Neurohormonal and Metabolic Effects of α_2-Adrenoceptor Agonists and in Combination with Benzodiazepine Receptor Agonist in Cats

（α_2-アドレナリン受容体作動薬およびベンゾジアゼピン受容体作動薬との併用がネコの神経内分泌および代謝に及ぼす影響）

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2007

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Chapter 1

General introduction

The α_2-adrenoceptor agonists, xylazine and medetomidine are used widely in many species of animals; dogs, cats, cattle, horses, pigs, sheep, and goats [1-5]. The main purposes of using α_2-adrenoceptor agonists are sedation, premedication of anethtesia, analgesia and muscle relaxation in veterinary medicine [5]. Xylazine is also used as a diagnostic agent for congenital or acquired hyposomatotropism in dogs and cats [6]. In addition, α_2-adrenoceptor agonists are useful for gastrointestinal endoscopy or surgery because of reducing gastrointestinal motility [5]. While, emetics, bradycardia, and arrhythmia are known as undesirable effects [5,7,8]. Furthermore, hyperglycemic effect induced by hypoinsulinemia was also reported in some species [9-13].

The influences of xylazine, medetomidine or other α_2-adrenoceptor agonists on the cardiopulmonary function have been investigated in many reports [4,14-17]. On the other hand, the neurohormonal and metabolic effects of medetomidine and xylazine is investigated poorly in cats. Although hyperglycemic effect of α_2-adrenoceptor agonists were reported in many species including humans, hyperglycemic effect of only xylazine was reported in cats [10]. In spite of the fact that α_2-adrenoceptor agonists potentially influence on the neuroendcrine system, there is no report to prove the effects of these agents on different endocrine and metabolic disease such as diabetes mellitus and hyperthyroidism. First, for the appropriate use of medetomidine and xylazine, although enough data on the effects of α_2-adrenoceptor agonists in healthy cats are needed.

It has been reported that hyperglycemic effect induced by α_2-adrenoceptor agonists resulted from decrease of insulin secretion by pancreas beta cell through the α_2-adrenoceptor-mediated action [18]. However, Ambrisko and Hikasa [9] reported that other factors except for the action mediated by α_2-adrenoceptors may be also involved in the hyperglycemic effect in dogs. Similar to the dogs, it is possible that other factors in additon to the decrease of insulin secretion may cause hyperglycemia in cats. Therefore, glucagon, cortisol and non-esterified fatty acid which are responsible to the glucose regulation, may be involved in the hyperglycemic effect of medetomidine and xylazine. In addition to the insufficient studies of the effect of α_2-adrenoceptor agonists on the feline metabolism, the studies showing the dose-responses and comparing the differences between medetomidine and xylazine are hardly done.

Moreover, Catecholamines also contributed to the glucose regulation via parasympathetic nerve system [19,20]. The effect of medetomidine and xylazine on the catecholamines release may play an important role not only neurohormonal function but also glucose metabolism. In dogs, catecholamines release was reported to be suppress by both medetomidine and xylazine [9]. Although similar changes are predicted in cats, there are no reports of the effects of α_2-adrenoceptor agonists on the blood catecholamines levels in the cats. The dose-responses of medetomidine and xylazine on the blood catecholamines levels have not been reported and compared in cats.

In chapter 1, the dose-responses of neurohormonal and metabolic effects of medetomidine and xylazine were examined and compared in cats. This study on the hyperglycemic, other metabolic and neurohormonal effects of medetomidine in healthy cats is the first report to author's knowledge.

The combination of medetomidine and other drugs has been studied to obtain the adequate sedative effect and suppression of undesirable effects, because of the potent effect of medetomidine on the cardiopulmonary functions [20-22]. It has been reported that

midazolam and/or butorphanol are used in combination with medetomidine in dogs [23,24]. These combinations are useful to obtain effective sedation safely. However, most of studies on the combination of medetomidine and other drugs have been performed for the effect on the cardiopulmonary functions. Thus, the research of the effect on the neurohormonal and metabolic variables at blood levels was not enough, especially in cats.

Nishimura et al [25] reported the influence of midazolam in combination with medetomidine on the blood glucose and insulin regulation in laboratory pigs. It was indicated that the combination of medetomidine and midazolam suppressed the hyperglycemia induced by medetomidine compared with same dose of medetomidine alone. However, midazolam, a benzodiazepine receptor agonist, was not reported to decrease blood glucose concentration in laboratory pigs. No study revealed that suppression of hyperglycemia induced by the combination of medetomidine and midazolam was due to whether the effect of midazolam alone or in combination with medetomidine. Therefore, it needs to investigate the neurohormonal effect of medetomidine alone and in combination with midazolam in healthy cats for appropriate use.

In chapter 2, the effects of midazolam in combination with medetomidine on neurohormone and metabolism at blood levels of variables, especially glucose regulation was investigated in healthy cats. In addition to the combination of medetomidine and midazolam, the experiments about the effects of medetomidine or midazolam alone to reveal the neurohormonal and metabolic effects of midazolam were performed in this chapter.

Chapter 2

Neurohormonal and metabolic effects of medetomidine compared with xylazine in healthy cats

Introduction

In veterinary practice, the α_2-adrenoceptor agonists medetomidine and xylazine are used mainly for sedative, muscle relaxant, and analgesic purposes [5]. These agents are also effective as emetics in small animal practice [8]. In addition, xylazine is used to stimulate the release of growth hormone for the diagnosis of congenital or acquired growth hormone deficiency [26]. Medetomidine is a more selective and specific α_2-adrenoceptor agonist than xylazine: the α_2/α_1 selectivity ratios are 1620 and 160, respectively [27]. In spite of this difference, the both 2 agents may be used similarly in practice.

Surgical stressors such as pain, blood loss, excitement, and underlying pathological conditions are well known to induce neurohormonal and metabolic changes in animals that are characterized by increases in blood levels of glucose, cortisol, catecholamines, and nonesterified fatty acids (NEFAs) and a decrease in blood insulin levels [28]. Since α_2-adrenoceptor-mediated actions are closely coordinated with these events, medetomidine and xylazine may interfere with the neurohormonal and metabolic response induced by stressors during and after anesthesia and surgery. For appropriate use, veterinarians need to know the neurohormonal and metabolic effects of medetomidine and xylazine in animals. Both agents induce hyperglycemia, hypoinsulinemia, inhibition of catecholamine release, and lipolysis in beagle dogs, but the hyperglycemic effect of medetomidine, in contrast to that of xylazine, is not dose-dependent [9].

There are limited reports as to the effects of xylazine on the blood glucose and insulin levels in cats [10], and specific time-dependent and dose-dependent data on the neurohormonal and metabolic effects of medetomidine and xylazine are lacking in cats, to author's knowledge. Therefore, this study aimed to investigate and compare the effects of these 2 agents on the blood levels of epinephrine, norepinephrine, cortisol, glucose, insulin, glucagon, and NEFAs in cats.

Materials and methods

Animals

Five healthy mixed-breed cats of both sexes, of mean age 3.4 ± 1.34 yrs (mean ± SD), and of mean weight 5.05 ± 0.23 (mean ± SD) kg were used. The cats were housed in my laboratory for at least 1 mo before the experiment and fed a standard commercial dry cat food. Routine hematologic examination was performed before the experiment; all values were within normal physiological ranges [29]. The experimental protocols were approved by the Animal Research Committee of Tottori University, Tottori, Japan.

Experimental protocols

The experiment involved 11 treatment groups in which each cat was given an intramuscular injection of physiological saline solution (2.0 mL/animal) as the control agent, 20, 40, 80, 160, or 320 μg/kg of a 1% solution of medetomidine hydrochloride (Domitor; Meiji Seika, Tokyo, Japan), or 0.5, 1, 2, 4, or 8 mg/kg of a 2% solution of xylazine hydrochloride (Celactal; Bayer, Tokyo, Japan). The groups will be referred to as control, MED-20, -40, -80, -160, and -320, and XYL-0.5, -1, -2, -4, and -8. Five cats were used repeatedly, with at least 1 wk between treatments, in each of the 11 groups, according to a modified randomized design, as follows: cat 1, control, XYL-0.5, MED-20, XYL-4,

MED-160, XYL-2, MED-80, XYL-8, MED-320, XYL-1, and MED-40; cat 2, XYL-1, MED-320, XYL-8, MED-160, XYL-4, MED-20, XYL-0.5, MED-80, control, MED-40, and XYL-2; cat 3, MED-20, XYL-0.5, control, XYL-8, MED-320, XYL-1, MED-40, XYL-4, MED-160, XYL-2, and MED-80; cat 4, XYL-1, MED-40, control, MED-20, XYL-0.5, MED-80, XYL-2, MED-320, XYL-8, MED-160, and XYL-4; and cat 5, MED-80, XYL-2, MED-40, XYL-1, MED-160, XYL-4, control, XYL-0.5, MED-20, XYL-8, and MED-320. The cats were fasted for 12 h before injection. Food and water were also withheld during the experiment and offered again 1 h after the last blood sampling of the day. The experiments were performed in a room with air temperature set at 25°C.

Sample collection

Blood samples (2.0 mL) were collected from the jugular vein by means of a 23-gauge needle with a 2.5-mL disposable syringe at the following 10 times: time 0 (before injection of the agent) and 0.25, 0.5, 1, 2, 3, 4, 5, 6, and 24 h after injection. An aliquot of 0.5 mL from each sample was mixed with aprotinin (Trasylol; Bayer, Leverksen, Germany) for glucagon measurement; the remaining 1.5 mL was mixed with ethylene diamine tetraacetic acid. Both samples were centrifuged immediately at 4 °C, and then the plasma was separated and kept frozen at –80 °C until analyzed for concentrations of catecholamines (epinephrine and norepinephrine), cortisol, glucose, insulin, glucagon, and NEFAs.

Analytical methods

Glucose and NEFA concentrations were determined with the use of commercially available kits (Glucose CII-test Wako and NEFA C-test Wako; Wako Junyakukogyo, Osaka, Japan). Glucose was analyzed by the mutarotase–glucose oxidase method, and NEFAs were analyzed by the acyl-coenzyme A (CoA) synthetase–acyl-CoA oxidase method. The intra-assay coefficients of variation (CVs) were < 2% and < 3% and the limits of

quantification 700 mg/dL and 2 mmol/L, respectively. The glucose and NEFA concentrations were measured with a spectrophotometer (Auto Sipper Photometer U-1080; Hitachi, Tokyo, Japan).

Insulin and glucagon concentrations were measured by double-antibody radioimmunoassay (RIA) with the use of the commercially available kits I-AJ16 (Eiken Chemical Company, Tokyo, Japan) and Glucagon kit Daiichi (TFB Stock Company, Tokyo, Japan), respectively. The intra-assay CVs were < 10% and 2.6% to 5.3%, respectively; the interassay CV with the glucagon kit was 2.4% to 3.6%. The limits of detection and quantification were 5 to 320 μU/mL for insulin and 15.6 to 4000 pg/mL for glucagon.

Cortisol was measured by single-antibody RIA with the use of a commercially available kit (Gamma Coat Cortisol; Nihon Sheering, Chiba, Japan). The intra-assay CV was 3.5% to 5.0% and the interassay CV 4.2% to 8.7%. The limits of detection and quantification were 0.23 to 60 μg/dL.

Catecholamines were extracted on activated alumina according to the method described by Bouloux et al [30] and measured by means of high-performance liquid chromatography (LaChrom; Hitachi) and an electrochemical detector (Coulochem II; ESA, Chelmsford, Massachusetts, USA). As an internal standard, 3,4-dihydroxybenzylamine (DHBA; Sigma Chemical Company, St. Louis, Missouri, USA) was used. The percentage recovery of authentic DHBA standard was 64% to 77%.

Data evaluation

All data obtained were analyzed together with Prism statistical software (version 4; GraphPad Software, San Diego, California, USA). One-way analysis of variance for repeated measures was used to examine the time effect within each group and the group effect at each time point. When a significant difference was found, the Tukey test was used to compare the means.

The normalized area under the curve (AUC) was calculated for each biochemical variable. The AUC was measured by calculating the sum of the trapezoids formed by the data points and the x-axis from 0 to 6 h. The difference between the mean AUC of the control group and the AUC of a certain individual was defined as the normalized AUC. The normalized AUC data were plotted against dose of medetomidine or xylazine, and simple linear regression analysis was applied. When a significant difference was found, the effect of the drug on the plasma level of the examined biochemical was claimed to be dose-related.

Mean values are presented with error of the mean in parenthesis. The level of significance in all tests was set at $P < 0.05$.

Results

For all of the variables, there were no significant differences between the groups at baseline (time 0).

Glucose values increased greatly after administration in all groups except the control group (Figure 1). A dose-dependent response of the peak glucose level was also found in the XYL groups at 2 h post-administration. The maximum mean value was 383 ± 80 (mean ± SD) mg/dL with XYL-8 and 371 ± 61 (mean ± SD) mg/dL with MED-320. The linear regression of the normalized AUC data from 0 to 6 h was significant in both groups (Figure 2), indicating that both drugs induced hyperglycemia in a dose-dependent manner. However, time-related changes differed: the glucose values in the XYL groups returned to baseline gradually after the peak at 2 h, whereas the values in the MED groups tended to plateau near the peak and then return gradually to baseline in a dose-dependent manner. The linear regression of the normalized AUC data from 0 to 2 h was significant in the XYL groups but not in the MED groups, indicating that medetomidine, in contrast to xylazine, did not cause a dose-related increase in plasma glucose concentration, especially during the early phase after

administration (Figure 3). Similar results were obtained with linear regression of the normalized AUC data from 0 to 3 h.

Compared with the baseline value, the mean concentration of norepinephrine was increased, but not significantly, in the control group at 2 h after saline administration. The mean concentration was significantly decreased in the XYL-1 group at 1 and 2 h after drug administration and tended to decrease in the other XYL and MED groups (Figure 4). The normalized AUC data from 0 to 6 h were lower in the MED and XYL groups than in the control group (Figure 5), significantly so in the MED-40, MED-160, XYL-1, XYL-2, and XYL-4 groups. However, the AUC data for the highest-dosage groups (MED-320 and XYL-8) were not further reduced when compared with those for the MED-160 and XYL-4 groups, respectively. The linear regression of the normalized AUC data was not significant for either treatment group, indicating that neither medetomidine nor xylazine induced a dose-dependent suppression of norepinephrine release within the tested dosages.

Compared with the baseline value, the mean concentration of epinephrine was significantly increased in the control group at 2 h after saline administration. The mean concentration tended to decrease in all MED and XYL groups (Figure 6). The normalized AUC data from 0 to 6 h tended to be lower in the MED and XYL groups than in the control group (Figure 7) and were significantly lower in the MED-160 and MED-320 groups. The linear regression of the normalized AUC data was not significant for either treatment group, indicating that neither medetomidine nor xylazine induced a dose-dependent suppression of epinephrine release within the tested dosages.

The mean concentration of cortisol had increased, though not significantly, from 1.42 ± 1.37 to 3.40 ± 2.79 (mean ± SD) μg/dL at 0.5 h after administration of saline; it then returned to baseline (Figure 8). In all MED groups, the concentration tended to decrease by 1 h after administration (for example, from 1.40 ± 0.75 to 0.28 ± 0.13 (mean ± SD) μg/dL in the MED-80 group). In the XYL-2, -4, and -8 groups, the concentration tended to decrease by 15

min to 1 h after administration (for example, from 1.24 ± 1.03 to 0.64 ± 0.43 (mean ± SD) μg/dL at 0.5 h in the XYL-2 group), whereas in the XYL-0.5 and -1 groups the concentration initially increased and then gradually returned to baseline. However, overall, there were no significant differences between the control and MED groups or between the control and XYL groups.

In both drug groups, the insulin concentration decreased immediately after administration of medetomidine or xylazine and gradually returned to baseline (Figure 9). The decrease was significant ($P < 0.05$) for 2 h in the MED-320 group. The slopes of the recovery phases indicated that medetomidine suppressed the plasma insulin concentration in a dose-dependent manner. The higher doses of xylazine delayed recovery from the insulin suppression. However, the linear regression of the normalized AUC data from 0 to 6 h was not significant for either treatment group, indicating that neither medetomidine nor xylazine induced a dose-dependent inhibition of insulin release within the tested dosages.

The NEFA concentrations decreased after administration of both drugs and then gradually returned to baseline (Figure 10). Higher doses of medetomidine and xylazine tended to delay recovery from the NEFA suppression. The linear regression of the normalized AUC data from 0 to 6 h was not significant for either treatment group.

The glucagon concentration did not significantly change during the experiments in any of the groups treated with either drug (Figure 11). The glucagon/insulin ratio increased and then returned to baseline with both drugs (data not shown). These findings depended on the changes in plasma insulin concentration.

