The Stress Related Neuroendocrine and Metabolic Effects of Alpha-2 Adrenergic Agents and Their Combinations with Injectable Analgesics in Dogs

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Chapter 1

General introduction

The α_2-adrenoceptor agonists, medetomidine and xylazine, are widely used in veterinary practice for different purposes. They are mainly used as sedative, muscle relaxant and analgesic agents in anaesthesia of different species [1,2]. By reducing gastric and intestinal motility, α_2-agonists are useful for gastrointestinal surgery or endoscopy [1]. These drugs are also reliable emetics for small animals [3,4]. In addition, xylazine is used as a diagnostic agent for congenital or acquired hyposomatotropism in dogs and cats [5].

Although α_2-agonists are multipotent drugs, they should be used carefully, because unexplained and sometimes fatal accidents may be associated with their use in the healthy small animal patient, even without painful intervention [6,7]. These are usually associated with the cardiovascular side effects of these drugs [1,2]. However, whether the neuroendocrine and metabolic effects of α_2-agonists are involved in the causes of these accidents are not fully understood. There are limited data on the usage of medetomidine and xylazine for anaesthesia of patients with cardiovascular or respiratory illness [1,8]. In spite of the fact that α_2-agonists strongly interfere with the neuroendocrine system, there is still no report to prove the effects of these agents on different endocrine or metabolic diseases such as diabetes mellitus, Cushing and Addison diseases. Numerous studies have shown that α_2-agonists such as xylazine and clonidine decrease plasma catecholamine and cortisol levels [9-11], inhibit insulin release [12,13] and lipolysis [14,15], and increase plasma glucose [16,17] and glucagon

[18] levels in various species. However, specific data on the effects of medetomidine and xylazine especially time and dose relations, are still insufficient in dogs. The effects of different dosages of medetomidine and xylazine on basal plasma cortisol and nonesterified fatty acid (NEFA) levels have not been examined in dogs yet. The purpose of this study was to investigate and compare the effects of medetomidine and xylazine on some stress related neurohormonal and metabolic variables (catecholamines, cortisol, glucose, insulin, glucagon and NEFA) at blood levels in dogs.

Medetomidine can reduce stress response to surgery as assessed by the attenuation of plasma catecholamine, adrenocorticotrophic hormone and cortisol levels [19]. Stress is a generalised response of the body to various factors, called stressors. Pain, blood loss, excitement, and underlying pathological conditions may all act as stressors in the surgical patient. The endocrine and metabolic stress response is characterised by the increase of catecholamine, cortisol, glucose, and NEFA blood levels, and the decrease of insulin levels [20]. Adrenoceptors play an important role in the co-ordination of these events therefore α_2-adrenergic agents may interfere to the pathophysiology of stress response before, during and after anaesthesia. That's why there is an increasing interest in using medetomidine as a pre-anaesthetic adjuvant or as a part of balanced anaesthesia [19].

If necessary, the actions of medetomidine can be reversed by α_2-adrenoceptor antagonists, such as the highly receptor specific atipamezole, or the less specific yohimbine [2]. The use of these antagonists may also have adverse effects, like hypotension, tachycardia, over alertness, and the absence of analgesia [2,21]. But weather the acceleration of stress response after antagonising may contribute to the fatal outcome of some patients is not fully understood. The stress-related hormonal and metabolic effects of antagonising an α_2-agonist by an antagonist, had already been

reported in horse [22], cattle and sheep [23]. However, such study has not yet been published in dogs. The purpose of this study was to investigate and compare the reversal effects of three different doses of atipamezole, and a single dose of yohimbine on stress-related hormonal and metabolic responses following medetomidine administration in dogs.

Other analgesics have endocrine and metabolic effects also [24,25]. Because analgesics act on similar receptor systems than the physiological stress-response there are certain analogy between the endocrine effects of analgesics and stress-response. The purpose of anaesthesia is to induce optimal painless condition and attenuate stress-response in the majority of cases for veterinary anaesthesia. However, critically ill patients sometimes need sympathetic support and suppressing their otherwise insufficient endocrine stress-response by some analgesics can be disadvantageous [26]. Proper management of the endocrine status is crucial for the surgical patient, therefore examination of the endocrine effects of different analgesics and anaesthetics are in the focus of many recent research projects. For this purpose we compared the neurohormonal and metabolic effects of opioid drugs (butorphanol and fentanyl) and ketamine.

Alpha-2 adrenoceptor agonists are often used in combination with opioids to produce sedation, analgesia and balanced anaesthesia for different species [27-30]. Such a combination is advantageous because the sedative and analgesic effects are more than additive between these drugs [31-33]. This can be explained with the synergistic interaction in antinociception between the α_2-adrenoceptors and the μ opioid receptors in the spinal cord [34]. Especially the medetomidine-butorphanol combinations became widely used in the canine practice because of their few side effects [35,36]. In contrast, fentanyl is rarely used in combination with medetomidine because of the profound

respiratory depression it may cause [37]. Medetomidine is also often combined with ketamine to provide balanced anaesthesia in dogs [38] and cats [39]. There are insufficient data on the stress-related neurohormonal and metabolic effects of these combinations. Therefore, we finally examined the effects of balanced anaesthesia with medetomidine in combination with butorphanol, fentanyl and ketamine in dogs.

Chapter 2

Neurohormonal and metabolic effects of medetomidine compared with xylazine in beagle dogs

The α_2-adrenoceptor agonists, medetomidine and xylazine, are widely used in veterinary practice for different purposes. They are mainly used as sedative, muscle relaxant and analgesic agents in anaesthesia of different species [1,2]. By reducing gastric and intestinal motility, α_2-agonists are useful for gastrointestinal surgery or endoscopy [1]. These drugs are also reliable emetics for small animals [3,4]. In addition, xylazine is used as a diagnostic agent for congenital or acquired hyposomatotropism in dogs and cats [5].

Although α_2-agonists are multipotent drugs, they should be used carefully, because unexplained and sometimes fatal accidents may be associated with their use in the healthy small animal patient, even without painful intervention [6,7]. These are usually associated with the cardiovascular side effects of these drugs [1,2]. However, whether the neuroendocrine and metabolic effects of α_2-agonists are involved in the causes of these accidents are not fully understood. There are limited data on the usage of medetomidine and xylazine for anaesthesia of patients with cardiovascular or respiratory illness [1,8]. In spite of the fact that α_2-agonists strongly interfere with the neuroendocrine system, there is still no report to prove the effects of these agents on different endocrine or metabolic diseases such as diabetes mellitus, Cushing and

Addison diseases. Numerous studies have shown that α_2-agonists such as xylazine and clonidine decrease plasma catecholamine and cortisol levels [9-11], inhibit insulin release [12,13] and lipolysis [14,15], and increase plasma glucose [16,17] and glucagon [18] levels in various species. However, specific data on the effects of medetomidine and xylazine especially time and dose relations, are still insufficient in dogs. The effects of different dosages of medetomidine and xylazine on basal plasma cortisol and nonesterified fatty acid (NEFA) levels have not been examined in dogs yet. Such study may provide useful information for the usage of α_2-agonists under different pathologic conditions or stress associated with surgical intervention.

The purpose of this study was to investigate and compare the effects of medetomidine and xylazine on some stress related neurohormonal and metabolic variables (catecholamines, cortisol, glucose, insulin, glucagon and NEFA) at blood levels in dogs. This study also aimed to examine the dose relation of the effects induced by the two drugs. Because both medetomidine and xylazine exert their actions mainly on α_2-adrenoceptors, we hypothesised that there are no differences between their effects on the blood levels of the examined variables.

Materials and methods

Animals

Nine healthy beagle dogs of either sex, weighing from 9 to 14 kg and ageing from 1 to 4 years, were used. All dogs were housed in our laboratory for at least one month before the experiment, and fed standard dry dog food. Routine haematological and plasma biochemical tests had been performed before the experiment. All values were within the normal physiological range. One day before the experiment, the animals were placed into separate cages in the experimental room controlled at 25 °C by air conditioning. Food and water were withheld for 12 h before the drug injection, and water was offered again after complete recovery from sedation. The feeding time was always 9 o'clock p.m. after the last blood sampling of that day. The experimental protocols were approved by the Animal Research Committee of Tottori University.

Experimental protocol

Two experiments were included in this study. The first experiment consisted of 7 groups with an intramuscular treatment in each. The treatments were physiological saline solution (0.5 mL); 10, 20, 40 and 80 μg/kg medetomidine HCl (1 % solution, Domitor, Meiji Seika Kaisha Ltd., Tokyo, Japan); and 1 and 2 mg/kg xylazine HCl (2 % solution, Celactal, Bayer, Tokyo, Japan). Five beagle dogs were used repeatedly in each of the 7 groups at weekly intervals according to a randomised design. The second experiment was designed to provide additional data about the effects of 4 and 8 mg/kg xylazine, especially on blood glucose. Four other beagle dogs were used for the 2 additional groups at a week interval in randomised order. The treatments will be referred to as MED-10, -20, -40 and -80 for medetomidine, and XYL-1, -2, -4 and -8 for xylazine treated groups.

