian Journal of Veterinary Research 66, 42–49.
33. Kanda T, Hikasa Y (2008) Neurohormonal and metabolic effects of medetomidine compared with xylazine in healthy cats. Canadian Journal of Veterinary Research 72, 278–286.
34. Murahata Y, Hikasa Y (2012) Comparison of the diuretic effects of medetomidine hydrochloride and xylazine hydrochloride in healthy cats. American Journal of Veterinary Research 73, 1871–1880.
35. Ueoka N, Hikasa Y (2008) Antagonistic effects of atipamezole, flumazenil and 4-aminopyridine against anaesthesia with medetomidine, midazolam and ketamine combination in cats. Journal Feline Medicine and Surgery 10, 47–54.
36. Gaynor JS, Muir WW (2002) Handbook of veterinary pain management. 1st ed. St Louis: Mosby Year Book Inc, pp. 49.

37. Desborough JP (2000) The stress response to trauma and surgery. British Journal of Anaesthesia 85, 109–117.
38. Ambrisko TD, Hikasa Y (2003) The antagonistic effects of atipamezole and yohimbine on stress-related neurohormonal and metabolic responses induced by medetomidine in dogs. Canadian Journal of Veterinary Research 67, 64–67.
39. Ambrisko TD, Hikasa Y, Sato K (2005) Influence of medetomidine on stress-related neurohormonal and metabolic effects caused by butorphanol, fentanyl, and ketamine administration in dogs. American Journal of Veterinary Research 66, 406–412.
40. Zaki S, Ticehurst K, Miyaki Y (2009) Clinical evaluation of Alfaxan-CD® as an intravenous anaesthetic in young cats. Australian Veterinary Journal 87, 82–87.
41. Kanda T, Hikasa Y (2008) Effects of medetomidine and midazolam alone or in combination on the metabolic and neurohormonal responses in healthy cats. Canadian Journal of Veterinary Research 72, 332–339.
42. Fagerholm V, Haaparanta M, Scheinin M (2011) α_2-Adrenoceptor regulation of blood glucose homeostasis. Basic and Clinical Pharmacology and Toxicology 108, 365–370.
43. Benson GJ, Grubb TL, Neff-Davis C, Olson WA, Thurmon JC, Lindner DL, Tranquilli WJ, Vanio O (2000) Perioperative stress response in the dog: Effect of pre-emptive administration of medetomidine. Veterinary Surgery 29, 85–91.
44. Ko JC, Mandsager RE, Lange DN, Fox SM (2000) Cardiorespiratory responses and plasma cortisol concentrations in dogs treated with medetomidine before undergoing ovariohysterectomy. Journal of American Veterinary Medical Association 217, 509–514.
45. Adams HA (1997) [Endocrine reactions following S-(+)-ketamine]. Anaesthesist 46, Suppl 1: S30–37.

46. Kun A, Pataricza J, Krassói I, Szécsi M, Hohn J, Varró A, Papp JG (2002) Low 4-aminopyridine concentration-induced contraction is mediated by neuronal noradrenaline in canine saphenous vein. Vascular Pharmacology 39, 7–11.

47. Ebner J, Wehr U, Baumgartner C, Erhardt W, Henke J (2007) Partial antagonization of midazolam-medetomidine-ketamine in cats-atipamezole versus combined atipamezole and flumazenil. Journal of Veterinary Medicine A, Physiology, Pathology, Clinical Medicine 54, 518–521.