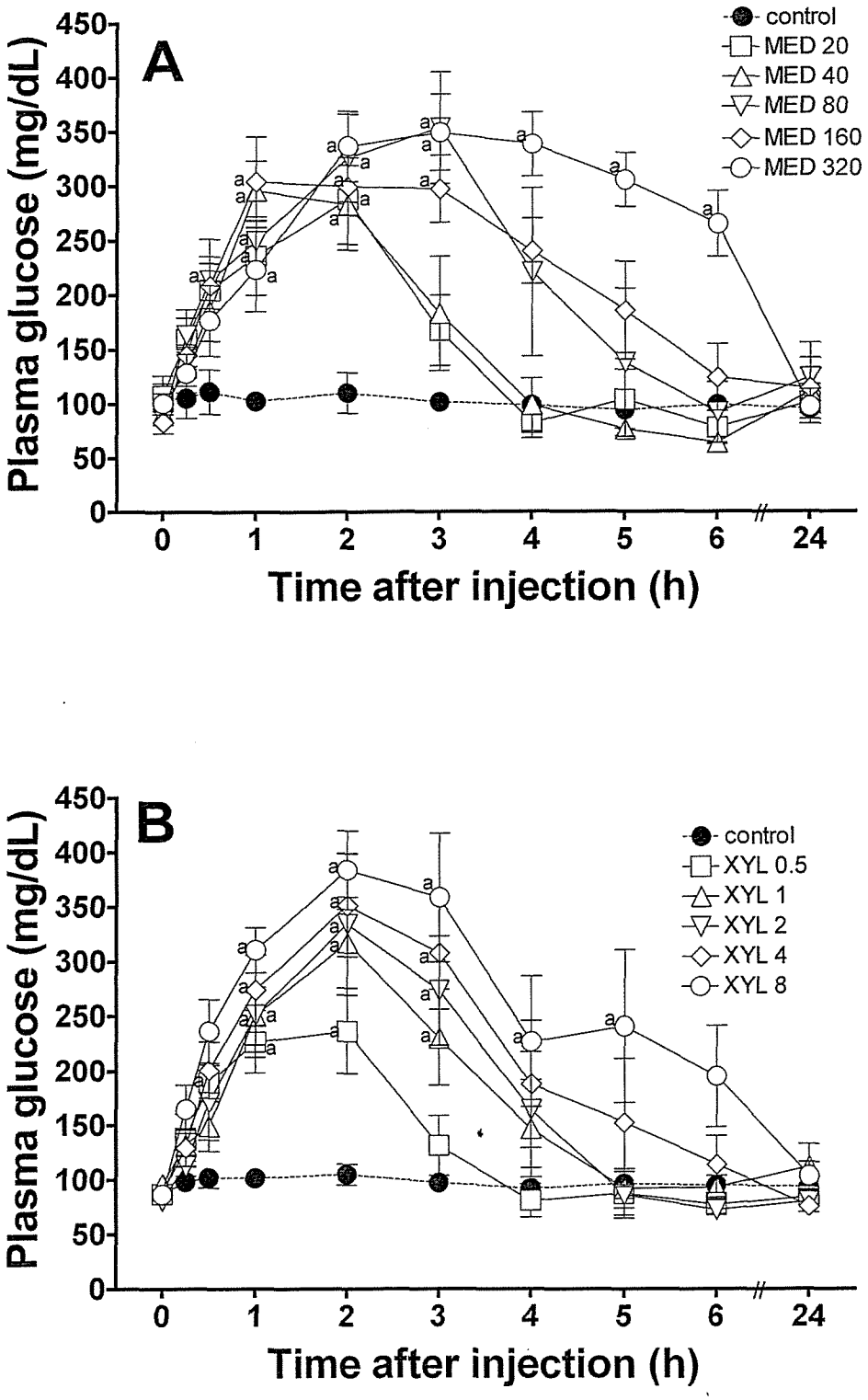

Figure 1. Plasma glucose concentrations after the administration of various doses of A) medetomidine (MED; μg/kg) and B) xylazine (XYL; mg/kg) to cats. Each point and vertical bar represent the mean and standard error ($n = 5$); a — significantly different ($P < 0.05$) from the value at time 0 (before drug administration).

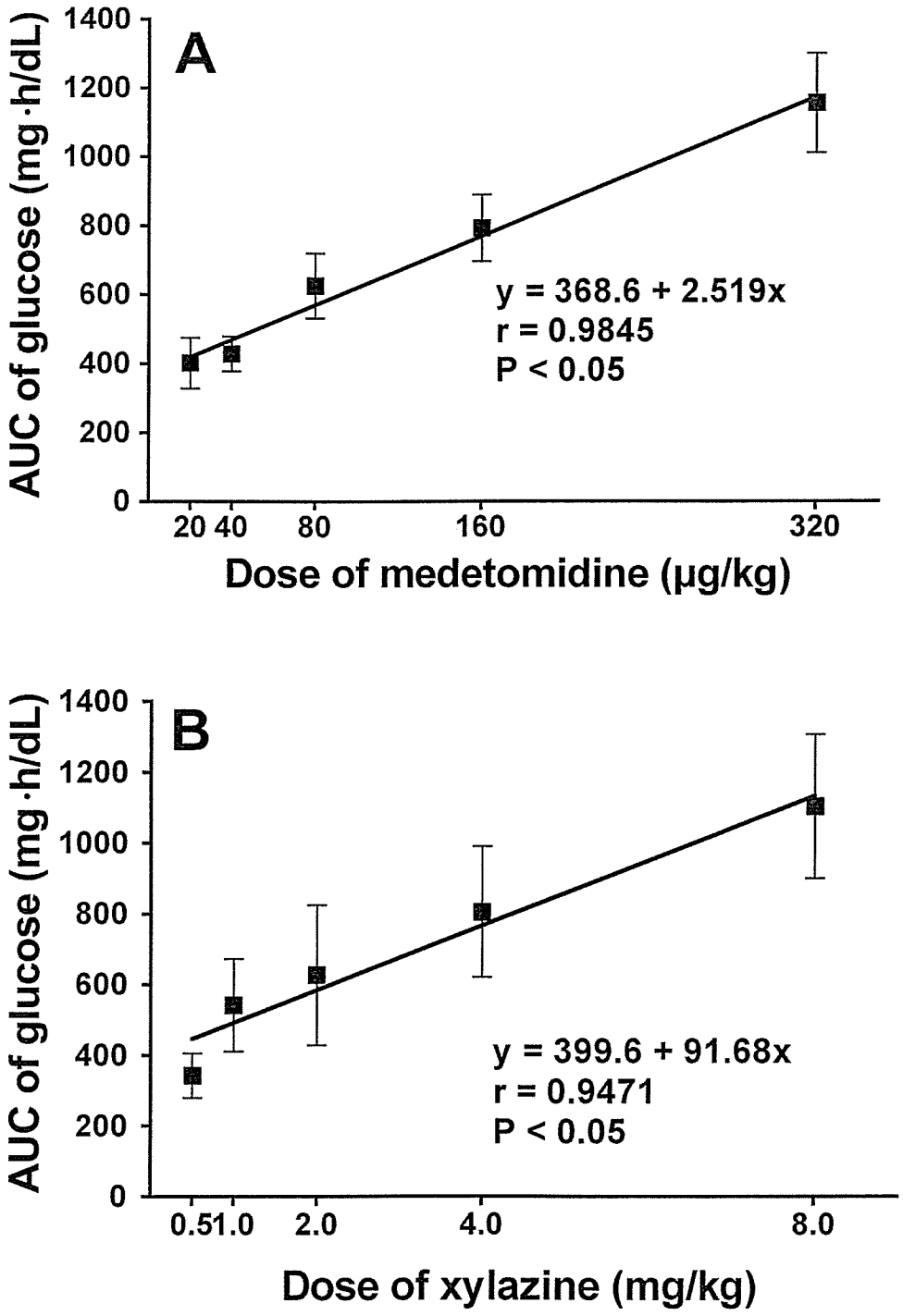

Figure 2. Normalized area-under-the-curve (AUC) data from 0 to 6 h for the plasma glucose concentration after administration of A) medetomidine or B) xylazine. Simple linear regression analysis was applied. Each point and vertical bar represent the mean and standard error ($n = 5$).

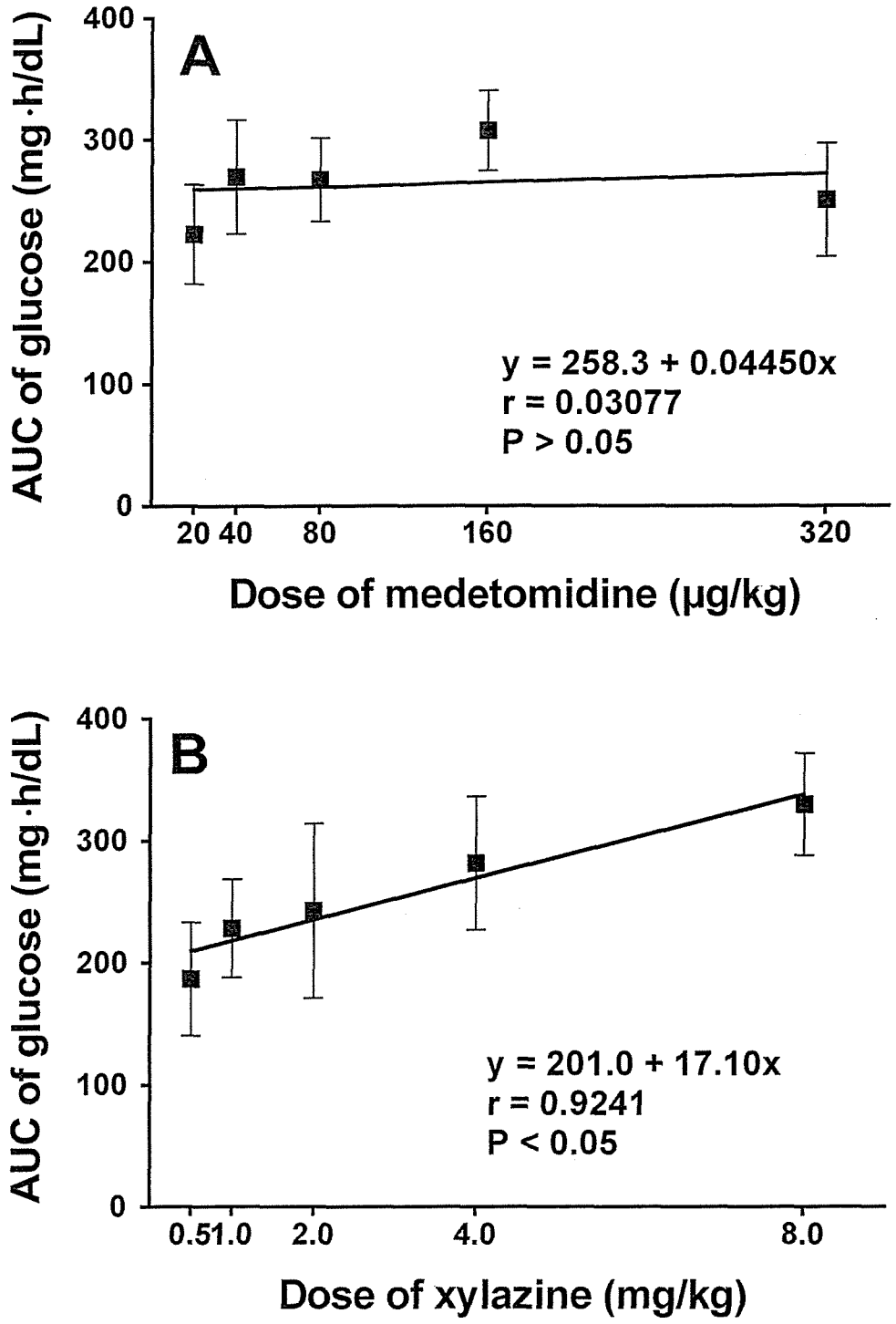

Figure 3. Normalized AUC data from 0 to 2 h for the plasma glucose concentration after administration of A) medetomidine or B) xylazine. Simple linear regression analysis was applied. Each point and vertical bar represent the mean and standard error (n = 5)

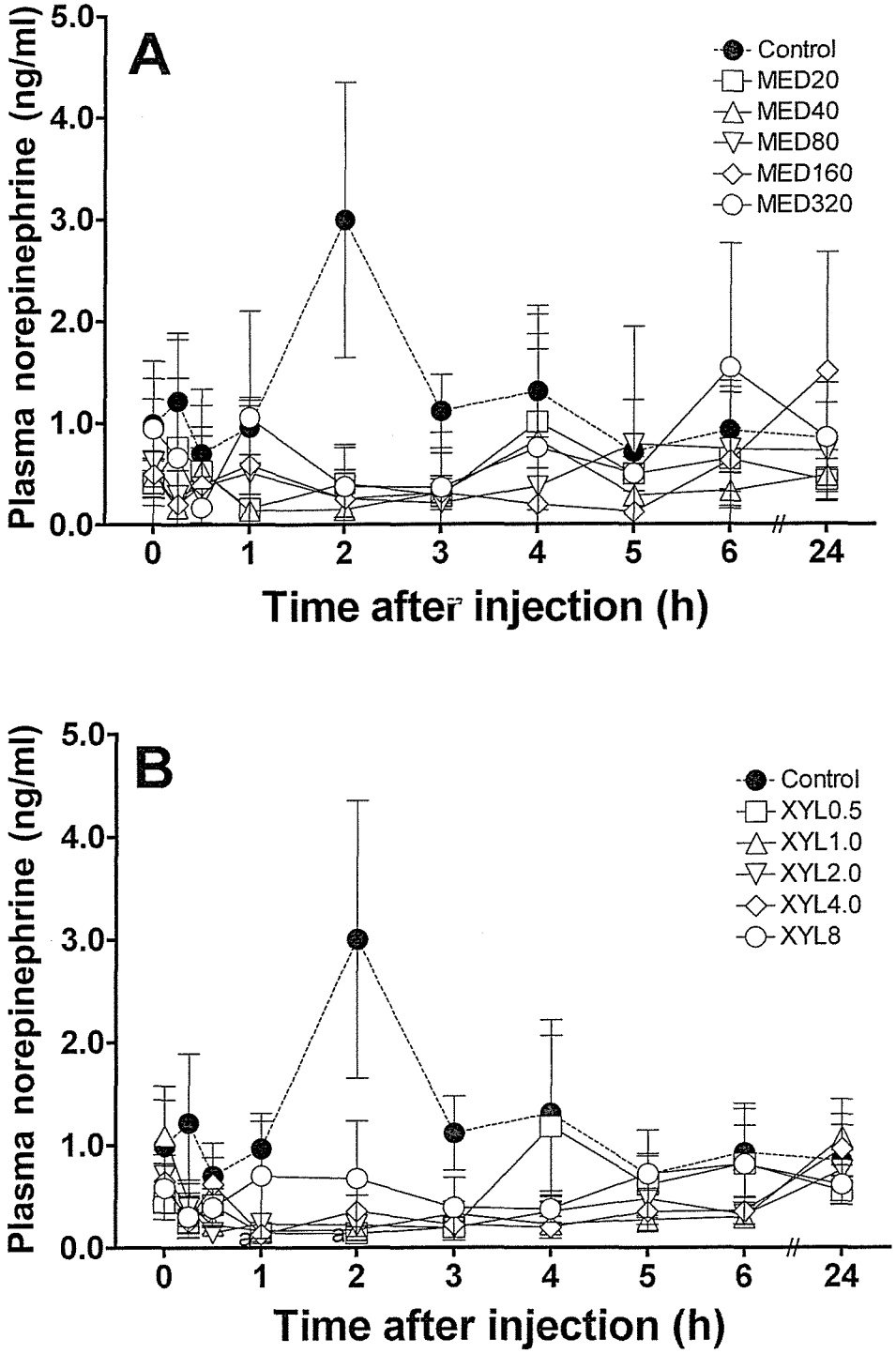

Figure 4. Plasma norepinephrine concentrations after the administration of A) medetomidine or B) xylazine to the cats (n = 5). Meanings of points, bars, and "a" as for Figure 1.