The base of the comparison of selected dosages in this study was that in our preliminary investigations the MED-10 and -20 showed similar level and duration of sedation induced by XYL-1 and –2, respectively. Consequently, the sedative effect when assessed by lateral and thoracic recumbency position, lasted for 65 ± 10 and 61 ± 6 min (mean $\pm$ SEM) in MED-10, and XYL-1, 112 ± 14 and 116 ± 18 min in MED-20 and XYL-2, 210 ± 16 and 181 ± 29 min in MED-40 and XYL-4, and 270 ± 35 and 209 ± 24 min in MED-80 and XYL-8 groups, respectively. Therefore, these pairs of MED and XYL produced similar duration of sedation in this study. However, higher dosages of xylazine induced more alertness, sometimes anxiety and muscle rigidity. That is why 4 and 8 mg/kg xylazine dosages are not recommended for clinical practice.

This study was designed to model clinical conditions (except for XYL-4 and -8). Because α_2-agonists are more often used intramuscularly [7], this route was preferred in our study. The femoral biceps muscle was used for injections. The concentrations of the medicines were kept at commercially recommended levels (1 % for medetomidine and 2 % for xylazine) as they are normally used in practice. Subsequently, the injected volumes were different between the treatments. This might have affected the speed and completeness of absorption, but this bias was also present under clinical conditions.

Instrumentation

Under local anaesthesia with 2 % lidocaine (Xylocaine, Fujisawa Pharmaceutical Co., Ltd. Osaka, Japan), a 16-gauge central venous (CV) catheter was introduced into the jugular vein up to the central vein area. The CV catheter was flushed with 0.5 mL of heparinized physiological saline solution, capped, and fixed. The catheter was placed in the evening before the experiment and removed after the last blood sampling. There was no remarkable inflammatory sign at the catheterised site during the course of the experiment.

Sample collection

In the first experiment, blood samples were collected from the CV catheter for the following 10 times: 0 (initial value before drug administration), 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after drug injection. The initial 1 mL of blood collected from the CV catheter was discarded to avoid contamination by heparin. The following 5 mL of blood was used as a sample. One mL of each blood sample was mixed with Trasylol (Bayer, Leverkusen, Germany) separately for glucagon measurement, and the remaining 4 mL blood was mixed with EDTA for other measurements. Both samples were centrifuged immediately at 4 °C, then the plasma was separated and frozen at -80 °C and measured within 3 months. Plasma catecholamines (norepinephrine and epinephrine), cortisol, glucose, insulin, glucagon, and NEFA were measured in all samples.

The blood samples for the second experiment (XYL-4 and -8) did not include Trasylol samples for glucagon measurements at any time, and the EDTA blood samplings at 12 h were omitted.

Analytical methods

Catecholamines were extracted on activated alumina according to the method described by Bouloux *et al* [40], and measured by a high performance liquid chromatography (LaChrom, Hitachi Ltd. Tokyo, Japan) combined with an electrochemical detector (Coulochem II, ESA. Inc. Chelmsford, USA). Cortisol was measured by single antibody radioimmunoassay (RIA) technique using a commercially available kit (I-AE16, Eiken Chemical Co. Ltd. Tokyo, Japan). Insulin and glucagon were measured by double antibody RIA technique (I-AJ16, Eiken Chemical Co. Ltd. Tokyo, Japan, and Glucagon kit Daiichi, TFB Stock Company, Tokyo, Japan). Glucose and NEFA values were determined by use of a spectrophotometer (Auto Sipper Photometer U-1080, Hitachi Ltd. Tokyo, Japan).

Data evaluation

All data obtained were analysed together using the StatView software. 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's test was used to compare the means.

Quadratic curves were fitted to the data of each group at the recovery period, and the slope was used to compare the speed of recovery among the treatments. When the time effect was significant by ANOVA and Tukey's test (norepinephrine, epinephrine, glucose, insulin and NEFA), the normalised area under curve (AUC) was calculated. The AUC was measured by calculating the sum of the trapezoids formed by the data points and the x-axis from 0 h to 6 or 8 h. The difference between the mean AUC of the control group and the AUC of a certain individual was defined as the normalised AUC. The normalised AUC data were plotted versus dose, in either XYL or MED treatment, and simple linear regression analysis was applied in each. When significance was found, the effect of XYL or MED on the plasma level of the examined biochemical was claimed to be dose-related.

The normalised AUC data was also used for general comparison of medetomidine and xylazine. The AUC data of every variable were divided into two groups, medetomidine and xylazine, regardless of the applied dose. These two groups were compared with unpaired *t*-test. This method provides an overall comparison between the potency of the effects of medetomidine and xylazine treatments. The level of significance of all tests was set at $p<0.05$.

Results

The norepinephrine levels significantly decreased at 0.5 h after the injection of medetomidine (Figure 1). This effect was the shortest in MED-10 in which norepinephrine levels decreased until 1 h and then returned to its initial value gradually. The norepinephrine values in MED-20 at 0.5 to 3 h were significantly lower than the initial value. The norepinephrine release in both MED-40 and -80 treatments was similarly suppressed until 4 h, and then slowly returned to baseline. These findings, the results of the slope of the recovery phase and the regression analysis of the normalised AUC data, all indicated that medetomidine suppressed the norepinephrine release dose-dependently. The norepinephrine values decreased after the xylazine treatments, and remained significantly low until 2 h in XYL-1, 3 h in XYL-2, 4 h in XYL-4 and 6 h in XYL-8 groups (Figure 1). The 24 h value increased significantly over baseline in XYL-8. The slopes of the recovery phases and the regression analysis of the AUC determinations proved that xylazine also decreased plasma norepinephrine levels dose-dependently. The overall comparison of the AUC data by *t*-test indicated that MED and XYL suppressed norepinephrine level with a similar degree.

The mean values of epinephrine in all treatment groups (except for XYL-1) decreased to a significantly low level at 0.5 h and 1 h post-injection and gradually returned to baseline (Figure 2). There was a tendency to decrease at 0.5 and 1 h in XYL-1, but it was not significant. The suppression of epinephrine release was similar in both MED-80 and XYL-8 according to the AUC results, and the significantly decreased levels continued until 4 h in both groups. The linear regression of AUC data was significant in the MED groups, indicating that MED decreased the plasma epinephrine levels dose-dependently. Although the effect of XYL on plasma epinephrine was not significant by regression analysis, the AUC data of epinephrine were significantly

different between XYL-1 and XYL-8 by Tukey's test. The slopes of the recovery phases also decreased dose-dependently in both MED and XYL. Comparing the AUC data by *t*-test we found that MED more potentially suppressed epinephrine release than XYL.

Glucose values increased significantly after injection in all groups except for both MED-10 and control groups (Figure 3). Both 1 and 2 h-values (124 ± 4 and 123 ± 9; mg/dL, mean ± SEM) in MED-10 group tended to increase from the baseline value (103 ± 11), but it was not significant. In the medetomidine groups, the highest glucose level shifted to the right as the dosage increased. Namely, the glucose level peaked at 2 h (161 ± 8) in MED-20, at 3 h (147 ± 10) in MED-40 and at 4 h (151 ± 19) in MED-80. The regression analysis of the AUC data was non-significant (Figure 4). Therefore, medetomidine did not show dose-related increase in blood glucose level, and higher doses of medetomidine resulted in even lower glucose levels than MED-20. On the other hand, this was not the case in the xylazine groups. Xylazine increased glucose values dose-dependently. Especially, the glucose levels in XYL-4 and XYL-8 groups were greatly increased when compared with the medetomidine groups. The regression analysis of the AUC data was also highly significant (Figure 4). However, just like in medetomidine groups, the highest glucose levels tended to shift right as the dosage of xylazine increased. The peaks were observed at 2 h in both XYL-1 and XYL-2 (126 ± 7 and 164 ± 12), and at 4 h in both XYL-4 and XYL-8 groups (256 ± 56 and 281 ± 39) after injection. The overall comparison of the AUC data showed that the hyperglycaemic effect of XYL was significantly greater than that of MED.

The insulin values significantly lowered at 0.5 h after injection of MED or XYL, and then gradually returned to baseline in all treatment groups (Figure 5). Significant decreases in the insulin level lasted for 2 and 3 h in MED-40 and MED-80 groups, and for 2 h in XYL-4 and XYL-8 groups. The slopes of the recovery phases indicated that

the plasma insulin levels returned to baseline in a dose-related manner after MED injection, and the slope was smaller in XYL-8 than XYL-1, -2 and -4 groups. The regression analysis of the AUC data was not significant in either MED or XYL treatment. The overall comparison also failed to prove significant difference between MED and XYL on plasma insulin levels. The high variances among the data may explain why the regression analysis was non-significant. This may result from the biases in absorption after intramuscular injections in this experiment.

NEFA significantly decreased at 0.5 h in all groups treated with medetomidine and xylazine, and gradually returned to baseline (Figure 6). The NEFA levels significantly decreased until 1, 2, 3 and 4 h in the MED-10, -20 -40 and -80 groups, respectively. On the other hand, NEFA levels were significantly low until 2 h in all XYL treated groups. The NEFA levels in XYL-4 and -8 tended to decrease until 6 and 8 h respectively, but it was not significant. There were significant increases over baseline in some of the MED groups at 6 to 12 h and at 24 h in XYL-8. The regression analysis of the AUC data and the slope data of the recovery phases indicated that the effects of both MED and XYL on plasma NEFA levels were dose-related. The overall comparison failed to prove significant difference between MED and XYL treatments in the degree of NEFA suppression.

Both cortisol and glucagon values did not change significantly during the course of experiment in any of the treatment groups (data is not shown).
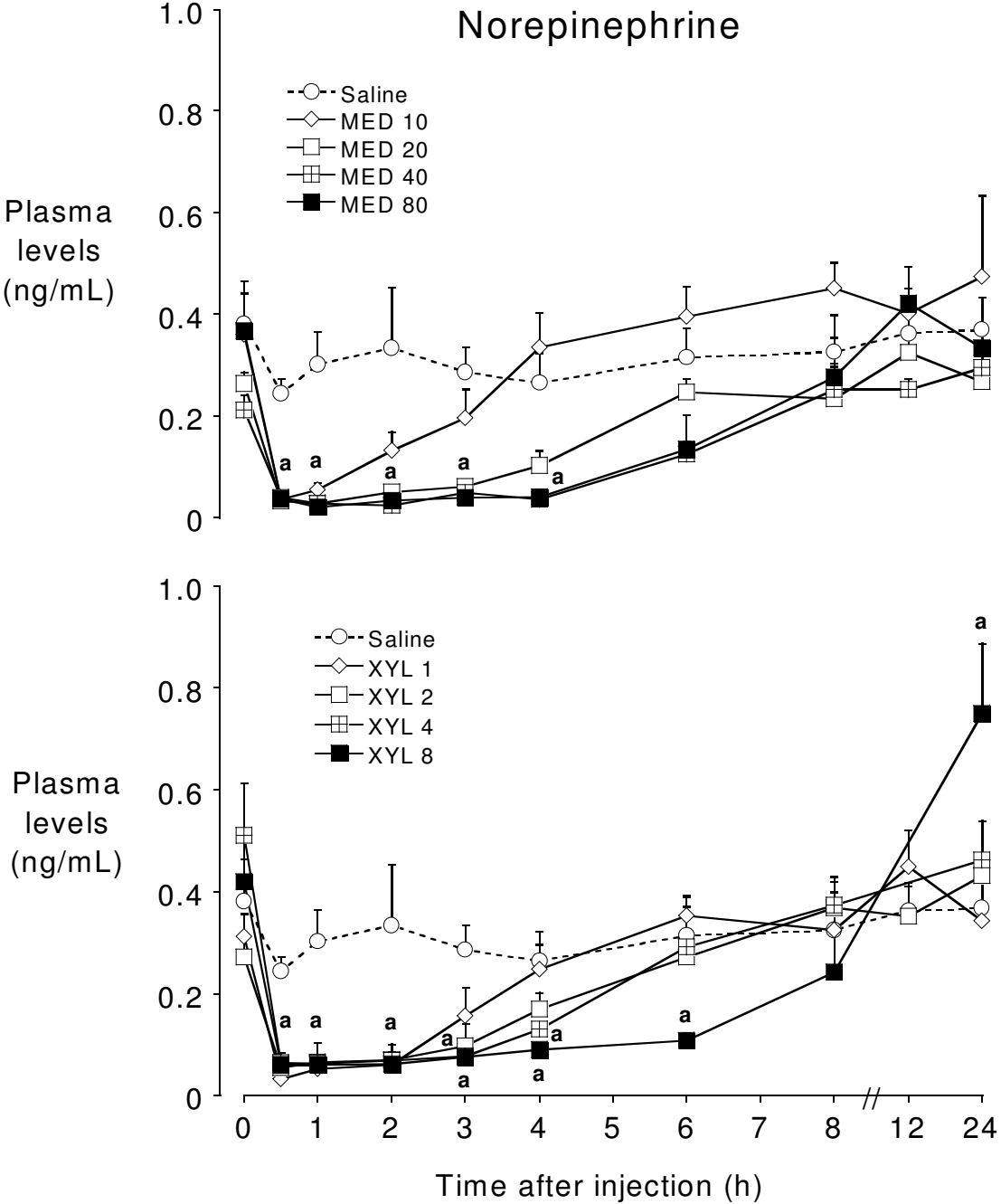