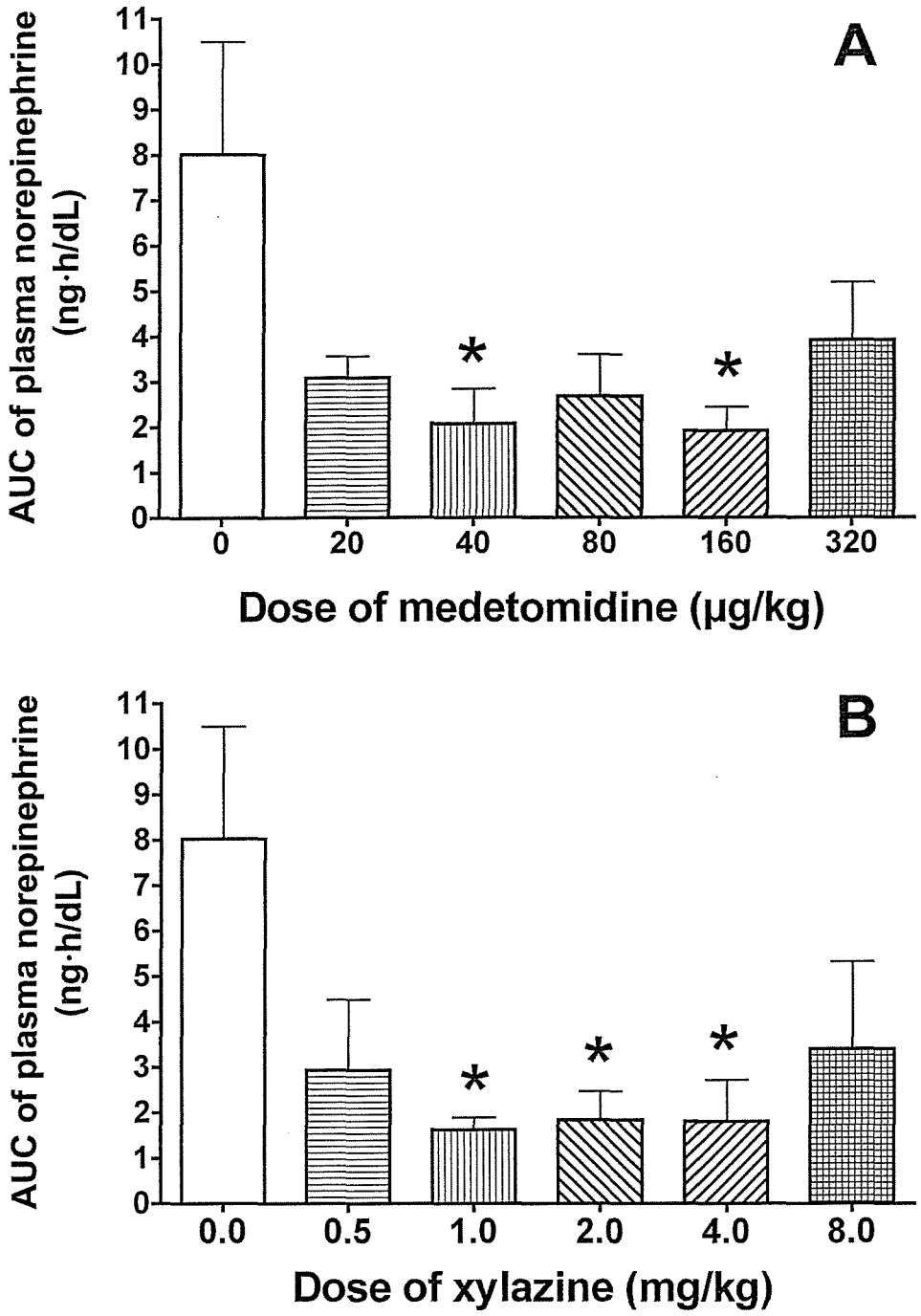

Figure 5. Normalized AUC data from 0 to 6 h for the plasma norepinephrine concentration after administration of A) medetomidine or B) xylazine. Each bar and vertical bar represent the mean and standard error ($n = 5$). Asterisks indicate a significant difference ($P < 0.05$) from the value at time 0.

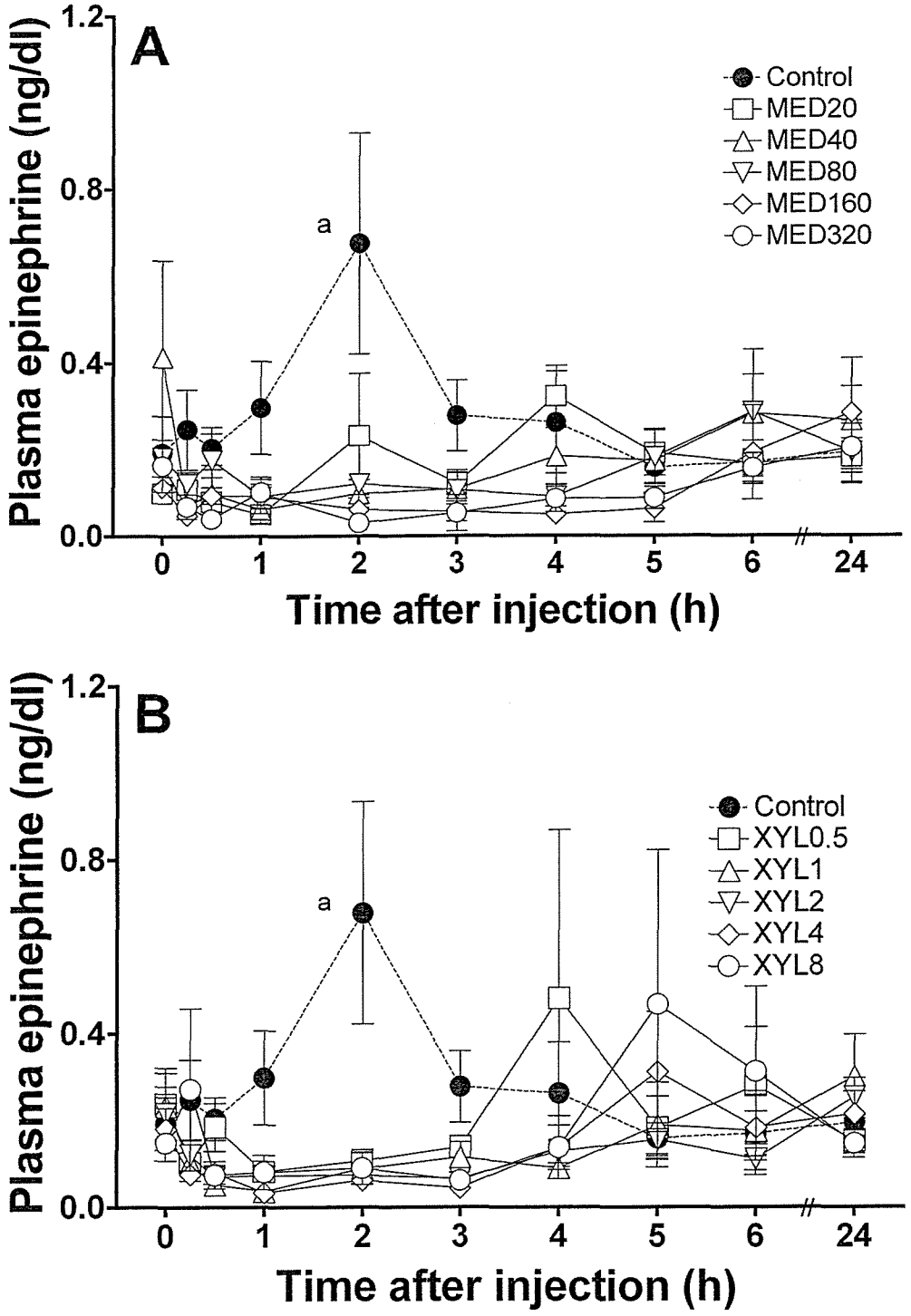

Figure 6. Plasma epinephrine concentrations after the administration of A) medetomidine or B) xylazine to the cats (n = 5). Meanings of points, bars, and "a" as for Figure 1.

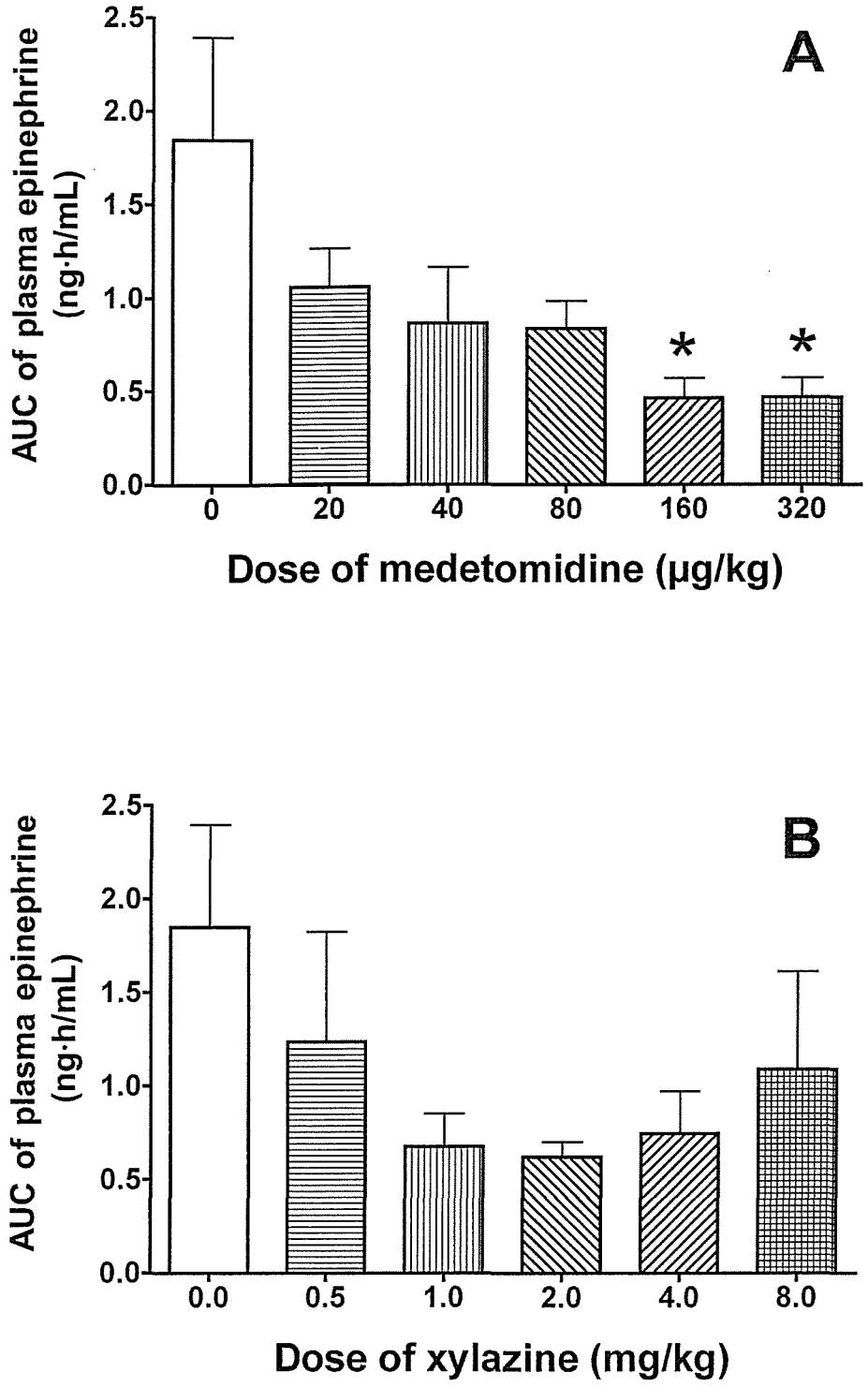

Figure 7. Normalized AUC data from 0 to 6 h for the plasma concentration after administration of A) medetomidine or B) xylazine. Each bar and vertical bar represent the mean and standard error ($n = 5$). Asterisks indicate a significant difference ($P < 0.05$) from the value at time 0.

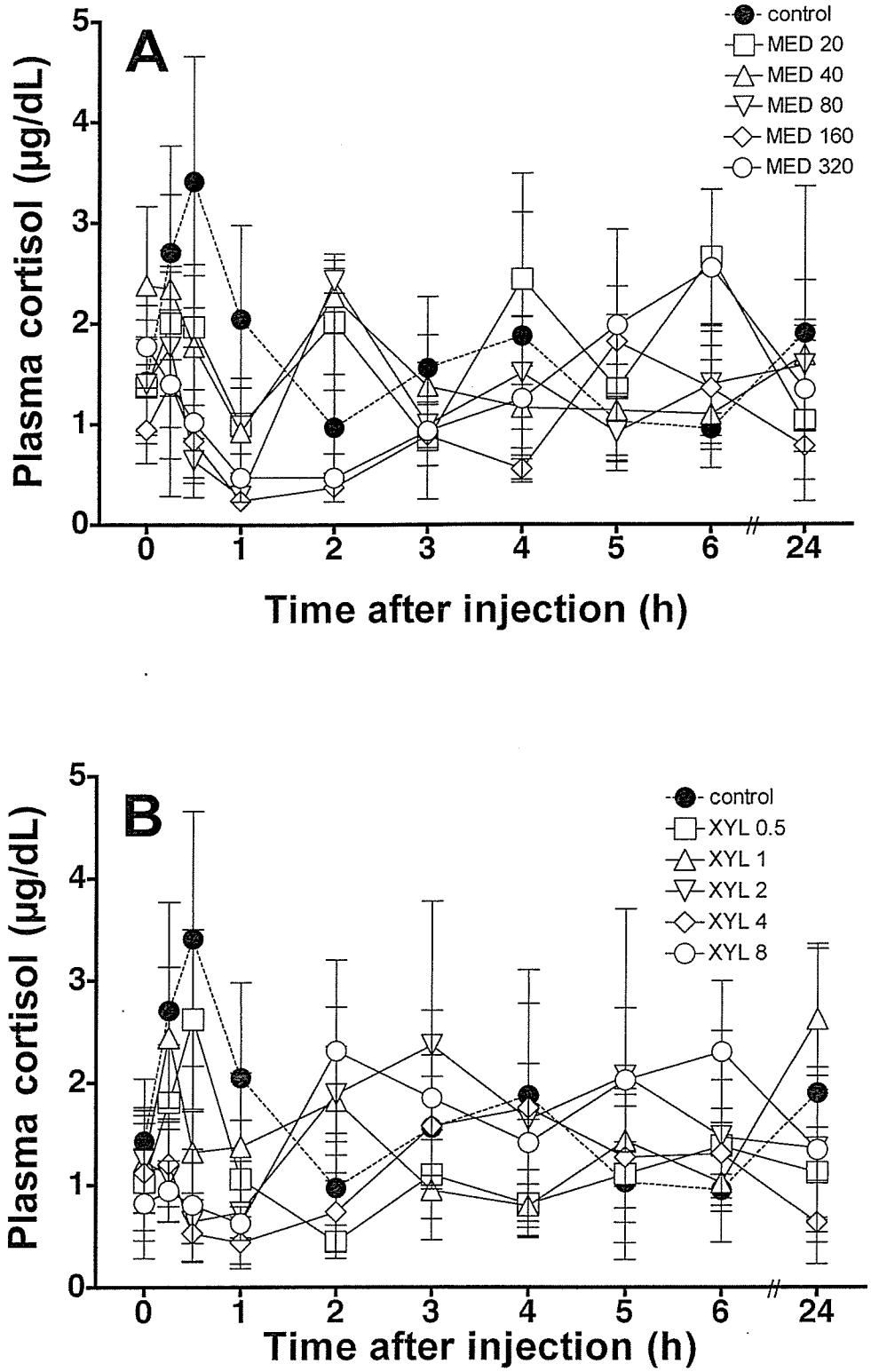

Figure 8. Plasma cortisol concentrations after the administration of A) medetomidine or B) xylazine to the cats (n =5). Meanings of points and bars as for Figure 1.

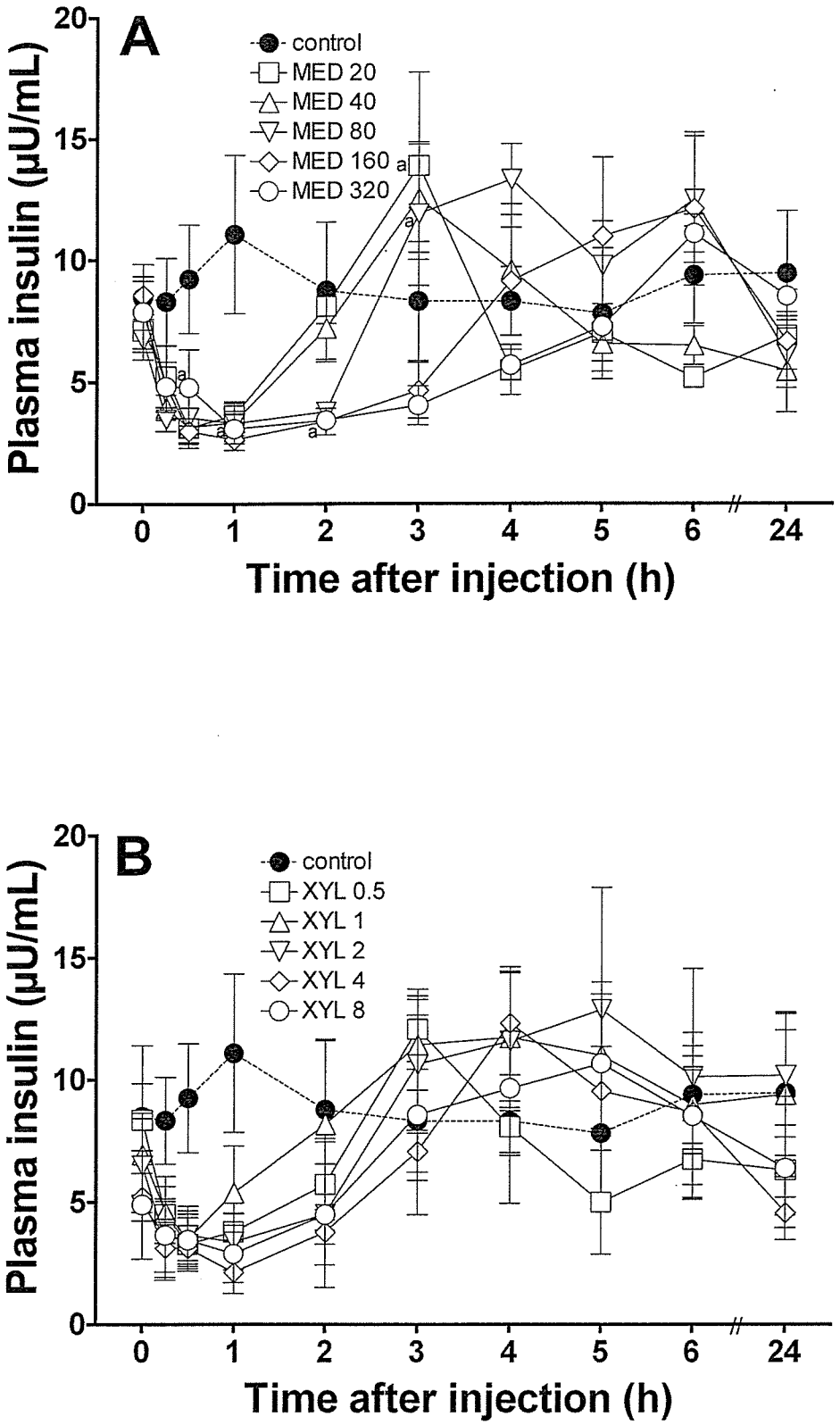

Figure 9. Plasma insulin concentrations after the administration of A) medetomidine or B) xylazine to the cats (n =5). Meanings of points, bars, and "a" as for Figure 1.

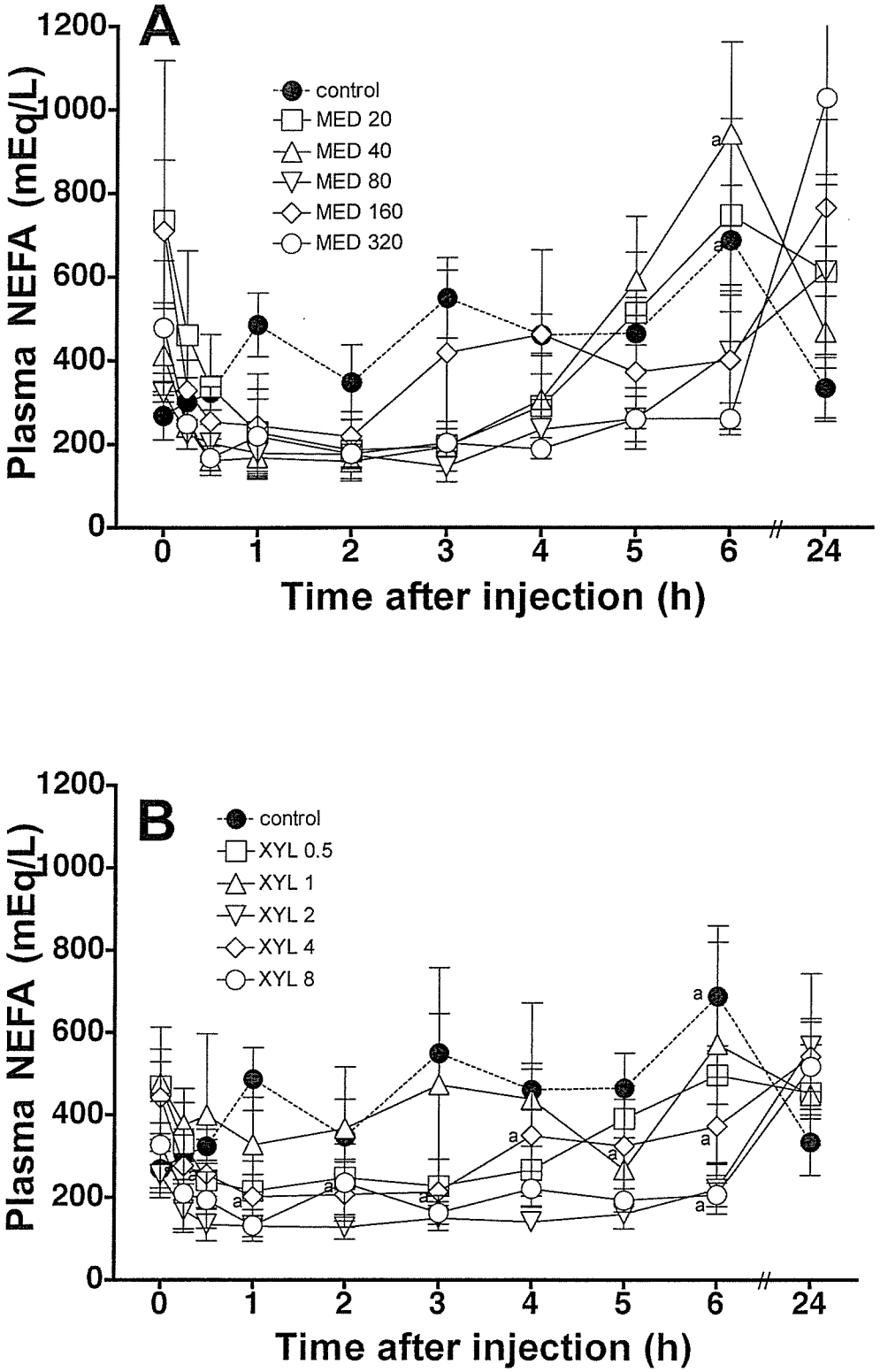

Figure 10. Plasma NEFA concentrations after the administration of A) medetomidine or B) xylazine to the cats (n =5). Meanings of points, bars, and "a" as for Figure 1.

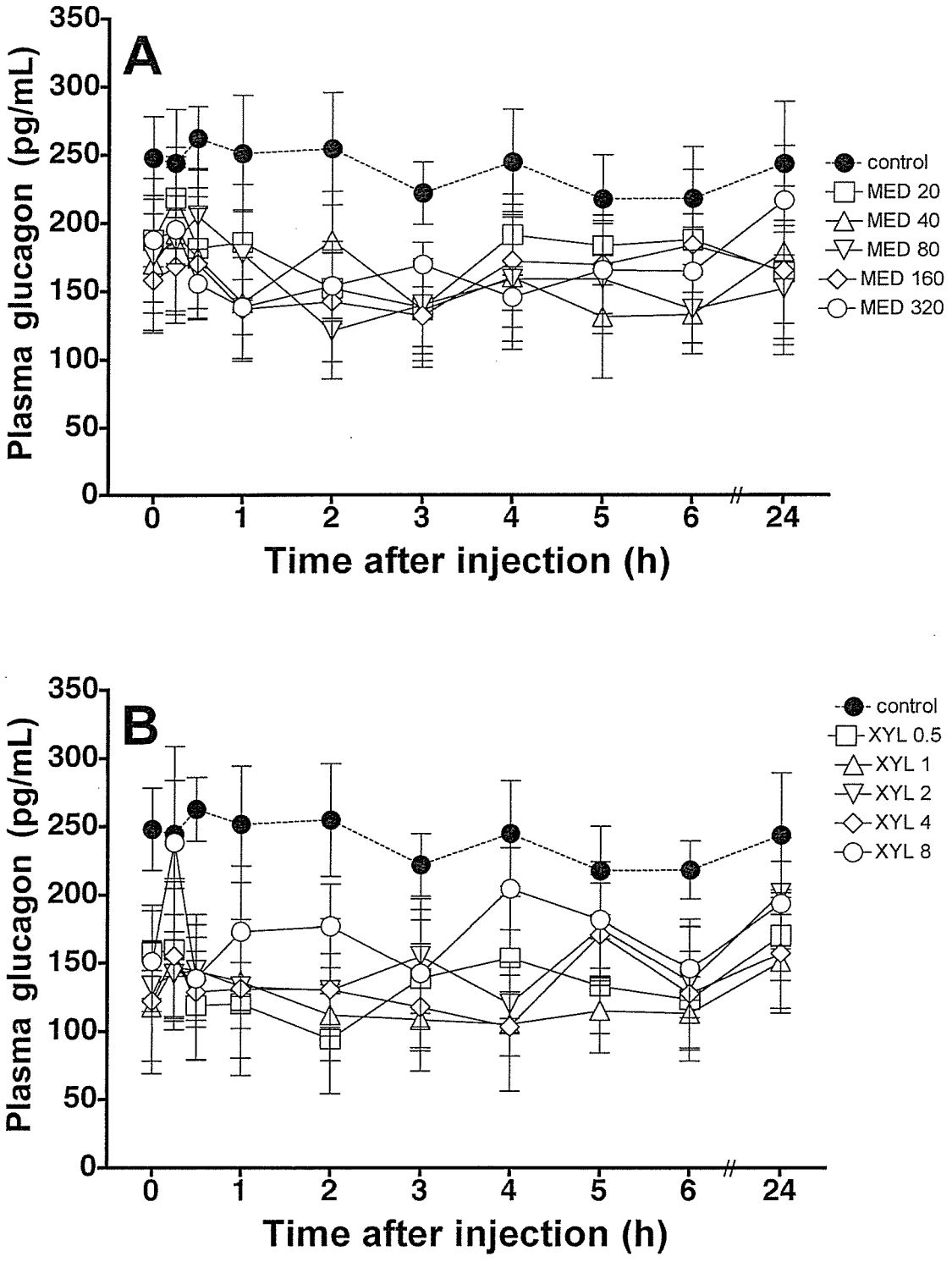

Figure 11. Plasma glucagon concentrations after the administration of A) medetomidine or B) xylazine to the cats (n =5). Meanings of points and bars as for Figure 1.

Discussion

Induction of hyperglycemia by medetomidine or xylazine has been reported in various animals, including dogs [9,11,12]. In this study, it was found that the hyperglycemia induced in cats by these drugs was greater than the previously reported values for dogs [9]. Hyperglycemia was reported to occur easily in cats as a result of acute stress, such as with restraint [31], and to be due to elevation of plasma concentrations of stress-related hormones, such as cortisol and catecholamines. However, in this experiment, only a slight, nonsignificant increase in cortisol level was observed in the control group, and it apparently was insufficient to cause an increase in glucose level. In addition, this study did not find elevations of cortisol and catecholamine levels in either drug-treated group, in spite of the remarkable hyperglycemia.

The mechanism of hyperglycemia induction by α_2-adrenoceptor agonists is understood to be mainly via inhibition of insulin secretion through actions of the agonists on α_2-adrenoceptors in the β cells of the pancreas [32,33]. The present results in cats showed that, although the suppression of the plasma insulin concentration induced by medetomidine and xylazine was to the same degree as in dogs, the elevation of the plasma glucose concentration was much greater than in dogs. The increases in glucose level were also higher in cats than in dogs soon after drug administration. These findings suggest that factors in addition to inhibition of insulin secretion may be involved in α_2-adrenoceptor-induced hyperglycemia in cats.

The effects of α_2-adrenoceptor agonists on plasma glucose and insulin concentrations have been reported in a variety of animals [9,13,34-38]. Clonidine and medetomidine were reported to increase plasma glucose levels in a dose-dependent manner in cattle [13,35] and rats [34,36]. Furthermore, 2.2 mg/kg of xylazine administered intramuscularly to dogs

caused an increase in plasma glucose concentration and a decrease in insulin concentration [37]. The results of these studies were similar to the present results.

When 10 and 20 μg/kg of medetomidine was administered intravenously to beagle dogs, the plasma glucose concentration tended to increase, but not significantly, and it remained within the normal physiological range [38]; the investigators observed a peak of about 90 mg/dL 3 h after administration of 20 μg/kg. In another study in beagle dogs, intramuscular injections of 10 to 80 μg/kg induced an increase in plasma glucose concentration and a peak of 122 mg/dL at 3 h after administration of 20 μg/kg [9]. In these 2 studies, the plasma insulin concentration decreased similarly. However, the hyperglycemic response was not dose-dependent [9]. In the present study, although the decrease in the plasma insulin concentration in cats was similar to that reported in dogs [9], the elevation in the plasma glucose concentration was remarkably greater in the cats than in dogs. Some factors besides the decrease in the plasma insulin concentration may be responsible for the difference between dogs and cats in the degree of hyperglycemia. It has been suggested that glucose production with high activity of rate-limiting glycogenic enzymes was greater in feline liver than in canine liver, although the plasma glucose concentrations under physiologically normal conditions were similar [39]. It is also possible that the increase in the plasma glucose concentration related to the decrease in the plasma insulin level is greater in cats than in dogs. This may be one of the reasons for the remarkable hyperglycemia induced by α_2-adrenoceptor agonists in cats.

A previous study found that in beagle dogs the hyperglycemic effect was dose-dependent with xylazine but not with medetomidine [9]. In the present study in cats, it was found that both drugs induced remarkable hyperglycemia in a dose-dependent manner. However, the hyperglycemic response in the early phase (up to 3 h after injection) was not dose-dependent with medetomidine but was with xylazine. To author's knowledge, this is the first report of a difference in hyperglycemic response between medetomidine and

xylazine in cats. The decreases in insulin concentration after administration of medetomidine and xylazine were similar in this study. Although the changes were not dose-dependent, high doses of both drugs tended to prolong the inhibition of insulin release. Therefore, the present results in cats suggest that the difference between the 2 drugs in the hyperglycemic response cannot be explained only by α_2-adrenoceptor-mediated inhibition of insulin release.

The increase in plasma glucose concentration was reported to be more significant when xylazine was administered via a peripheral vein rather than the lateral ventricle, indicating that xylazine-induced hyperglycemia is mediated through actions on peripheral sites rather than the central nervous system [10]. Furthermore, those investigators found that xylazine acted more rapidly and potent on hyperglycemia when it was infused into the femoral rather than the portal vein in cats. They suggested that xylazine-induced hyperglycemia was not produced by a direct action on the liver. Glucose metabolism in the liver is regulated by both anabolic action, which is accelerated by insulin, and catabolic action, which is accelerated by glucagon. The glucagon/insulin ratio is an indication of glucose metabolism in the liver. The ratio was found to increase after xylazine administration in cats, which meant relative acceleration of gluconeogenesis over glycolysis [10]. Similar results in cats given either xylazine or medetomidine were obtained in this study.

Other have reported that higher doses of clonidine increased glucose release from bovine and canine liver slices *in vitro* [35,40]. The subtype of α-adrenoceptors mediating this action seemed to be α_1 rather than α_2, because prazosin, a specific α_1-adrenoceptor antagonist, blocked the release of glucose more effectively than yohimbine, an α_2-antagonist [40]. Both medetomidine and xylazine also have an effect on α_1-adrenoceptors, xylazine more so than medetomidine. Imidazoline-receptor agonists were reported to increase the secretion of insulin [41]. Medetomidine has an affinity for the imidazoline receptors, unlike xylazine [8]. However, the decrease in plasma insulin concentration was similar with medetomidine and

xylazine in this study. Therefore, some of the differences between medetomidine and xylazine in their direct actions in the liver or in their actions that are mediated by α_1-adrenoceptors, imidazoline receptors, or both may be partially involved in the hyperglycemic responses to the 2 agents in cats.

The elevation in plasma glucose concentration was apparent at 15 min after drug administration in this study. It was assumed that this acute hyperglycemia in cats might be partially due to the changing cortisol concentration. The cortisol concentration is regulated by both the peripheral system, in the adrenal cortex, and the central nervous system, through the release of corticotropin-releasing hormone and adrenocorticotropic hormone (ACTH). The effect of α_2-adrenoceptor agonists on the secretion of cortisol has been reported for various animals. In dogs given medetomidine or xylazine intramuscularly, there was no significant change in the plasma cortisol concentration [9]. In calves given 40 μg/kg of medetomidine, there was a slight, nonsignificant elevation in plasma cortisol concentration, whereas in cows and sheep given the same dosages there were increases of approximately 4 times and 6 to 8 times, respectively [12]. In humans, oral administration of clonidine, 0.45 mg/kg for 3 d, decreased the plasma cortisol level [42], whereas 0.1 to 0.2 mg/kg for 4 d did not cause a significant decrease [43]. In dogs, premedication with medetomidine reduced or delayed the elevation in plasma cortisol concentration induced by ovariohysterectomy in dogs [44], and sedation with xylazine diminished the increase after intradermal testing [45].