Figure 1. Norepinephrine plasma levels following the administration of medetomidine (MED μg/kg, IM) and xylazine (XYL mg/kg, IM) in dogs. Superscript 'a': significantly different from the initial value ($p < 0.05$). Each points and vertical bars represent the mean and the standard error of mean (SEM) (n = 4 to 5).
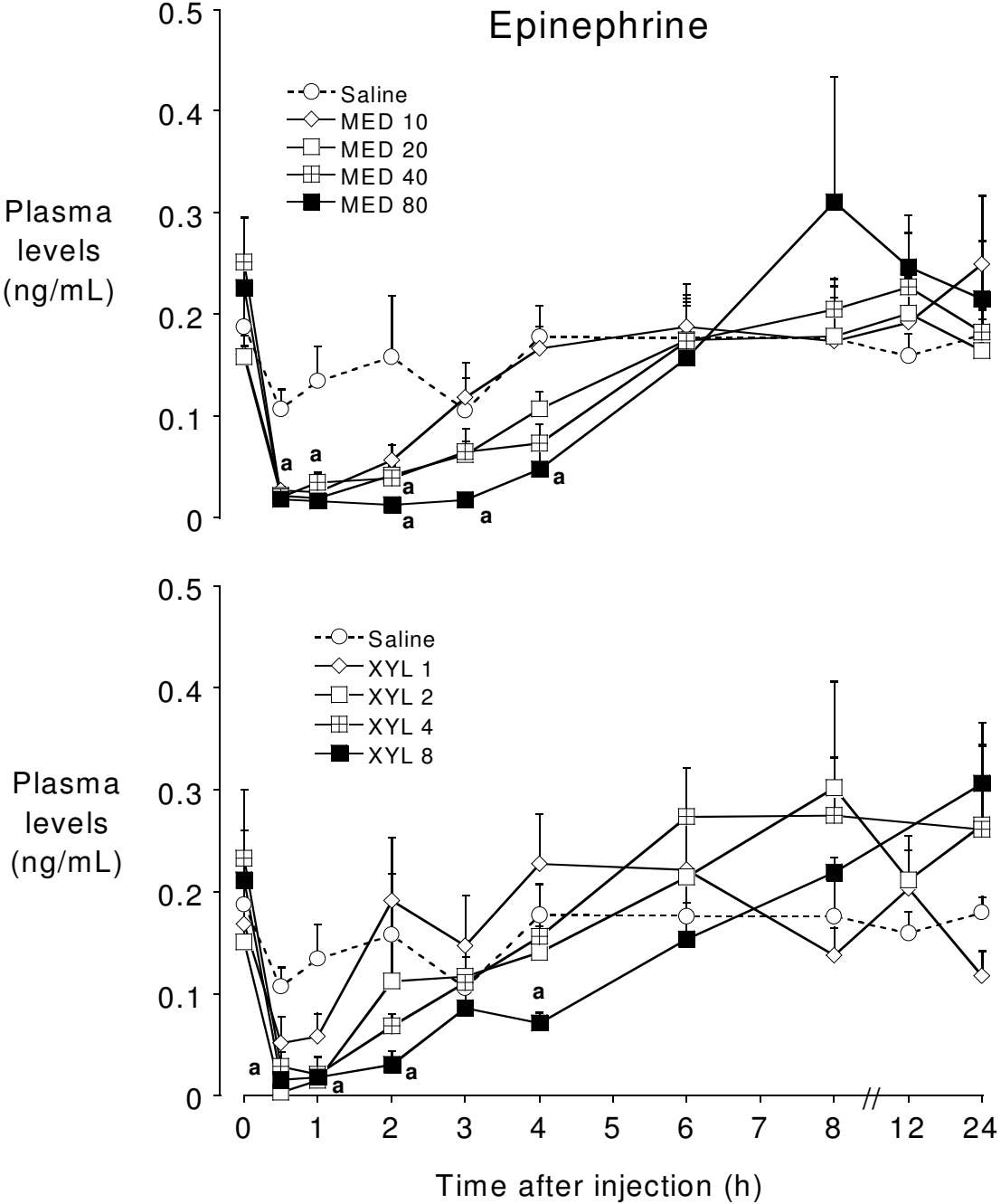
Figure 2. Epinephrine plasma levels following the administration of medetomidine (MED µg/kg, IM) and xylazine (XYL mg/kg, IM) in dogs. Superscript 'a': significantly different from the initial value ($p < 0.05$). Each points and vertical bars represent the mean and SEM (n = 4 to 5).
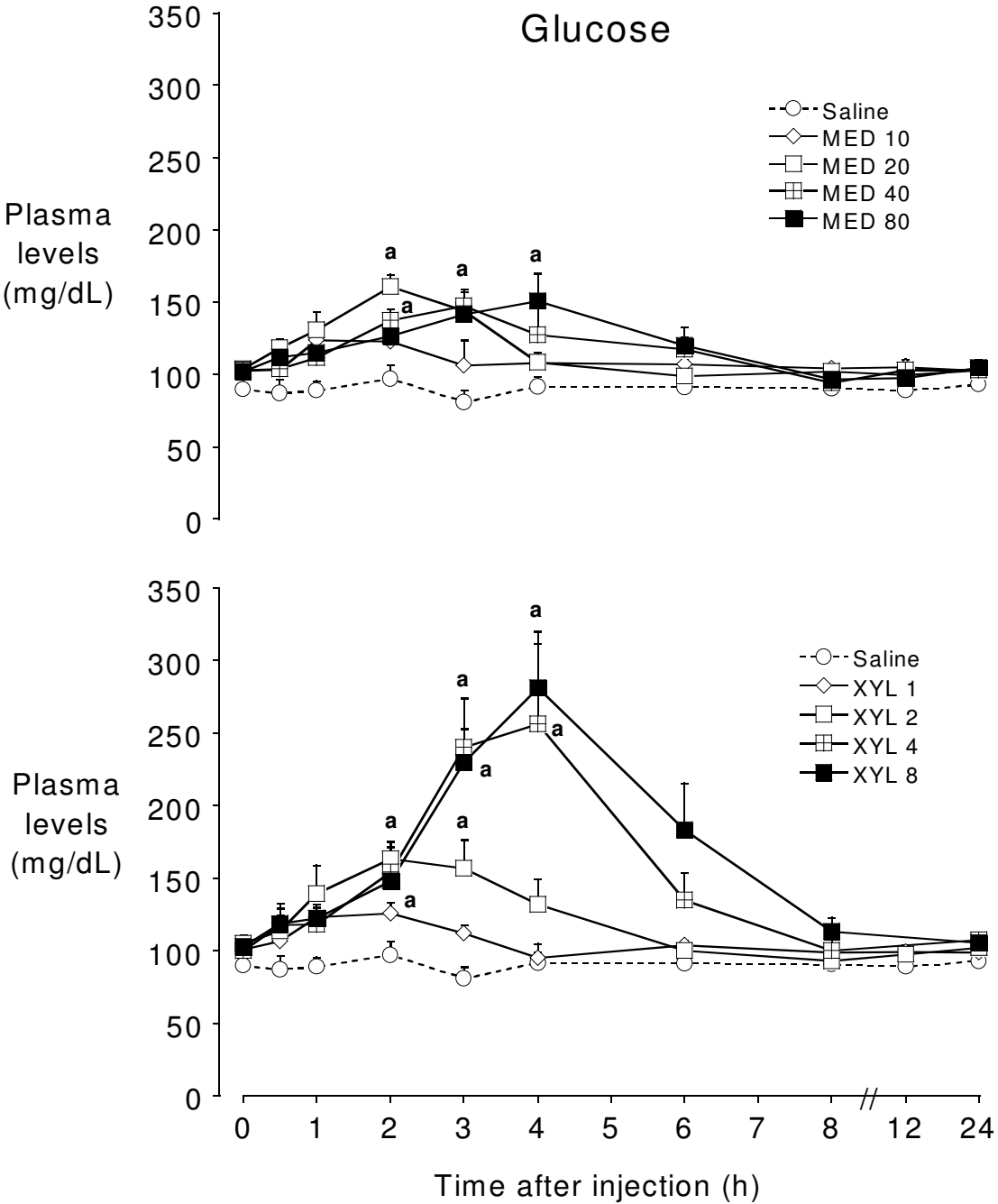
Figure 3. Glucose plasma levels following the administration of medetomidine (MED µg/kg, IM) and xylazine (XYL mg/kg, IM) in dogs. Superscript 'a': significantly different from the initial value (*p* < 0.05). Each points and vertical bars represent the mean and SEM (n = 4 to 5).
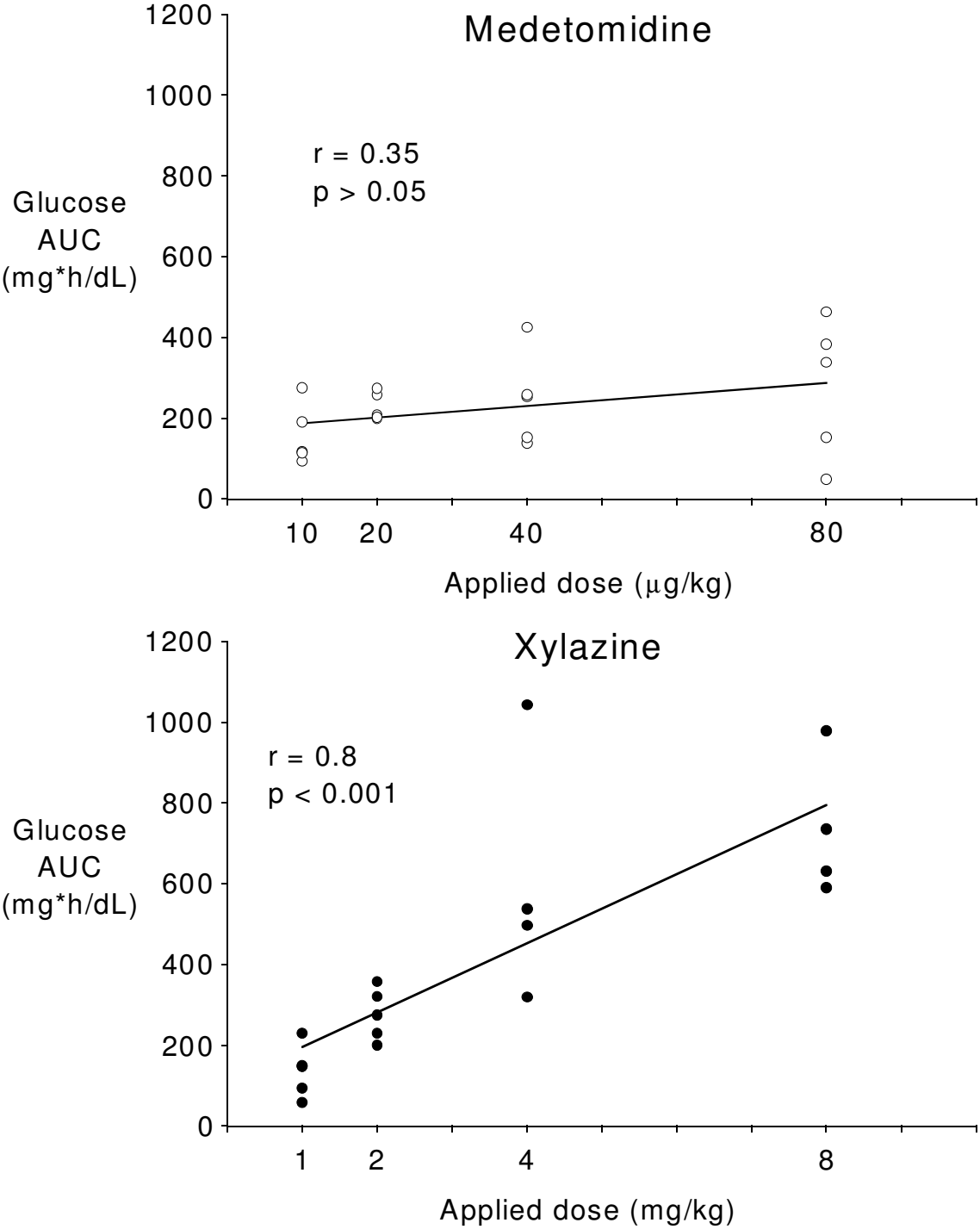
Figure 4. The normalised area under curve (AUC, 0-8 h) data of the plasma glucose graphs are plotted versus the dose of medetomidine and xylazine. Simple linear regression analysis was applied. *p*< 0.001 proves that the hyperglycaemic effect of xylazine was dose dependent.

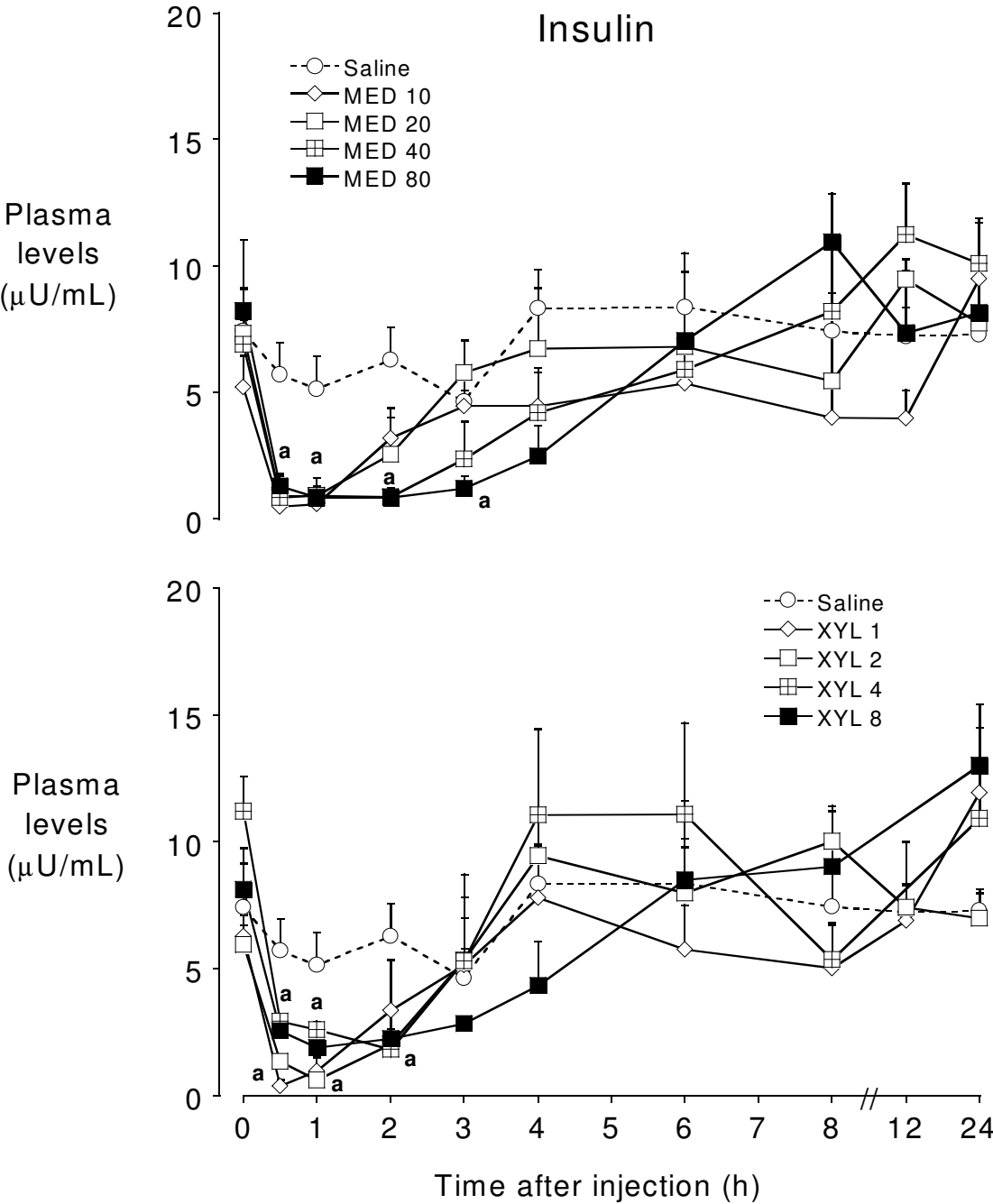**Figure 5. Insulin plasma levels following the administration of medetomidine (MED μg/kg, IM) and xylazine (XYL mg/kg, IM) in dogs. Superscript 'a': significantly different from the initial value ($p < 0.05$). Each points and vertical bars represent the mean and SEM (n = 4 to 5).**

Figure 6. Nonesterified fatty acid (NEFA) plasma levels following the administration of medetomidine (MED μg/kg, IM) and xylazine (XYL mg/kg, IM) in dogs. Superscript 'a': significantly different from the initial value ($p < 0.05$). Each points and vertical bars represent the mean and SEM (n = 4 to 5).
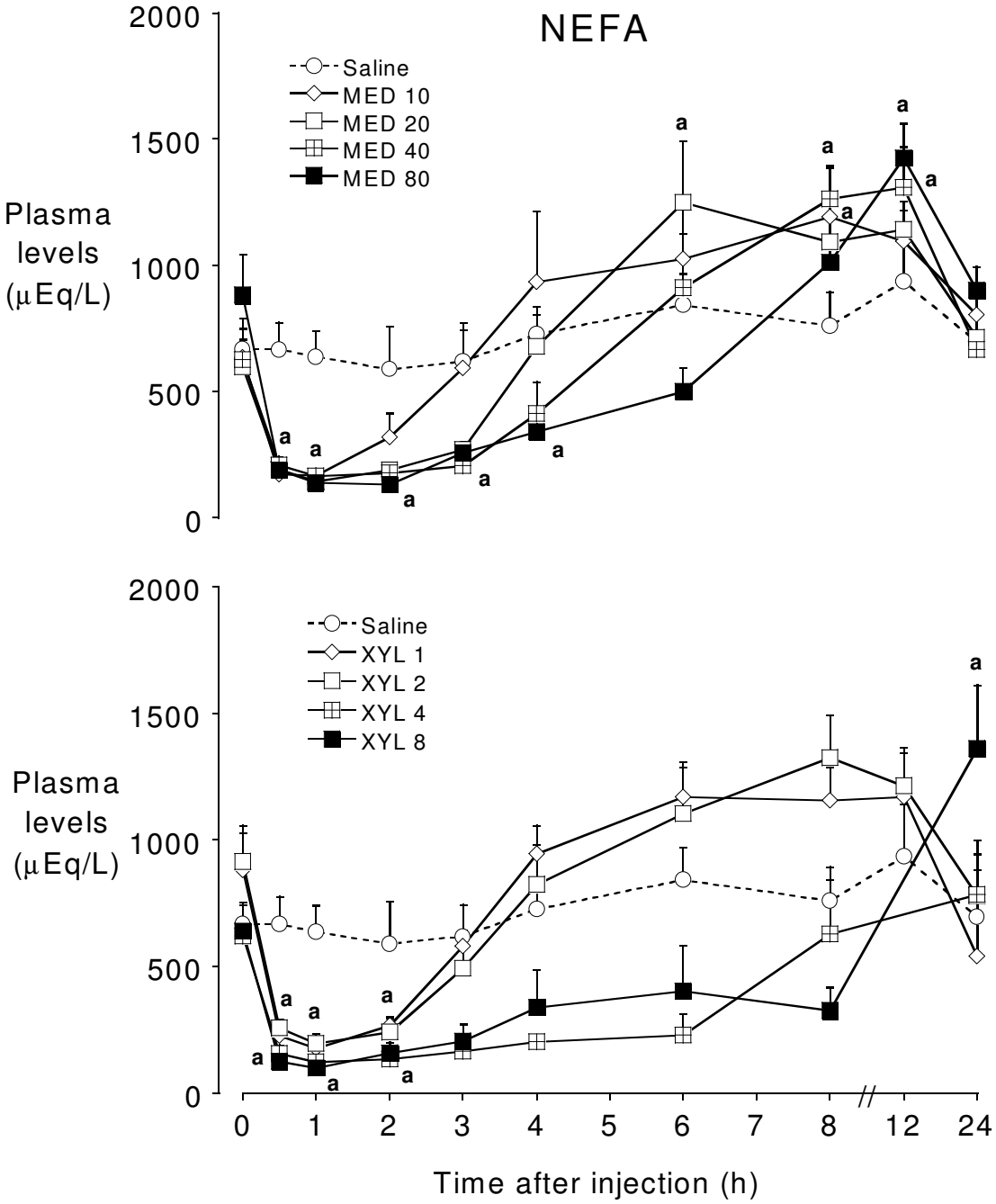
Discussion

The α_2-adrenoceptor agonists are well known to inhibit the sympathetic outflow in the central nervous system through their actions on α_2-adrenoceptors, hence decreasing the level of circulating catecholamines [2,11]. Our results support this theory because the plasma catecholamine levels decreased after an injection of either medetomidine or xylazine. There are several data demonstrating the inhibition of catecholamine release at blood levels associated with the usage of α_2-agonists. For example, Benson *et al* [19] found that medetomidine administered pre-operatively reduced the catecholamine levels in both ovariohysterectomised and non-operated dogs under isoflurane anaesthesia. Talke *et al* [41] reported that dexmedetomidine administered to human patients postoperatively using a computer-controlled infusion technique decreased the plasma levels of catecholamines during infusion. Benson *et al* [9] also reported that xylazine injected after onychectomy reduced plasma concentrations of catecholamines in cats. In addition, Flacke *et al* [42] reported that treatment with clonidine reduced the catecholamine level in dogs anaesthetised with fentanyl-enflurane-nitrous oxide. However, there were no reports to compare the effects of medetomidine and xylazine at different dosages on the plasma catecholamine levels in dogs under basal conditions. In the present study, both medetomidine and xylazine suppressed norepinephrine release dose-dependently with similar potency (Figure 1). On the other hand, medetomidine dose-dependently suppressed the epinephrine secretion. Xylazine also tended to inhibit epinephrine release dose-dependently (Figure 2). However, the potency of medetomidine in reducing the plasma epinephrine levels was greater than that of xylazine.