These studies indicate that α_2-adrenoceptor agonists such as medetomidine, xylazine, and clonidine can inhibit cortisol secretion. However, it is unclear whether this effect is specific for α_2-adrenoceptors or other receptors. Recent studies suggest that imidazoline receptors, but not α_2-adrenoceptors, may be involved in the inhibition of cortisol secretion from the adrenal cortex. An *in vitro* study revealed that the imidazoline – α_2-adrenergic agents medetomidine, detomidine, and atipamezole all inhibited the secretion of cortisol from porcine adrenocortical cells [46]. As medetomidine and detomidine are selective

α_2-adrenoceptor agonists and atipamezole is a selective α_2-adrenoceptor antagonist, this effect is unrelated to these agents' actions on α_2-adrenoceptors. The selective α_2-adrenoceptor agonist D-medetomidine and its enantiomer, L-medetomidine, which is ineffective clinically, were found to be equally effective in inhibiting ACTH-stimulated corticosterone secretion from adrenocortical cells of rats [47]. These findings also indicate that imidazoline receptors may be involved in the suppression of cortisol secretion. The present experiments in cats revealed that the plasma cortisol concentration from 15 min to 1 h after administration of either medetomidine or xylazine tended to be suppressed, whereas it tended to be increased at 0.5 h after administration of saline. However, these findings were not significant. Therefore, the present results suggest the possibility that both medetomidine and xylazine have an inhibitory effect on the elevation of the plasma cortisol concentration in cats. These effects would be useful for relief from a variety of stressors.

The α_2-adrenoceptor agonists are well known to inhibit sympathetic outflow in the central nervous system through their actions on α_2-adrenoceptors, hence decreasing the level of circulating catecholamines. A reduction in plasma catecholamine levels associated with the use of α_2-adrenoceptor agonists has been reported for dogs [9], humans [48,49], horses [50,51], and goats [52]. However, there were no previous reports about the effects of medetomidine and xylazine on plasma catecholamine levels in cats. The present study in cats revealed that both drugs reduced the plasma epinephrine and norepinephrine concentrations. A previous report in dogs described a dose-dependent decrease in plasma epinephrine and norepinephrine concentrations with both drugs [9]. In the cats in the present study, however, the suppression of catecholamine release was not dose-dependent for either drug. In the XYL-8 group, the reductions tended to be smaller than in the other XYL groups. As xylazine has a low α_2/α_1 selectivity ratio of 160 [27], a high dose of xylazine (as in the XYL-8 group) may act on α_1-adrenoceptors that mediate the stimulation of sympathetic outflow and thus be less effective in reducing the plasma epinephrine and norepinephrine

concentrations. In contrast, since the α_2/α_1 selectivity ratio of medetomidine is 1620, its action on α_1-adrenoceptors may not have been present in the MED groups in this study. However, medetomidine also has an affinity for imidazoline receptors. Imidazoline-related drugs are able not only to inhibit norepinephrine release through the α_2-adrenoceptor-mediated mechanism but also to induce norepinephrine release through indirect mechanisms related to I1 imidazoline receptors [53]. Therefore, actions of medetomidine on both α_2-adrenoceptors and imidazoline receptors may have been involved in the changes in plasma epinephrine and norepinephrine concentrations in the present study.

It is also possible that the action of α_2-adrenoceptor agonists on the cardiovascular system may be responsible for the smaller suppression of catecholamine release with high doses of medetomidine and xylazine. The α_2-adrenoceptor agonists can produce hypotensive and bradycardic effects through the central nervous system, whereas they induce vasoconstriction via peripheral α_2-adrenoceptors in both the arterial and venous vasculature. Thus, α_2-adrenoceptor agonists show a biphasic effect on blood pressure. Hypotension activates the sympathetic system through the arterial baroreceptor reflex, causing an increase in epinephrine and norepinephrine concentrations [54]. In humans, an overdose of xylazine can induce hypotension [55]. Such differences in the sympathetic system among the dosages of medetomidine and xylazine might influence the suppression of epinephrine and norepinephrine release. The precise mechanisms by which the higher doses of medetomidine and xylazine did not further reduce the catecholamine concentrations are not clear.

In the present study, the plasma glucagon level did not change significantly after administration of either medetomidine or xylazine to the cats, indicating that it is not related to the hyperglycemic effects of these agents. This finding is in agreement with a previous report on dogs [9].

The plasma NEFA concentration decreased similarly after administration of medetomidine and xylazine in this study. To author's knowledge, this is the first report

outlining the effect of these drugs on the plasma NEFA concentration in cats. The suppression was similar to that previously reported for dogs [9] and cattle [56]. Suppression of lipolysis in cats may be mediated by both central and peripheral α_2-adrenoceptors, as has previously been reported for dogs [57] and humans [58].

In conclusion, both medetomidine and xylazine induced remarkable hyperglycemia in cats compared with that reported for dogs. The hyperglycemic response to medetomidine during the initial 3 h was not dose-dependent, in contrast to the response to xylazine. Both drugs suppressed epinephrine and norepinephrine release, but the suppression was also not dose-dependent at the tested dosages. Both agents inhibited insulin release and lipolysis, with similar potency, and tended to suppress cortisol release. The glucagon level did not change significantly in any treatment group. These results suggest that the effects of medetomidine and xylazine on glucose metabolism and catecholamine release may not be due only to the actions mediated by α_2-adrenoceptors.

Chapter 3

Effects of medetomidine and midazolam alone or in combination on the metabolic and neurohormonal responses in healthy cats

Introduction

A selective α_2-adrenoceptor agonist, medetomidine is widely used for sedation, analgesia, or muscle relaxation in veterinary medecine [5]. However, it induces undesirable effects such as hyperglycemia, hypoinsulinemia, emesis, and bradyarrhythmias in dogs and cats [9,59-61]. The medetomidine-induced hyperglycemia is extremely greater in cats than dogs [61]. Unfortunately, there are no reports indicating why medetomidine-induced hyperglycemia is greater in cats. In feline practice, diabetes mellitus, hyperthyroidism, and hypertention are often observed. Such disorders largely influence the feline metabolic and neurohormonal function. Therefore, the neurohormonal and metabolic effects of sedative agents should be understood for appropriate use in feline practice. Above all, hyperglycemia induced by α_2-adrenoceptor agonists as well as diabetes mellitus is accompanied by concurrent hypoinsulinemia. Thus, medetomidine-induced hyperglycemia may affect the feline metabolism similar to diabetes mellitus. Recently, the neurohormonal and metabolic effects of medetomidine at different dosages in cats were studied. And, it was found that medetomidine induced hyperglycemia, hypoinsulinemia, and the inhibition of catecholamines release and lipolysis [61]. Sedative effect should be adequate for various purposes in feline practice. For example, cats are needed to be immobile for opthalmologic or otologic treatments and examinations. Treatments for wounds or biopsy also need to be done under

good sedation. High doses of medetomidine increase the sedative effect, but also produce larger metabolic and nerohormonal effects such as hyperglycemia in cats [61]. Therefore, medetomidine may be used in the combination with other drugs to obtain adequate sedation with minimal neurohormonal and metabolic effect.

Midazolam is a water-soluble benzodiazepine that is used as an anxiolytic in human medicine [62]. Midazolam alone does not typically produce sedation in healthy cats, but it induces ataxia, restlessness, and abnormal behavior that are more difficult to approach and restrain, and does not induce profound sedation [63]. A combination of medetomidine with midazolam has been reported to enhance the sedative and analgesic actions of the individual drug in rats [64] and pigs [65], and to produce deep sedation in dogs [66]. This combination has been reported to greatly reduce the anesthetic induction dose of sodium thiopental and propofol in dogs [67]. The influence of medetomidine-midazolam combination on the cardiopulmonary function or the electroencephalogram has been also reported in dogs and sheep [68-70]. However, to the best of author's knowledge, there are no reports on the neurohormonal and metabolic effects of medetomidine-midazolam combination in cats. As previously reported in laboratory pigs [25], it is also postulated in cats that midazolam in combination with medetomidine may reduce hyperglycemic effect induced by medetomidine alone.

To evaluate the advantage of the combination of medetomidine and midazolam in cats, this study was conducted to investigate the effects of medetomidine and midazolam alone or in their combination on the neurohormonal and metabolic variables (glucose, insulin, cortisol, catecholamines, and nonesterified fatty acid (NEFA)). This study was also designed to model clinical conditions. The clinically recommended dose of medetomidine as a sedative-analgesic in cats has been reported to be 0.04 to 0.08 mg/kg intramuscularly [71]. Also, a previous study has revealed that an intravenous administration of 0.05 and 0.5 mg/kg midazolam after 3 mg/kg ketamine had beneficial effects on behavioral responses in cats [63].

It caused a greater proportion of cats to assume a laterally recumbent position with head down compared with ketamine alone. In addition, doses of midazolam of 0.5 mg/kg or above reduced muscle rigidity observed in cats which received ketamine alone, and greatly diminished a nociceptive response in cats [72]. Based on previous findings described above, intramuscular doses of 40 μg/kg medetomidine and 0.5 mg/kg midazolam as a combination were determined in this study.

Materials and Methods

Animals

Five healthy mixed-bleed cats of both sexes (2 intact males, 2 intact females, and 1 neutered male), aged 4.8 ± 3.2 (mean ± SD) yrs, and weighing 5.1 ± 0.8 (mean ± SD) kg were used. The cats were housed in author's laboratory for at least one month before the experiments and fed a standard commercial dry food. Routine hematological examination was performed prior to the experiment. All values were within normal physiological ranges [29]. The experimental protocols were approved by the Animal Research Committee of Tottori University.

Experimental protocols

Five cats were used repeatedly in each of the five groups according to a randomized latin square crossover design. There were at least one week between treatments for each cat. This experiment consisted of five groups. The treatments were physiological saline solution (2.0 ml/animal) (Control); 0.5 mg/kg midazolam HCl (Dormicum; Astellas Pharma, Tokyo, Japan) (MID0.5); 40 μg/kg medetomidine HCl (Domitor; Meiji Seika Kaisha, Tokyo, Japan) (MED40); 80 μg/kg medetomidine HCl (MED80); and 40 μg/kg medetomidine HCl and 0.5 mg/kg midazolam HCl (MED40-MID0.5) mixed in a syringe. The drugs were administered intramuscularly (IM) in all of groups. As the α_2-adrenoceptor agonists have been often used

intramuscular injection [73], this route was preferred in the present study. The quadricepts or semi-membranousus muscle was used for injection site.

The cats were fasted for 12 h before drug injection. Food and water were also withheld during the experiment, and offered again 1 h after the last blood sampling of the day. The experiments were performed in a room with air temperature set at 25 °C.

Instrumentation and sample collection

One day before experiment, a 17 gauge central venous catheter (EXCV catheter kit; Tyco Healthcare Japan, Tokyo, Japan) was introduced into the jugular vein under general anesthesia as follows. Prior to placement of the catheter, 6.6 to 8.8 mg/kg propofol (Rapinovet; Schering-Plough Animal Health, Osaka, Japan) was administered intravenously (IV) until adequate anesthesia was induced. After induction, anesthesia was maintained at a constant IV infusion rate of 0.22 to 0.44 mg/kg/min propofol. Lidocaine spray (Xylocaine pump spray 8%; Astra Zeneca, Osaka, Japan) was used for assistance of local analgesia at the catherization site of skin. The catheter was flushed with 1.5 mL of heparinized physiological saline solution, capped, and fixed. After fixing the catheter, the cat was placed in the individual cage to rest overnight. Blood samples were taken from the catheter at 0 (base line), 0.25, 0.5, 1, 2, 3, 4, 5, 6, and 24 h after drug injection. After every sampling, the cat was put back into the cage. Amount of blood withdrawn from each cat at once experiment was approximately 20 mL. Packed cell volume and the other routine hematological parameters were monitored through the experiments. After the single experiment, the catheter was removed and the cat were allowed to recover without any problems.

Measurements of behavioral responses and physical parameters

Cats were observed for behavioral and visible effects such as sedation, excitation, and vomiting for 6 h after injection of the drugs. The elapsed time from initiation of lateral

recumbency position to recovery for prone position by oneself was measured as one of behavioral responses for the sedative effect.

After every blood sampling, other technicians held a cat, and immediately measured physical parameters. Heart rate was monitored by using a stethoscope. Respiration rate was monitored by the movements of the thorax. Rectal temperature was measured by use of a digital thermometer.

Sample processing

A 2.0 mL of blood was collected at each time. A 0.5 mL volume from each blood sample was mixed with aprotinin (Trasylol; Bayer Pharmaceuticals Corporation, Leverksen, Germany) separately for glucagon measurement, and the remaining 1.5 mL blood was mixed with ethylenediamine tetraacetic acid (EDTA) for other measurements. Both samples were centrifuged immediately at 4 °C, then the plasma was separated and frozen at -80 °C and analyzed. Plasma glucose, insulin, cortisol, catecholamines (epinephrine and norepinephrine), glucagon, and NEFA were measured in all samples.

Analytical methods

Glucose and NEFA values were determined by enzyme assay technique using commercially available kits (Glucose CII-test Wako and NEFA C-test Wako; Wako Junyakukogyo, Osaka, Japan). Glucose was analyzed by the mutarotase-glucose oxidase method. NEFA was analyzed by the acyl-CoA synthetase-acyl-CoA oxidase method. Each coefficient of variation (CV) values of these kits were < 2 % at intra assay in glucose and < 3 % at intra assay in NEFA. The limits of quantification were 700 mg/dL in glucose and 2 mEq/L in NEFA. Glucose and NEFA were measured by use of a spectrophotometer (Auto Sipper Photometer U-1080; Hitachi, Tokyo, Japan). Insulin and glucagon were measured by double antibody radioimmunoassay (RIA) technique (I-AJ16; Eiken Chemical and Glucagon

kit Daiichi; TFB Stock Company, Tokyo, Japan). The CV values of these RIA kits were < 10 % at intra assay in insulin and 2.6 to 5.3 % at intra assay, 2.4 to 3.6 % at inter assay in glucagon. The limits of detection and quantification were 5 to 320 μU/mL in insulin and 15.6 to 4000 pg/mL in glucagon. Cortisol was measured by single antibody RIA technique (Gamma Coat Cortisol; Nihon Sheering, Chiba, Japan). The CV values of this kit were 3.5 to 5.0 % at intra assay and 4.2 to 8.7 % at inter assay. The limits of detection and quantification were 0.23 to 60 μg/dL. Catecholamines were extracted on activated alumina according to the method described by Bouloux et al [30], and measured by a high performance liquid chromatography (LaChrom; Hitachi, Tokyo, Japan) combined with an electrochemical detector (Coulochem II; ESA, Chelmsford, Massachusetts, USA). Internal 3-4-dihydroxybenzylamine (DHBA; Sigma, St. Louis, Missouri, USA) standard was used. The percentage recoveries of authentic DHBA standard were 64 to 77 %.

Data evaluation

All data obtained were analyzed together using statistical software (Prism v.4; Graphpad software, Inc. San Diego, USA). One-way analysis of variance (ANOVA) for repeated measures was used to examine the time effect within each group, and one-way ANOVA for group effect at each time point. When a significant difference was found, the Tukey test was used to compare the means.

The area under curve (AUC) was calculated for each biochemical variable. The AUC was measured by calculating the sum of the trapezoids formed by the data points and the x-axis. The level of significance in all tests was set at $P < 0.05$.

Results

Behavioral responses and physical parameters

A profound sedation was not observed in MID0.5 group. However, excitement-like behaviors such as ataxia behavior, salivation, and restlessness were observed. In the MED40, MED80, and MED40-MID0.5 groups, all cats showed a profound sedation and subsequently lateral recumbency. Vomiting was observed before profound sedation in all cats given the MED. The duration of lateral recumbency was 49 ± 11 (mean ± SEM) min in the MED40 group and 126 ± 14 (mean ± SEM) min in the MED80 group, whereas it was 88 ± 10 (mean ± SEM) min in the MED40-MID0.5 group. The duration of lateral recumbency in the MED40-MID0.5 group was significantly longer compared to the MED40 group ($P < 0.05$). However, there was no significant difference in the duration of lateral recumbency between the MED40-MID0.5 and the MED80 groups ($P > 0.05$).