The plasma cortisol levels are influenced by both the peripheral site at the adrenal cortex, and the central site through the release of corticotrophin-releasing factor (CRF)

and adrenocorticotrophic hormone (ACTH) in the brain. The effects of α_2-agonists on the plasma cortisol level have been assessed under different experimental conditions in a variety of species. Maze *et al* [43] found that an intramuscular injection of 80 μg/kg dexmedetomidine reduced the basal cortisol level and the cortisol release to ACTH stimulation at 3 h post-injection in dogs, and concluded that only high dosages of dexmedetomidine inhibit adrenal steroidogenesis. In humans, it was reported that 0.45 mg clonidine administered orally for 3 days reduced plasma cortisol level [44], but 0.1 and 0.2 mg clonidine for 4 days did not affect it under basal conditions [45]. Haas *et al* [46] reported that an intraperitoneal administration of 1 mg/kg clonidine significantly decreased the hypothalamic CRF-like immunoreactivity in rats. Furthermore, Taylor *et al* [47] reported in ponies that an intravenous anaesthesia using detomidine, ketamine and guaiphenesin decreased the cortisol level, but did not significantly change the ACTH concentration. On the other hand, there are several reports with respect to the influence of α_2-agonists on the cortisol release associated with surgical stimulation. Premedication with medetomidine was reported to reduce or delay the increase of plasma cortisol levels induced by ovariohysterectomy in dogs [19,48]. Sedation with xylazine diminished the increase in the plasma cortisol level after intradermal testing in dogs [10]. In addition, clonidine prevented the elevation of cortisol level during laparoscopy in humans [49]. These findings indicate that treatments with α_2-agonists such as medetomidine, xylazine or clonidine, show inhibitory effect on the release of cortisol. However, whether it is due to the α_2-adrenoceptor-mediated specific action, other receptor-mediated actions, or the result of non-specific effects by providing sedation and analgesia which reduce stress response, is unknown. In this respect, recent studies have evidenced that imidazoline receptors but not α_2-adrenoceptors may a play role in the direct inhibition of cortisol release in the adrenal cortex. An *in vitro* study

revealed that the imidazoline α_2-adrenergic agents, medetomidine, detomidine and atipamezole, all suppressed the release of cortisol from porcine adrenocortical cells [50]. As medetomidine and detomidine are selective α_2-agonists, and atipamezole is a selective α_2-antagonist, this effect is unrelated to their actions on α_2-adrenoceptors. Maze *et al* found [43] that the selective α_2-agonist, dexmedetomidine (*d*-medetomidine) and its clinically ineffective enantiomer, *l*-medetomidine were equally effective in blocking the ACTH-stimulated corticosterone secretion in adrenocortical cells of rats. This finding also supports that imidazoline receptors may also be responsible for inhibition of adrenal steroidogenesis.

In the present study, we examined the plasma cortisol level under basal conditions without surgical stress or ACTH stimulation, and found that both medetomidine and xylazine failed to significantly alter the plasma cortisol levels at the examined dosages. The possible explanation why an imidazoline derivative, medetomidine did not significantly decrease the cortisol level may be that its applied dose was too little to decrease cortisol release [43].

The most important finding of this study was that medetomidine did not increase the plasma glucose level in a dose-related manner in the examined dosages, whereas xylazine increased it dose-dependently (Figure 3). Our finding that the hyperglycaemic effect of medetomidine was not dose-dependent is the first report in dogs. On the other hand, the present study revealed that either medetomidine or xylazine decreased plasma insulin levels in all dosages used (Figure 5). The comparison of the AUC insulin data also suggested that the potencies of medetomidine and xylazine to suppress insulin release were similar. There are numerous reports as to the effects of α_2-agonists on the blood glucose and insulin levels in different species. Either clonidine or xylazine was found to dose-dependently increase blood glucose in cattle [16,17] and rats [51,52]. It

was reported in dogs that an intramuscular injection of 2.2 mg/kg xylazine increased blood glucose and decreased insulin level [12], which were in agreement with the present results. Similar results have also been reported in cats [13] and sheep [18]. Two reports are available on the effect of medetomidine in dogs. Burton *et al* [53] found that 10 and 20 μg/kg medetomidine administered intravenously to beagle dogs tended to elevate the plasma glucose level, but it did not reach the level of significance and remained within the normal physiological range. They observed a peak in glucose level of about 90 mg/dL at 3 h after 20 μg/kg medetomidine treatment. That is in contrast with our results because the glucose level in the MED-20 group of our study significantly elevated at 2 and 3 h post-injection up to the level of 161 ± 8 and 144 ± 14 mg/dL (mean $\pm$ SEM) respectively. In that study, they also found that the insulin levels decreased similarly to our results, and that those effects were not different between 10 and 20 μg/kg medetomidine treatment. In addition, Benson *et al* [19] have reported that an intramuscular injection of 15 μg/kg medetomidine induced a decrease in insulin level, but did not significantly alter the glucose level in the non-operated dogs under isoflurane anaesthesia. The insulin levels in their study [19] were similar to those observed in MED-10 and -20 of our study, but in contrast with our results, the plasma glucose level almost did not change 75 min after 15 μg/kg medetomidine injection in their study. It is not clear whether isoflurane anaesthesia in their study might influenced the glucose response. The fact that insulin plasma levels decreased after treatment and the glucose levels did not increase [19] suggests that insulin might not be the only factor controlling the plasma glucose levels after medetomidine treatment. Our results also suggest that the difference in the hyperglycaemic response between medetomidine and xylazine found in our study can not be explained only by the α_2-adrenoceptor-mediated inhibition of insulin release. There may be other factors to consider.

It is well known that α_2-agonists inhibit the insulin release through their actions on α_2-adrenoceptors in the pancreas β-cells [54,55]. Clonidine at high concentrations has been reported to increase the glucose release from bovine and canine liver slices *in vitro* [16,56]. The subtype of α-adrenoceptors involved in this action seems to be α_1 rather than α_2, because prazosin, a specific α_1-adrenoceptor antagonist blocked this glucose release more effectively than the α_2-antagonist, yohimbine [56]. As both xylazine and clonidine have similar α_2/α_1 selectivity ratios [57], high dosages of xylazine might exert some α_1-adrenoceptor-mediated effect similar to clonidine. This could be one reason for the extreme increasing of plasma glucose levels in XYL-4 and -8 groups of our study.

The central effects of α_2-agonists are also obscure. Xylazine administered intracerebroventricularly (ICV) at a dosage of 0.5 mg did not change the blood glucose level in a cat [13]. Clonidine administered ICV to rats dose-dependently increased the blood glucose level [52], but whether it was central effect or the result of systemic absorption remained unclear. On the other hand, central imidazoline receptors may have a role in the regulation of glucose metabolism, because ICV-injected agmatine, a putative endogenous ligand for imidazoline receptors, exerted anti-hyperglycaemic effect [58]. Medetomidine shows higher α_2/α_1 receptor selectivity than xylazine [57], but because of its imidazoline structure it may also have affinity to bind to the imidazoline receptors [43,50]. A possible action on the putative imidazoline receptors might explain why the glucose levels did not increase further after administration of high dosages of medetomidine in our study.

Other hormones such as growth hormone (GH) and glucagon may also influence the blood glucose level. Xylazine is known to increase the blood level of GH in dogs and cats [5]. However, GH values were not determined in the present study. On the other hand, the plasma glucagon levels did not change significantly in the treatment groups of

the present study, indicating that it may not be related to the hyperglycaemic effects of both medetomidine and xylazine. This was in agreement with previous reports that xylazine did not significantly change the plasma glucagon level in dogs [59] and cats [13]. Therefore, further work may be necessary to clarify the differences in the hyperglycaemic effects of medetomidine and xylazine in dogs.