No significant change in rectal temperature was not found in either control or the MED0.5 group. Rectal temperature decreased significantly ($P < 0.05$) at 1 to 5 h after administration in the MED40, MED80, and MED40-MID0.5 groups (Figure 12-A). The decrease of rectal temperature in the MED40-MID0.5 group tended to be greater than the MED40 group, but smaller than the MED80 group.

Heart rate decreased significantly ($P < 0.05$) at 0.25 to 2 h in either MED40 or MED40-MID0.5 group, and at 0.25 to 3 h in the MED80 group (Figure 12-B). There were no significant differences in heart rate between the MED40-MID0.5, and MED40 groups.

In the MID0.5 group, respiration rate tended to decrease at 0.5 h after administration, but not significantly (Figure 12-C). In the MED40 group, respiration rate decreased significantly ($P < 0.05$) at 2 to 4 h after administration. Similar tendency of decreased respiration rates were found in the MED80 group, but not significant. While, in the MED40-MID0.5 group, respiratory rate also tended to decrease, and to recover more rapidly than the MED40 group.

Glucose

Plasma glucose concentration increased after administration in the MED40, MED80, and MED40-MID0.5 groups. While, no significant change compared to baseline was observed in the control and MID0.5 groups (Figure 13-A). A significant increase of glucose value was found at 1 and 2 h after administration in the MED40 group, and the maximum value was 225 ± 72 (mean ± SD) mg/dL at 2 h. In MED80 group, glucose value increased significantly at 1 to 3 h after administration, and the maximum value was 267 ± 99 (mean ± SD) mg/dL at 2 h. Although glucose value tended to increase at 1 to 2 h in the MED40-MID0.5 group, no significant change was not found. The AUC (0 - 6 h) data of the MED40 and the MED80 groups were significantly greater compared to control group (Figure 13-A). The AUC (0 - 6 h) data of the MED40-MID0.5 group tended to be greater than that of control group, but no statistically significant difference was found. There was no significant difference in AUC value between the MID0.5 and control groups (Figure 2-B).

Insulin

Plasma insulin concentration in the MID0.5 group did not significantly change. In either MED40 or MED80 group, insulin concentration tended to decrease at 0.25 to 1 h after administration. After these suppressive changes, insulin concentration tended to increase over the baseline value in both groups (Figure 14-A). In the MED40-MID0.5 group, plasma insulin concentration decreased significantly at 0.25 to 1 h ($P < 0.05$), and subsequently increased significantly ($P < 0.05$) at 3 h after administration. The AUC (0 - 6 h) value did not significantly differ between the groups. However, AUC (0 - 1 h) value of plasma insulin concentration tended to be lower in the MED40, MED80, and MED40-MID0.5 groups than control group (Figure 14-B).

Glucagon

Glucagon levels of both the MID0.5 and control groups did not significantly change. In the MED40 and MED40-MID0.5 groups, plasma glucagon concentration tended to increase at 0.25 h, and then decrease at 2 or 3 h after administration, but not significant (Figure 15-A). In the MED80 group, glucagon concentration tended to decrease at 1 to 2 h after administration, but also not significant. The AUC (0 - 6 h) value of plasma glucagon did not reveal significant differences between any groups (Figure 15-B).

Cortisol

In MID0.5 group, cortisol value tended to increase at 0.5 h after administration, and then decrease gradually. Plasma cortisol level tended to slightly decrease at 1 h after administration in the MED40, MED80, and MED40-MID0.5 groups, but not significantly (Figure 16-A). Throughout the period of this experiment, cortisol level in the control group did not significantly change. The AUC (0 - 6 h) value of cortisol did not significantly differ among any groups (Figure 16-B).

NEFA

In the MED40, MED80, and MED40-MID0.5 groups, plasma NEFA concentrations decreased significantly ($P < 0.05$) at 0.5 to 3 h after drug administration (Figure 17-A). In the MID0.5 group, plasma NEFA concentration tended to decrease transiently at 0.25 h after administration, and then increase gradually. The AUC (0 - 4 h) values of NEFA concentration were significantly ($P < 0.05$) lower in the MED40, MED80, and MED40-MID0.5 groups compared to control (Figure 17-B).

Norepinephrine

In control group, there was no significant change throughout the period of this experiment. In the MID0.5 group, norepinephrine concentration increased significantly ($P < 0.05$) at 0.5 h after administration, and also tended to increase at 2 h. In the MED40 group, plasma norepinephrine concentration decreased significantly ($P < 0.05$) at 0.25 to 1 h after administration (Figure 18-A). In the MED80 group, norepinephrine concentration decreased significantly ($P < 0.05$) at 0.25 to 3 h after administration compared to baseline. Changes of norepinehrine concentration in the MED40-MID0.5 group were similar to those in the MED40 and MED80 groups, but not significant. The AUC(0 - 3 h) data of norepinephrine were lower significantly ($P < 0.05$) in the MED40, MED80, and MED40-MID0.5 groups compared to the MID0.5 group (Figure 18-B). The AUC (0 - 3 h) value was greater in the MED40-MID0.5 group than the MED40 group, but not significant.

Epinephrine

No significant change of plasma epinephrine concentration was found in all of groups throughout 24 h period of this experiment (Figure 19-A). However, epinephrine concentration tended to decrease from baseline at 0.25 h after administration in the MED40 group, at 0.25 to 0.5 h in the MED40-MID0.5 group, and at 0.25 to 2 h in the MED80 group. Whereas, in the MID0.5 group, epinephrine concentration tended to increase at 0.25 to 2 h after administration. There were no significant differences in AUC (0 - 6 h) values among any groups (Figure 19-B).

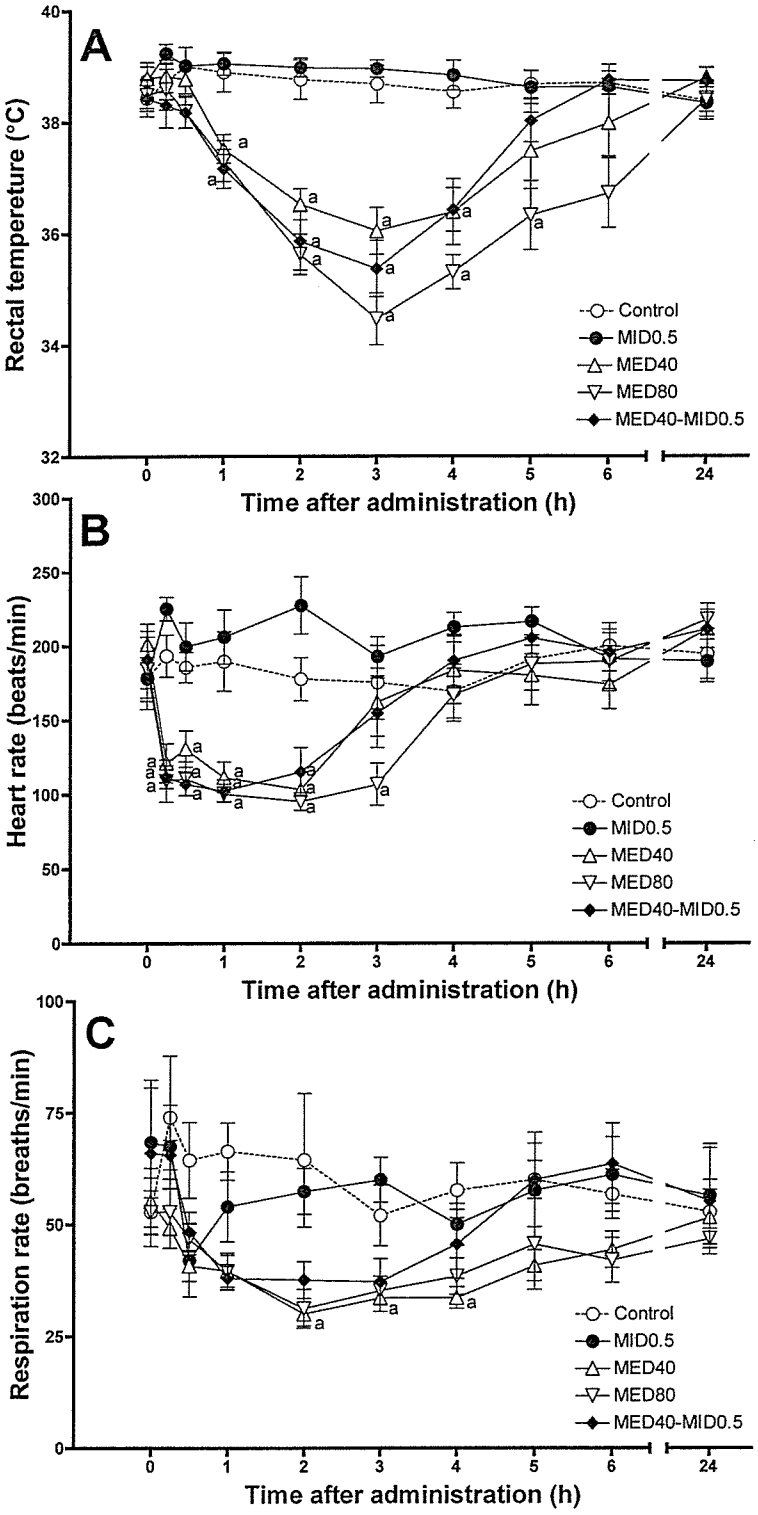

Figure 12. Rectal temperature (A), heart rate (B), and respiration rate (C) following the administration of medetomidine (MED μg/kg) and midazolam (MID mg/kg) in cats. a: significantly different from the initial value ($P < 0.05$). Each point and vertical bar represent the mean and SEM ($n = 5$).

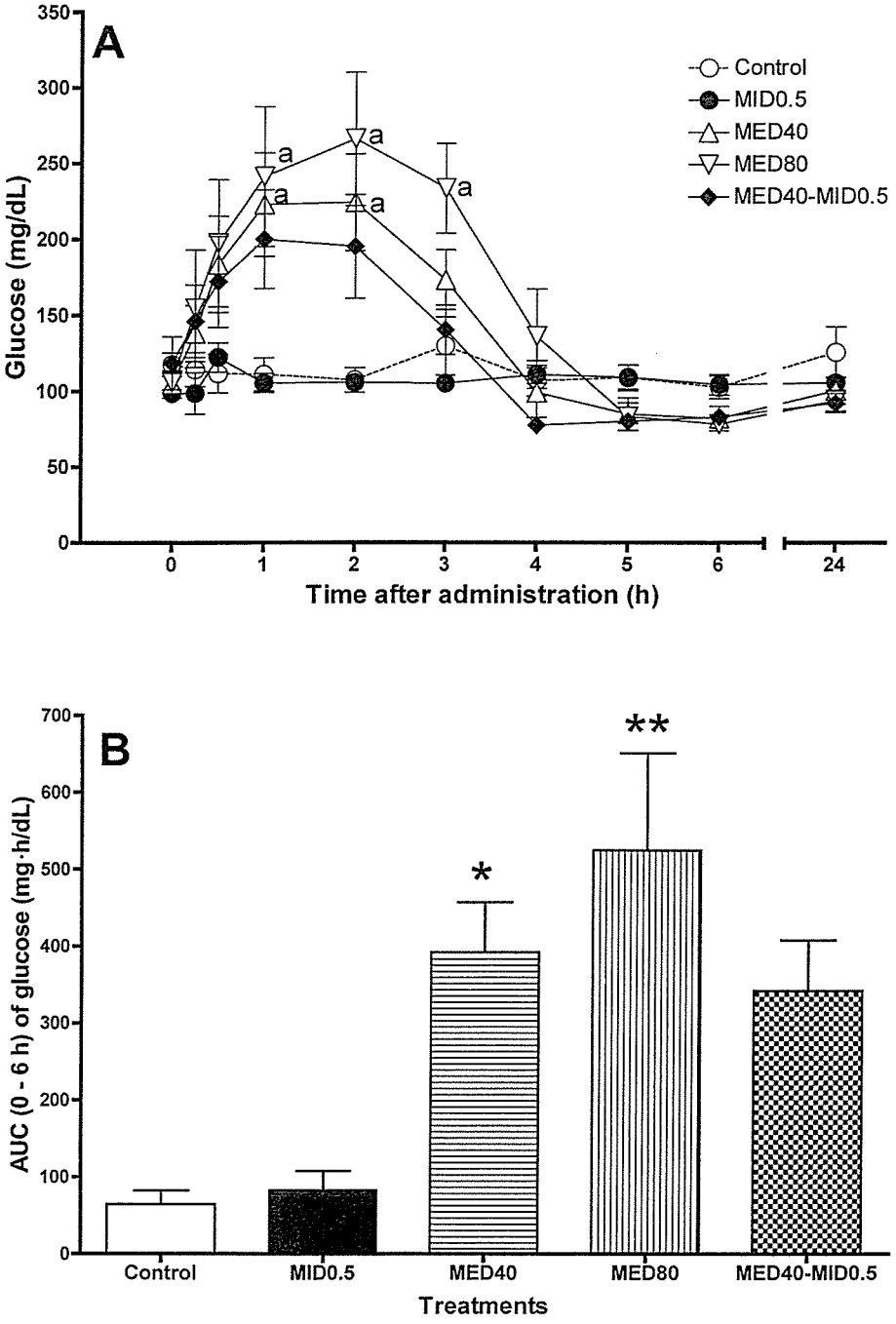

Figure 13. Plasma glucose levels following the administration of medetomidine (MED μg/kg) and midazolam (MID mg/kg) in cats (A). Each point and vertical bar represent the mean and SEM ($n = 5$). a: significantly different from the initial value ($P < 0.05$). The AUC (0 - 6 h) data of the plasma glucose graphs are plotted for the each treatment (B). Columns show the mean with vertical bars indicating SEM ($n = 5$). *, ** : significantly different from the control ($P < 0.05$, 0.01).

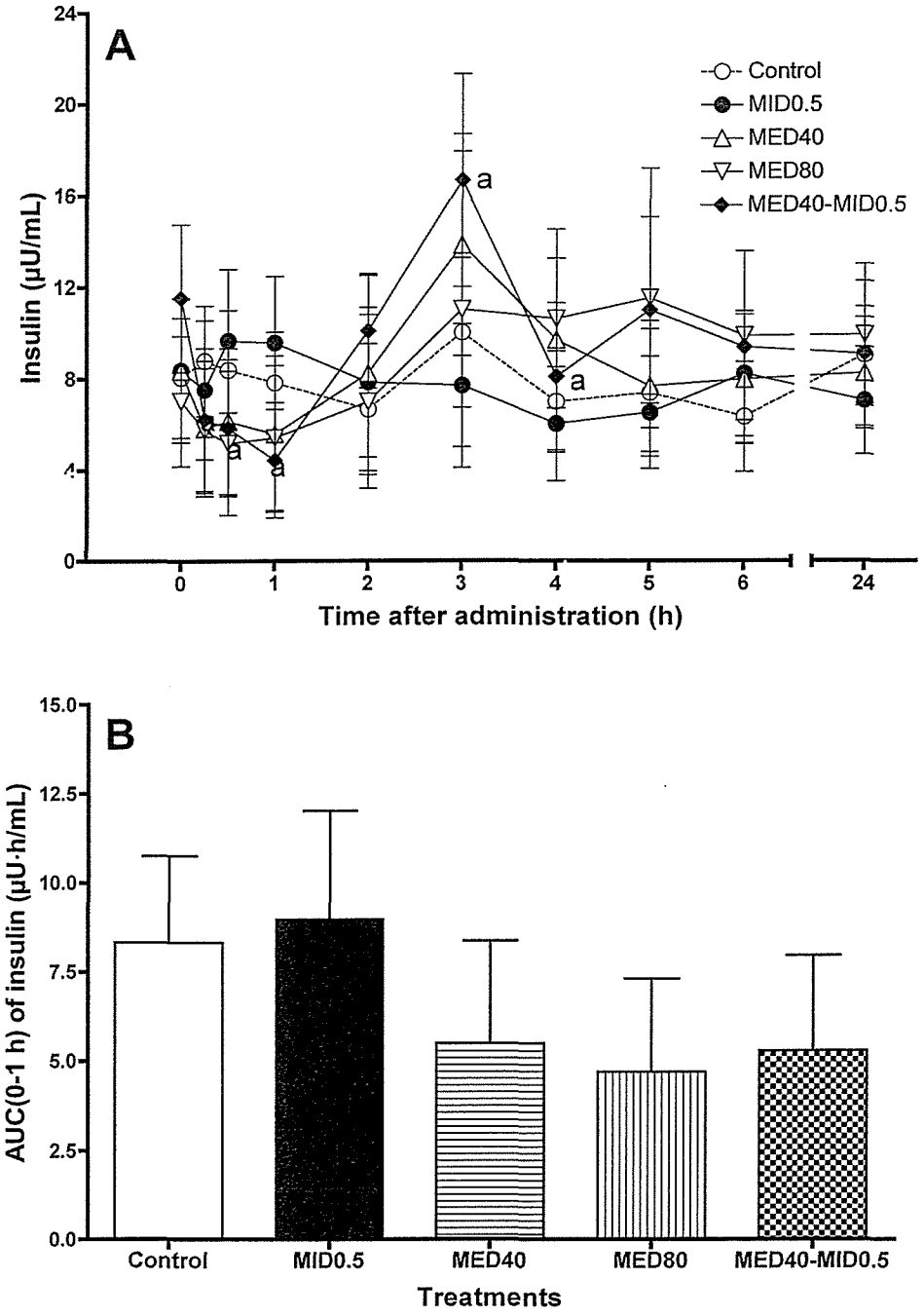

Figure 14. Plasma insulin levels following the administration of medetomidine (MED μg/kg) and midazolam (MID mg/kg) in cats (A). Each point and vertical bar represent the mean and SEM ($n = 5$). a: significantly different from the initial value ($P < 0.05$). The AUC (0 - 1 h) data of the plasma glucose graphs are plotted for the each treatment (B). Columns show the mean with vertical bars indicating SEM ($n = 5$).

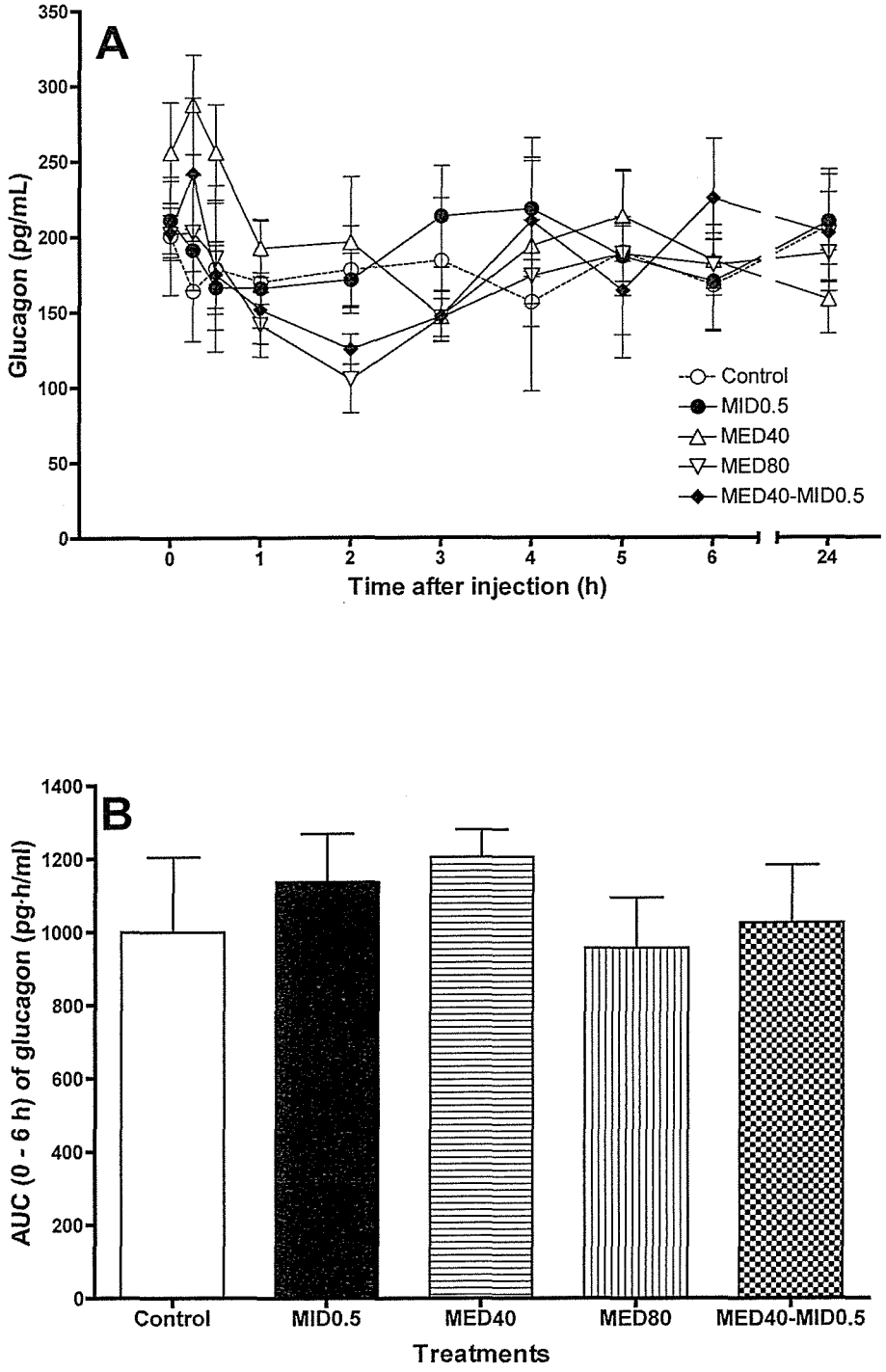

Figure 15. Plasma glucagon levels following the administration of medetomidine (MED μg/kg) and midazolam (MID mg/kg) in cats (A). Each point and vertical bar represent the mean and SEM (n = 5). The AUC (0 - 6 h) data of the plasma glucose graphs are plotted for the each treatment (B). Columns show the mean with vertical bars indicating SEM (n = 5).

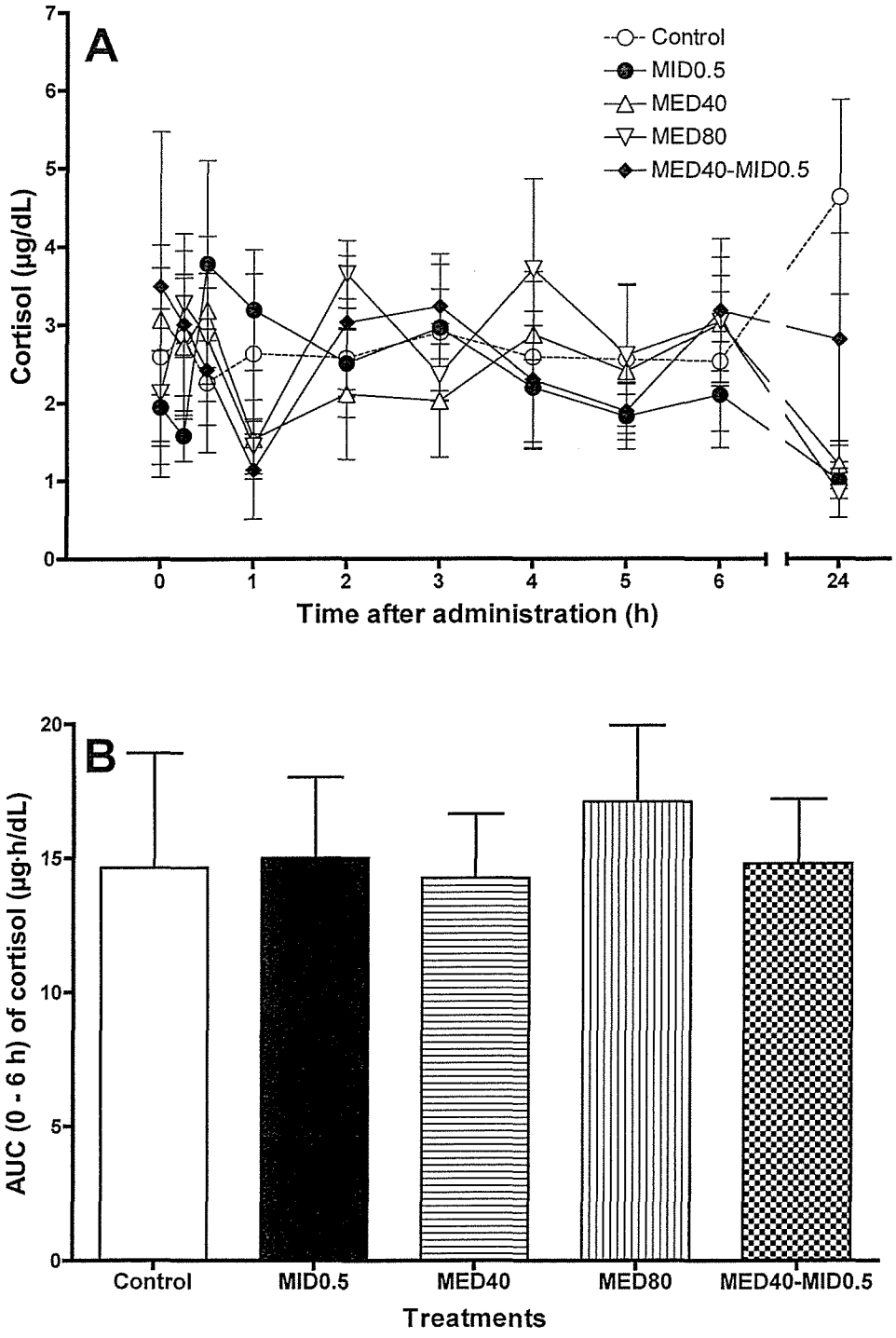

Figure 16. Plasma cortisol levels following the administration of medetomidine (MED µg/kg) and midazolam (MID mg/kg) in cats (A). Each point and vertical bar represent the mean and SEM (n = 5). The AUC (0 - 6 h) data of the plasma glucose graphs are plotted for the each treatment (B). Columns show the mean with vertical bars indicating SEM (n = 5).

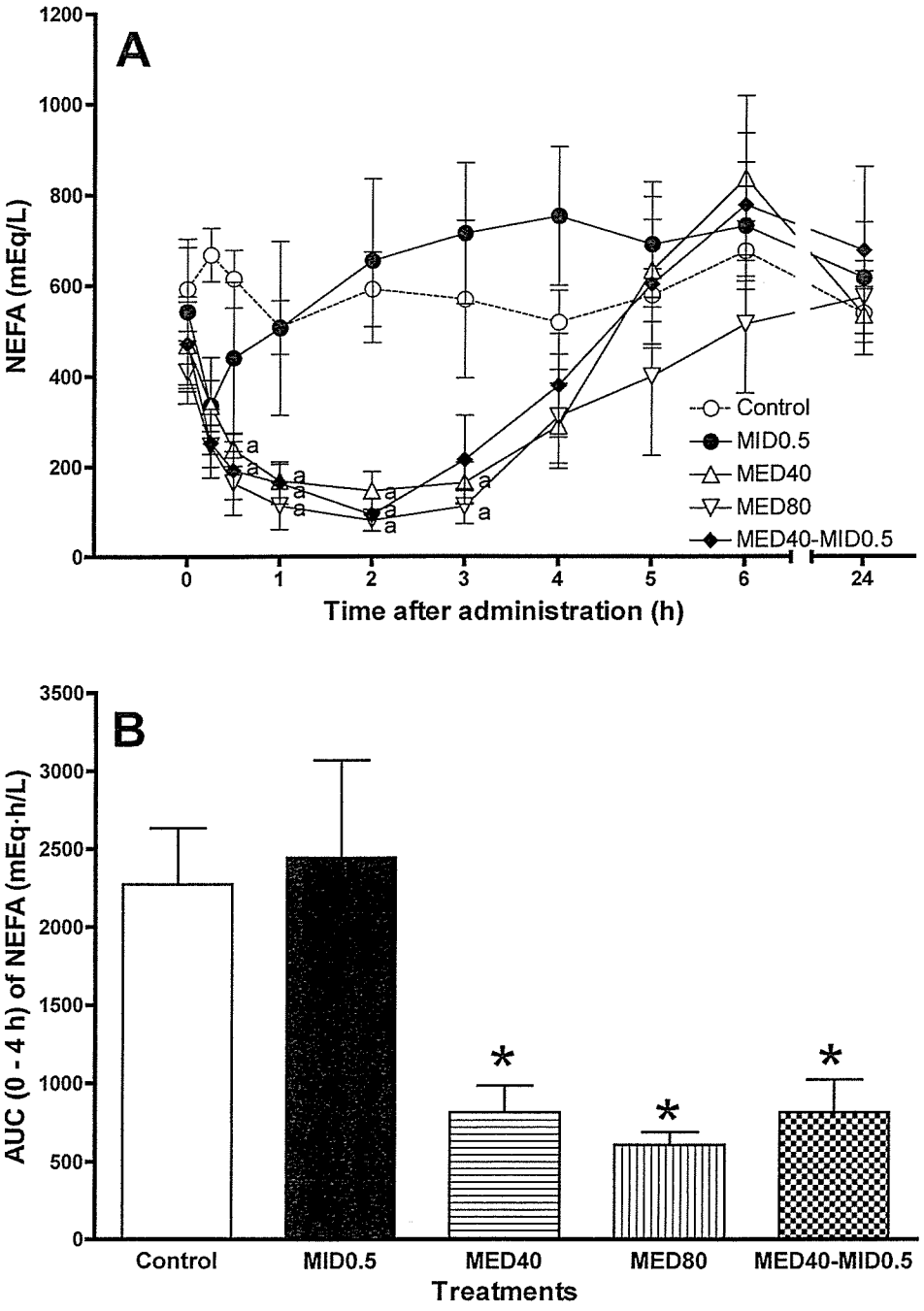

Figure 17. Plasma NEFA levels following the administration of medetomidine (MED μg/kg) and midazolam (MID mg/kg) in cats (A). Each point and vertical bar represent the mean and SEM ($n = 5$). a: significantly different from the initial value ($P < 0.05$). The AUC (0 - 4 h) data of the plasma glucose graphs are plotted for the each treatment (B). Columns show the mean with vertical bars indicating SEM ($n = 5$). * : significantly different from the control ($P < 0.01$).

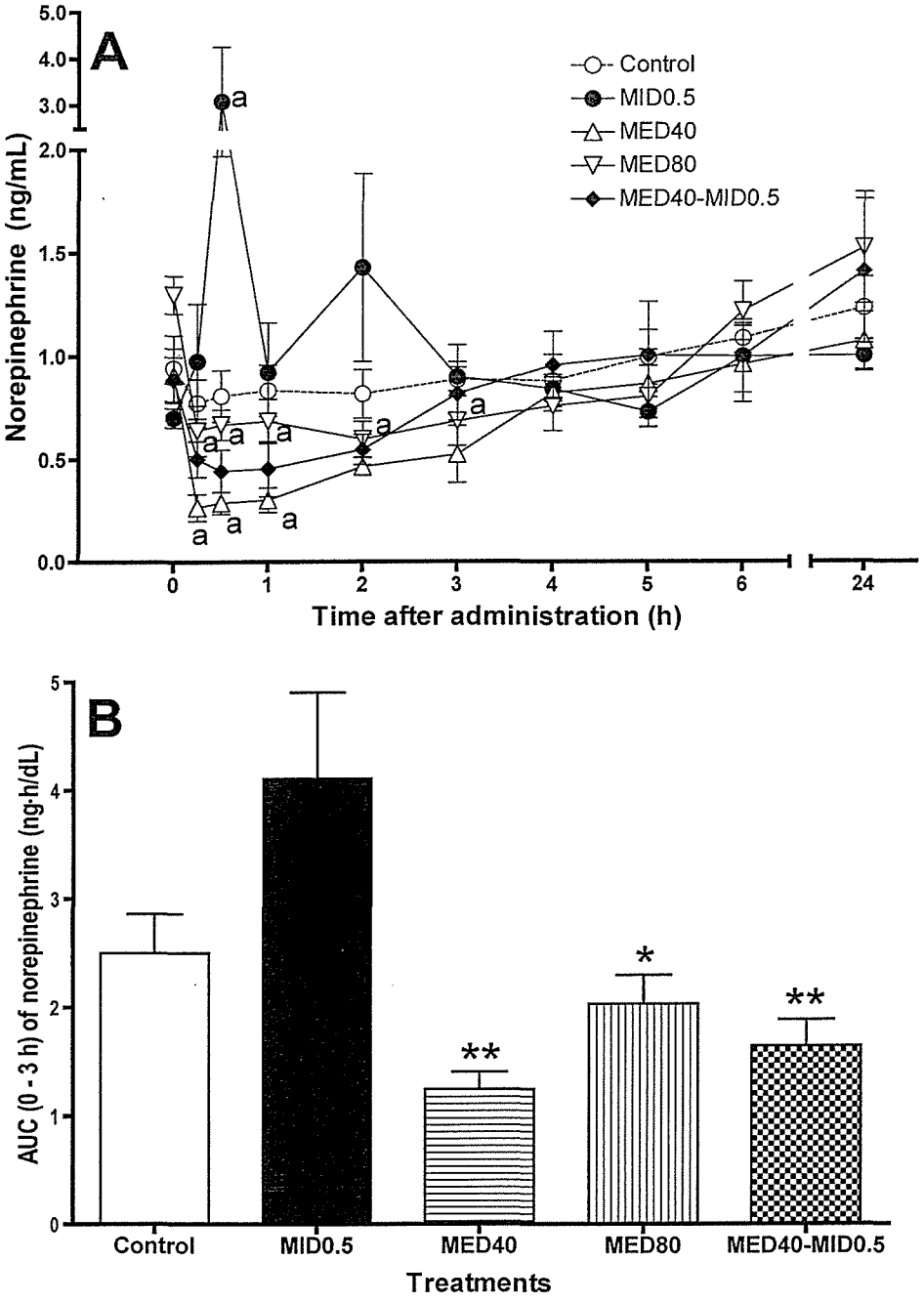

Figure 18. Plasma norepinephrine levels following the administration of medetomidine (MED μg/kg) and midazolam (MID mg/kg) in cats (A). Each point and vertical bar represent the mean and SEM (n = 5). a: significantly different from the initial value ($P < 0.05$). The AUC (0 - 3 h) data of the plasma glucose graphs are plotted for the each treatment (B). Columns show the mean with vertical bars indicating SEM (n = 5). *, **: significantly different from the MID0.5 ($P < 0.05$, 0.01).