Both medetomidine and xylazine similarly dose-dependently decreased the plasma NEFA levels in this study (Figure 6). According to the authors' knowledge this is the first report available on the effects of either medetomidine or xylazine on NEFA levels in dogs. The suppression of lipolysis in dogs may be mediated by both central and peripheral α_2-adrenoceptors [14,15].

In conclusion, the present study revealed that both medetomidine and xylazine similarly inhibited norepinephrine release and lipolysis dose-dependently. Medetomidine suppressed epinephrine release dose-dependently with greater potency than xylazine. The cortisol and glucagon levels did not change significantly in any treatment group. Both drugs suppressed insulin secretion with similar potency. Furthermore, this study demonstrated that the hyperglycaemic effect of medetomidine, in contrast with xylazine, was not dose-dependent at the tested dosages, and suggested that the effect of medetomidine on glucose metabolism may not be only due to α_2-adrenoceptor mediated actions.

Chapter 3

The antagonistic effects of atipamezole and yohimbine on stress-related neurohormonal and metabolic responses induced by medetomidine in dogs

Medetomidine, a potent and highly specific α_2-adrenoceptor agonist, is often used in veterinary practice as a sedative, analgesic and muscle relaxant [2]. Beside of these effects, medetomidine can reduce stress response to surgery as assessed by the attenuation of plasma catecholamine, adrenocorticotrophic hormone and cortisol levels [19]. Stress is a generalised response of the body to various factors, called stressors. Pain, blood loss, excitement, and underlying pathological conditions may all act as stressors in the surgical patient. The endocrine and metabolic stress response is characterised by the increase of catecholamine, cortisol, glucose, and NEFA (nonesterified fatty acid) blood levels, and the decrease of insulin levels [20]. Adrenoceptors play an important role in the co-ordination of these events, therefore α_2-adrenergic agents may interfere to the pathophysiology of stress response during and after anaesthesia. That's why there is an increasing interest in using medetomidine as a pre-anaesthetic adjuvant or as a part of balanced anaesthesia. If necessary, the actions of medetomidine can be reversed by α_2-adrenoceptor antagonists, such as the highly receptor specific atipamezole, or the less specific yohimbine [2]. The use of these

antagonists may also have adverse effects, like hypotension, tachycardia, over alertness, and the absence of analgesia [2,21]. But weather the acceleration of stress response after antagonising may contribute to the fatal outcome of some patients is not fully understood. The stress-related hormonal and metabolic effects of antagonising an α_2-agonist by an antagonist, had already been reported in horse [22], cattle and sheep [23]. However, such study has not yet been published in dogs.

The purpose of this study was to investigate and compare the reversal effects of three different doses of atipamezole, and a single dose of yohimbine on stress-related hormonal and metabolic responses following medetomidine administration in dogs. The examined variables were the plasma levels of norepinephrine, epinephrine, cortisol, glucose, insulin, glucagon, NEFA and lactate. We hypothesised that the reversal effects of atipamezole were dose-related and similar to the effects of yohimbine.

Materials and methods

Our experimental protocols were approved by the Animal Research Committee of Tottori University. Five healthy female beagle dogs, weighing from 9 to 13 kg and ageing from 2 to 5 years, were used in each of the five experimental groups in randomised order at one week interval. A day before the experiment, a 16-gauge central venous (CV) catheter was introduced into the jugular vein. Food and water were withheld for 12 hs before drug injection. The dogs in every experimental group received 20 µg/kg medetomidine HCl (1 mg/mL, Domitor; Meiji Seika Kaisha, Tokyo, Japan) intramuscularly (IM) as first treatment. This was followed 30 min later by a second IM treatment, namely: 0.5 mL physiological saline, 40, 120, or 320 µg/kg atipamezole HCl (5 mg/mL, Antisedan; Meiji Seika Kaisha, Tokyo, Japan), or 110 µg/kg yohimbine HCl (Sigma Chemical, St. Louis, USA). The yohimbine injection was prepared in our laboratory (1 mg/mL in aqueous solution). The experimental groups will be hereafter referred to as MED-SAL, MED-ATI 40, MED-ATI 120, MED-ATI 320 and MED-YOH 110, respectively.

The optimal dose of atipamezole was reported to be four to six fold the dose of medetomidine [21], and that of yohimbine was 110 µg/kg [1]. According to our experience 120 µg/kg atipamezole and 110 µg/kg yohimbine are able to antagonise the sedative effect of 20 µg/kg medetomidine with a similar potency. Both yohimbine and atipamezole are recommended for IM use under most circumstances [1] to attenuate the side effects of sudden reversal. Therefore, to model clinical conditions, we administered these drugs through IM route. Although, the speed and the completeness of absorption may differ among individuals, those differences should also be present under practical conditions.

Blood samples were collected 7 times from the CV catheter. The initial sample was taken at 0 h (before medetomidine injection), the second at 0.5 h (before antagonist injection), and at 1, 2, 3, 4, and 6 h after the injection of medetomidine. The sampling and analytical methods were similar as previously published [60]. Shortly catecholamines were measured by a high performance liquid chromatograph equipped with an electrochemical detector; cortisol, insulin and glucagon by radioimmunoassay techniques; and glucose, NEFA and lactate by use of a spectrophotometer.

One-way analysis of variance (ANOVA) for repeated measures was used to examine time effect within each group and one-way ANOVA for treatment effect at each time-point separately. When ANOVA was significant, Tukey's test was used for multiple comparisons of the means. Area under curve (AUC) was calculated by the trapezoidal method for each individual under the concentration-time curves of the measured variables. These AUC values were plotted versus doses of atipamezole, and linear regression analysis was applied to determine dose-dependency. The level of significance was set at $p < 0.05$ in each test.

Results

Vomiting occurred in 15 occasions (60 % incidence), 6.3 ± 1 (mean ± SEM) min following the medetomidine injections. All dogs become mildly sedated and laterally recumbent at 9.6 ± 1 min after medetomidine treatment. The first sign of arousal was observed at 76 ± 22 min following saline, 13 ± 3, 7 ± 1, and 5 ± 1 min after 40, 120, and 320 µg/kg atipamezole IM respectively, and 18 ± 5 min after yohimbine (110 µg/kg, IM) treatment. The middle and the large doses of atipamezole and the yohimbine treatments completely antagonised the sedative effects of medetomidine, while the animals remained sedated until 149 ± 29 min following the small dose of atipamezole injection. The arousal was smooth after atipamezole treatment, but hyper alertness, vocalisation, muscle tremor, defecation and temporary pain at the injection site occurred after yohimbine injection.

Both plasma norepinephrine (NE) and epinephrine (EPI) levels (Figure 7) significantly decreased after medetomidine injection, and there was no significant difference between the groups at 0 and 1 h. The plasma NE levels were significantly lower until 4 h in the MED-SAL group. Atipamezole dose-dependently antagonised these effects. Yohimbine also returned NE levels to baseline at 1 h, but increased them over baseline at 3, 4, and 6 h. On the other hand, plasma EPI levels (Figure 7) returned to the baseline values in every antagonist-treated group and were not significantly different from the baseline thereafter. The regression analysis confirmed that the effect of atipamezole on plasma EPI was not dose-dependent.

Neither medetomidine nor atipamezole treatments changed significantly the plasma cortisol levels (data not shown). In the yohimbine-treated group however, mean cortisol concentration significantly increased at 1 h (5.1 ± 0.6 µg/dL) compared with both 0 h (2.0 ± 0.6 µg/dL) and 0.5 h (1.0 ± 0.2 µg/dL) values.

Plasma glucose levels (Figure 8) tended to increase at 0.5 h after medetomidine injection in every group, but it was only significant in the MED-YOH 110 group. However, there was no significant difference between the groups at that time-point. The mean glucose level in the control group increased further up to about 170 mg/dL at 2 and 3 h, and then returned to baseline at 4 h. The glucose levels decreased after every antagonist treatment and were not significantly different from the 0 h values thereafter. However, in the MED-ATI 40 and MED-ATI 120 groups, plasma glucose slightly elevated again from baseline at 2 and 3 h. The effect of atipamezole on plasma glucose was dose-dependent.

The plasma insulin levels (Figure 8) decreased after medetomidine treatment, remained significantly lower until 2 h, and then increased over baseline at 4 and 6 h in the MED-SAL group. Atipamezole dose-dependently increased the insulin levels, but this effect in the MED-ATI 40 group was rather week. Insulin levels increased slower after yohimbine injection, but elevated above baseline at 2, 3, and 4 h.

The plasma NEFA levels (Figure 9) significantly decreased after medetomidine injection in every group, and remained lower than the baseline in the MED-SAL group until 3 h. Atipamezole dose-dependently increased the NEFA levels, but this effect was week in the MED-ATI 40 group. The NEFA levels were similar in both the highest dose of atipamezole and the yohimbine treatment groups.