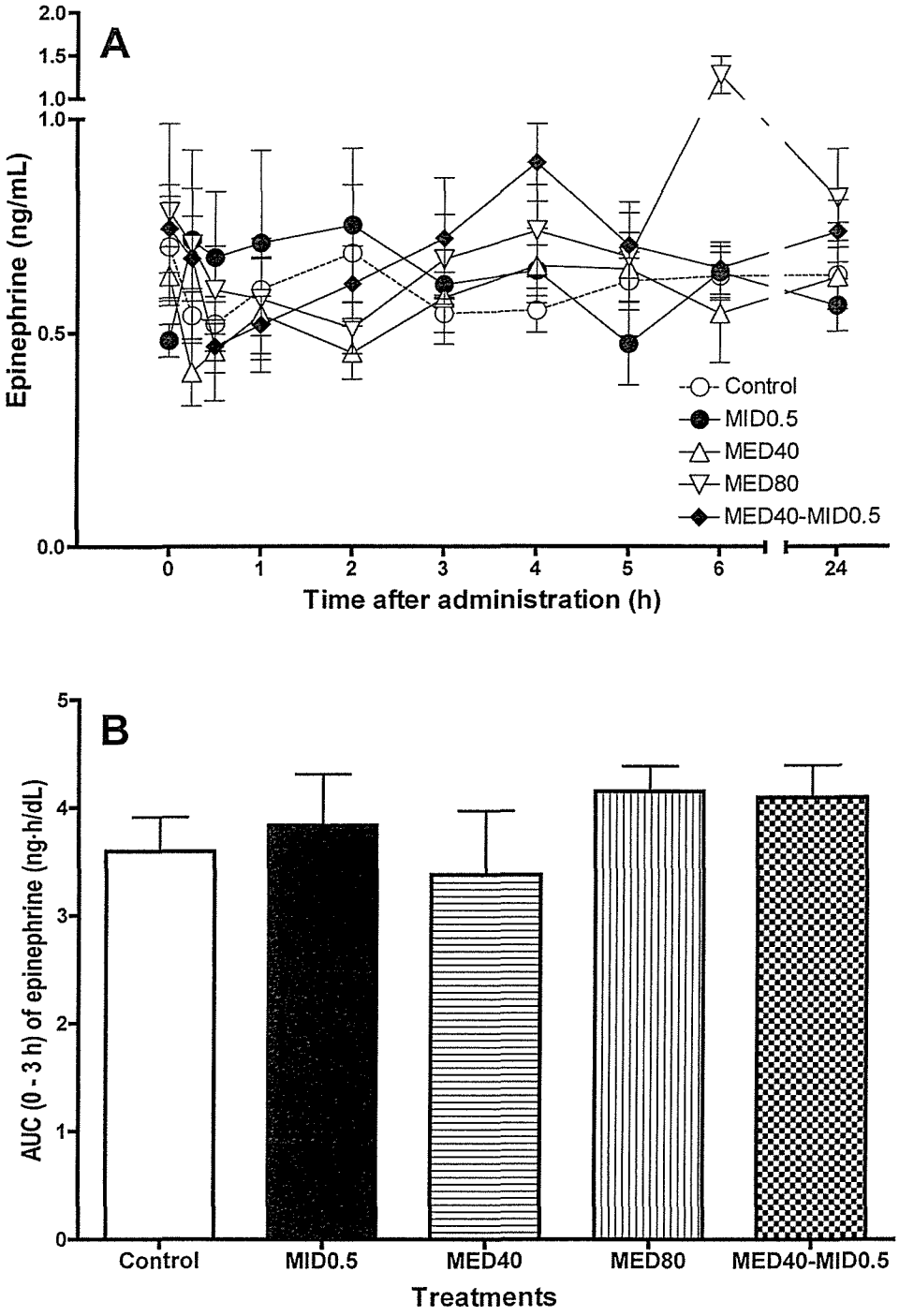

Figure 19. Plasma epinephrine levels following the administration of medetomidine (MED μg/kg) and midazolam (MID mg/kg) in cats (A). Each point and vertical bar represent the mean and SEM (n = 5). The AUC (0 - 6 h) data of the plasma glucose graphs are plotted for the each treatment (B). Columns show the mean with vertical bars indicating SEM (n = 5).

Discussion

The present study revealed that midazolam in combination with medetomidine significantly prolonged the duration of lateral recumbency induced by medetomidine in cats. Furthermore, the addition of midazolam to medetomidine reduced or did not enhance the adverse effects such as hypothermia, bradyarrhythmia, and bradypenia induced by medetomidine alone. Therefore, in spite of enhancement of the immobility duration, the combination of medetomidine and midazolam does not largely alter or partially diminish the medetomidine-induced adverse effects related to physical parameters measured in this study.

A previous study has revealed that medetomidine induces hyperglycemia in cats [58]. This is one of the undesirable effects for the use of medetomidine in feline practice. The hyperglycemia induced by medetomidine is accompanied by hyposinsulinemia similar to the diabetes mellitus. This hyperglycemic effect may limit the use of medetomidine in cats with metabolic and neurohormonal problems such as diabetes mellitus, ketosis, and glycosuria. Then, it was examined whether medetomidine-midazolam combination could influence the hyperglycemic effect of medetomidine. The present study revealed that medetomidine-midazolam combination tended to induce hyperglycemia, but not significantly, whereas either MED40 or MED80 induced apparent hyperglycemia. On the other hand, in the present experiment, MID0.5 alone did not produce a significant change of plasma glucose concentration in cats. These results indicated that MED40-MID0.5 combination can reduce the hyperglycemic effect induced by the same dose of medetomidine alone. This effect of midazolam in combination with medetomidine may be due to interactions between the α_2-adrenoceptors and benzodiazepine receptors or gamma-aminobutyric acid (GABA) receptors on the neurohormonal mechanisms related to hyperglycemia and hypoinsulinemia. However, the precise mechanism of this effect is unknown. This effect of medetomidine-midazolam combination on hyperglycemia was also reported in laboratory pigs [25]. To author's knowledge, this is the first report that medetomidine-midazolam

combination can reduce the increase in blood glucose levels induced by medetomidine alone in cats.

In this study, the decrease of plasma insulin concentration was not significantly different between MED40-MID0.5 and MID40. However, recovery from hypoinsulinemia induced by MED40-MID0.5 tended to be more rapid compared to MED40 alone. There are controversal reports on the effect of midazolam alone for insulin secretion from islet cells. Desborough et al [74] have reported that the use of midazolam was associated with a decrease in insulin secretion during upper abdominal surgery, and a decrease in blood glucose levels in humans. Desborough et al [75] also reported that midazolam did not directly inhibit glucose-stimulated insulin secretion from an *in vitro* rat islet preparation. In contrast, Cuparencu [76] reported that midazolam increased plasma insulin level in streptozotocin-induced diabetes in rats. In present present study, the midazolam alone did not significantly affect plasma insulin concentration in cats. On the other hand, an enhancement of the binding of GABA to low affinity receptor sites may give rise to many of the *in vivo* actions of the benzodiazepines [77]. GABA was also reported to inhibit insulin secretion from β cells at high concentrations of glucose [78, 79], and inhibit the release of glucagon from α cells [80]. These actions may also influence on the plasma glucose levels of cats given medetomidine-midazolam combination. The present results also indicated that the changes of glucagon levels induced by MED40-MID0.5 did not differ from those by MED40 alone. In addition, no significant differences in glucose levels were found between MID0.5 and control groups. Therefore, it seemed that the dose of midazolam used in this study did not affect the plasma glucagon and insulin levels.

Medetomidine and xylazine have been reported to inhibit norepinephrine release in either cats [61] or dogs [9]. The present results that MED40 and MED80 inhibited plasma norepinephrine release were in agreement with a previous result in cats [61]. Medetomidine-midazolam combination tended to reduce the medetomidine-induced

inhibition of norepinephrine release. This might be in part due to the effect of midazolam on norepinephrine release, because midazolam alone increased plasma norepinephrine levels. In contrast, it was reported that midazolam did not significantly change plasma norepinephrine concentrations in humans [81]. In this study, however, it is unknown whether this increase in norepinephrine concentration was induced by pharmacological effect of midazolam, or by excitement-like behavior associated with midazolam administration. This is the first report that midazolam increased plasma norepinephrine concentration in cats

Medetomidine, midazolam, and the combination of both drugs did not significantly change the plasma epinephrine concentration of cats in this study. The result as to the effect of midazolam was in agreement with a previous report in humans [81]. On the other hand, several studies reported that medetomidine reduced the plasma epinephrine concentration in cats [61] and dogs [9]. However, medetomidine has been also reported not to affect on the epinephrine level in humans [82]. Thus, the effect of medetomidine on the plasma epinephrine concentration was controversial.

In this study, there were no significant changes of plasma cortisol concentration in all of groups. The present results were similar to previous findings [61]. No significant differences were also found among the MED40, MED80, and MED40-MID0.5 groups. Therefore, these results indicated that the addition of midazolam does not affect the plasma cortisol concentration of cats given medetomidine.

Plasma NEFA concentration has been reported to be reduced by medetomidine in cats [61]. This response may be due to the suppression of lipolysis mediated by α_2-adrenoceptors [57, 58]. The results of this study confirmed a previous report in which medetomidine decreased plasma NEFA levels [61]. On the other hand, the changes of plasma NEFA concentrations in MED40-MID0.5 combination were similar to those in MED40 alone in this study. Therefore, the present results indicated that the addition of midazolam does not alter the inhibition of lipolysis induced by medetomidine in cats.

In conclusion, Medetomidine-midazolam combination reduced the hyperglycemia induced by medetomidine in cats. The decrease of plasma norepinephrine levels induced by medetomidine alone was diminished by the addition of midazolam. Midazolam alone increased plasma norepinephrine levels, but did not change glucose, insulin, glucagon, cortisol, epinephrine, and NEFA concentrations in cats. Therefore, this study revealed that medetomidine-midazolam combination produces minimal neurohormonal and metabolic changes without greater adverse effects when compared to medetomidine alone in healthy cats.

Chapter 4

General conclusion

In cats, both medetomidine and xylazine included remarkable hyperglycemia. It was greater in cats than dogs reported previously. It was found that hyperglycemia at early phase (0 to 2 hours after administration) in the medetomidine treated group was not dose-dependent. On the other hand, xylazine induced dose-dependent hyperglycemia through the experiments. The changes of insulin concentration did not show significant dose-dependency in either medetomidine or xylazine treated group. These results suggested the differences on the mechanism of hyperglycemia between medetomidine and xylazine, although both medetomidine and xylazine were α_2-adrenoceptor agonists. The differences on the affinity to each subtype of α-adrenoceptors between medetomidine and xylazine, and/or the affinity to the imidazoline receptor of medetomidine may contribute to differences on the mechanism of hyperglycemia. However, the present study could not reveal the precise mechanism.

Either medetomidine or xylazine decreased the concentration of norepinephrine. Higher dose of medetomidine (160, 320 μg/kg) significantly decreased the concentration of norepinephrine. While, xylazine tended to decrease the plasma norepinephrine concentration, but not significantly. Plasma NEFA concentration was also significantly decreased in both the medetomidine and xylazine treated groups. These results agree with a previous report in dogs.

In chapter 2, it was demonstrated that midazolam in the combination with medetomidine suppressed the hyperglycemia induced by medetomidine alone in cats. The combination of medetomidine and midazolam accelerated the recovery of insulin concentration compared to medetomidine alone. On the other hand, midazolam alone did not affect the plasma

concentrations of both glucose and insulin. Therefore, only when midazolam was administered in the combination with medetomidine, suppressed medetomidine-induced hyperglycemia was suppressed. The interactions among the α_2-adrenoceptors, imidazoline receptors, benzodiazepine receptors, and GABA receptors may be involved in these responses. However, the present study and any other reports did not reveal detailed mechanisms on the interactions of medetomidine and midazolam for hyperglycemic effect.

Plasma concentration of norepinephrine was decreased by medetomidine administration. The addition of midazolam suppressed the decrease of norepinephrine concentration induced by medetomidine. It may due to the effect of midazolam because midazolam alone increased plasma norepinephrine concentration.

In conclusion, the present study revealed that medetomidine unlike xylazine did not induce dose-dependent hyperglycemia at early phase in cats. Such hyperglycemia was greater in cats than dogs reported previously. This study revealed that midazolam in combination with medetomidine suppress the hyperglycemia induced by medetomidine alone in cats.

Summary

In chapter 1, the effects of xylazine and medetomidine on some of neurohormonal and metabolic variables were investigated and compared in healthy cats. Five cats were used repeatedly in each of 11 groups, which were treated with the physiological saline solution (control), 0.5, 1, 2, 4, and 8 mg/kg xylazine, and 20, 40, 80, 160, and 320 μg/kg medetomidine intramuscularly. Blood samples were taken for 10 times during 24 h from the jugular vein. Plasma glucose, insulin, cortisol, epinephrine, norepinephrine, glucagon, and nonesterified fatty acid concentrations were determined. Both xylazine and medetomidine induced remarkable hyperglycemia in a dose-dependent manner, but the hyperglycemic response of medetomidine from 0 to 3 h was not dose-dependent. Both xylazine and medetomidine suppressed epinephrine and norepinephrine release. In both xylazine and medetomidine groups, the suppression of catecholamine release was not dose-dependent at the tested dosages. Both agents inhibited insulin release and lipolysis with similar potency, and tended to suppress cortisol release. The glucagon levels did not change significantly in any of groups. These results suggested that the effects of medetomidine and xylazine on glucose metabolism and catecholamine release may not be due only to the actions mediated by α_2-adrenoceptors.

In chapter 2, the effects of medetomidine-midazolam combination on some neurohormonal and metabolic variables were investigated in healthy cats. Five cats were used repeatedly in each of 5 groups, which were treated with physiological saline solution (control), 0.5 mg/kg midazolam, 40 μg/kg medetomidine, 80 μg/kg medetomidine, and 40 μg/kg medetomidine and 0.5 mg/kg midazolam intramuscularly. Blood samples were taken for 10 times during 24 h from the catheter introduced into the juglar vein. Plasma glucose, insulin, glucagon, cortisol, nonesterified fatty acid (NEFA), norepinephrine, and epinephrine concentrations were determined. Additionally, duration of lateral recumbency, rectal

temperature, heart and respiration rates were examined. The combination of medetomidine and midazolam enhanced the duration of lateral recumbency and reduced the hyperglycemia induced by medetomidine alone. Recovery from hypoinsulinemia induced by medetomidine-midazolam combination tended to be more rapid compared to same dose of medetomidine alone. The decrease of plasma norepinephrine levels induced by medetomidine alone was diminished by the addition of midazolam to medetomidine. Midazolam alone did not significantly change plasma glucose, insulin, glucagon, cortisol, epinephrine, and NEFA concentrations, but increased norepinephrine concentration. This study revealed that the combination of medetomidine and midazolam produces minimal neurohormonal and metabolic changes when compared to medetomidine alone in cats.

Acknowledgements

The author wishes to acknowledge Dr. Yoshiaki Hikasa (Tottori Universiy) for his appropriate guidance as supervisor, critical review of the manuscript, and escorting the first steps as veterinary researcher. The author greatfully appreciate Dr. Takashi Takeuchi (Tottori University) and Dr. Masaru Okuda (Yamaguchi University) for their technical advises and suggestions on this study. The author thanks Dr. Kota Sato (Hokkaido University) for his valuable suggestions.

The author especially grateful to the students of the Laboratory of Veterinary Internal Medicine, Tottori University including Hajime Takahashi, Kanako Sato and Hiroe Kawamura for their indispensable technical assistance.

The author greatly indebted the the Japan Society for the Promotion of Science and Meiji Seika, Tokyo, Japan for supporting this study.

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