The plasma glucagon and lactate levels did not change significantly at any sampling time in this experiment (data not shown).
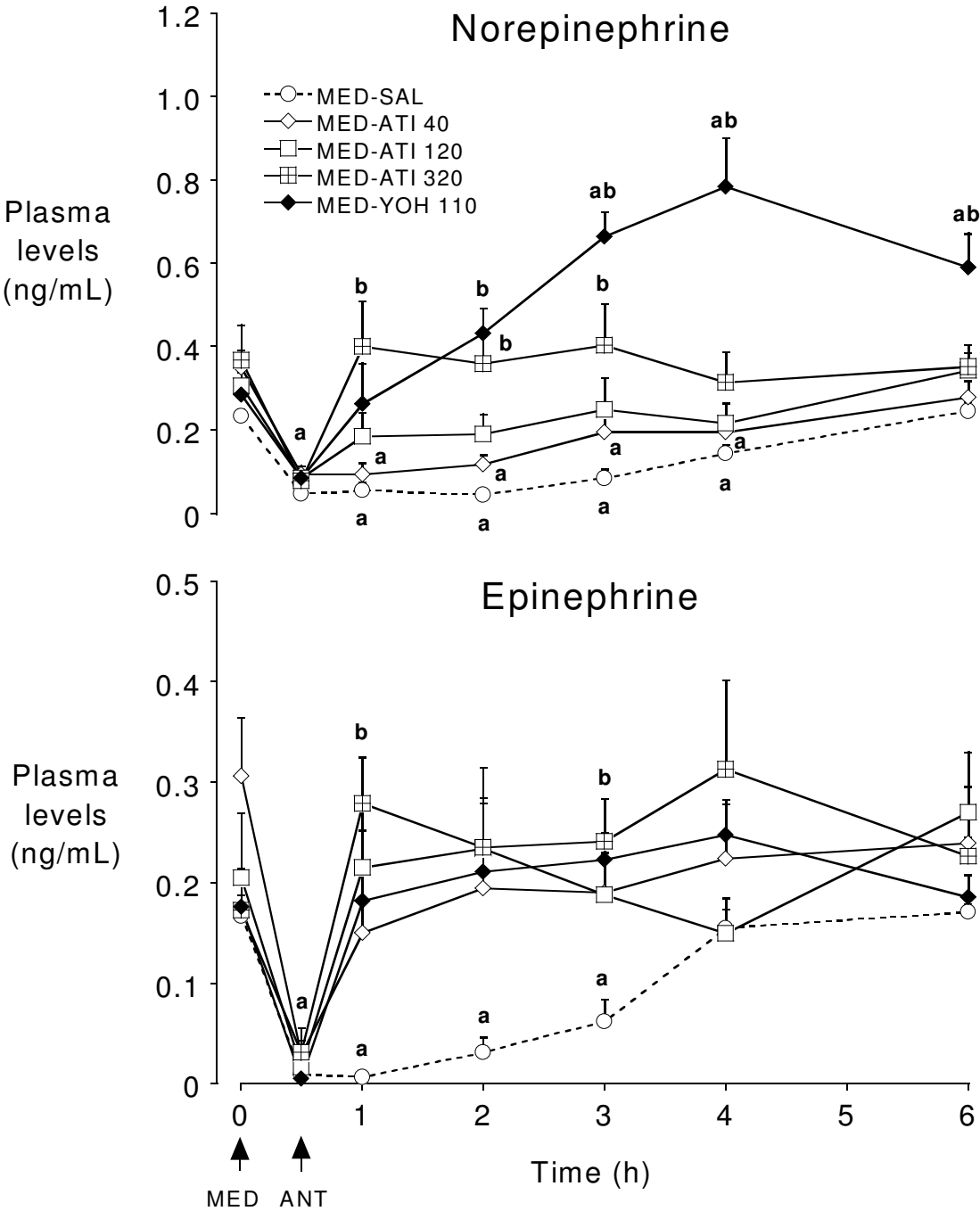
Figure 7. Effects of atipamezole (ATI 40, 120, 320 μg/kg, IM), yohimbine (YOH 110 μg/kg, IM) and saline (SAL, IM) on norepinephrine and epinephrine plasma levels, 30 min following medetomidine (MED 20 μg/kg, IM) administration in dogs. Arrows show the time of MED and antagonist (ANT) injections. Superscript 'a': significantly different from the initial, 0 h value. Superscript 'b': significantly different from the MED-SAL group at that time-point ($p < 0.05$). Each point and vertical bar represent the mean and SEM (n = 5).

Figure 8. Effects of atipamezole (ATI 40, 120, 320 μg/kg, IM), yohimbine (YOH 110 μg/kg, IM) and saline (SAL, IM) on glucose and insulin plasma levels, 30 min following medetomidine (MED 20 μg/kg, IM) administration in dogs. Arrows show the time of MED and antagonist (ANT) injections. Superscript 'a': significantly different from the initial, 0 h value. Superscript 'b': significantly different from the MED-SAL group at that time-point ($p < 0.05$). Each point and vertical bar represent the mean and SEM (n = 5).
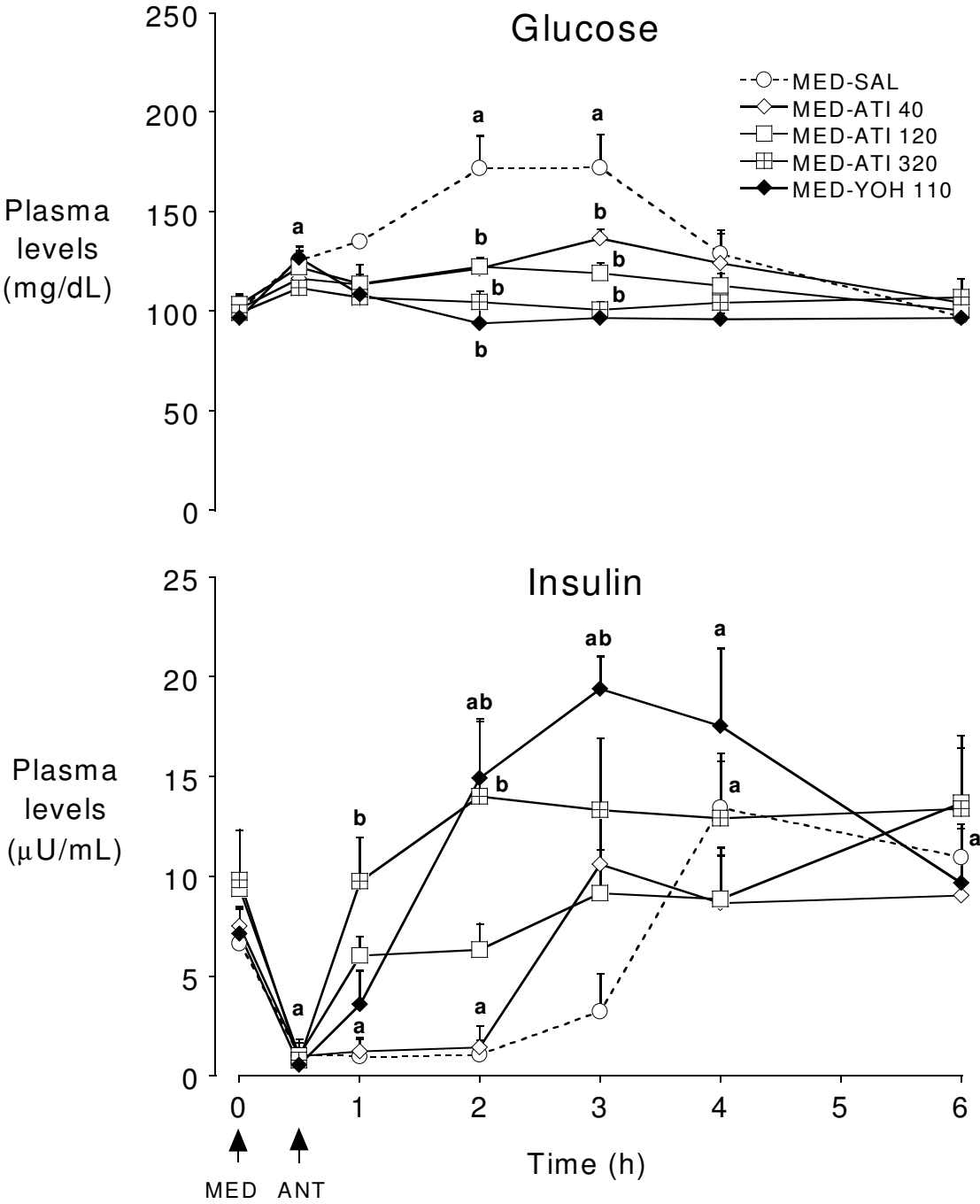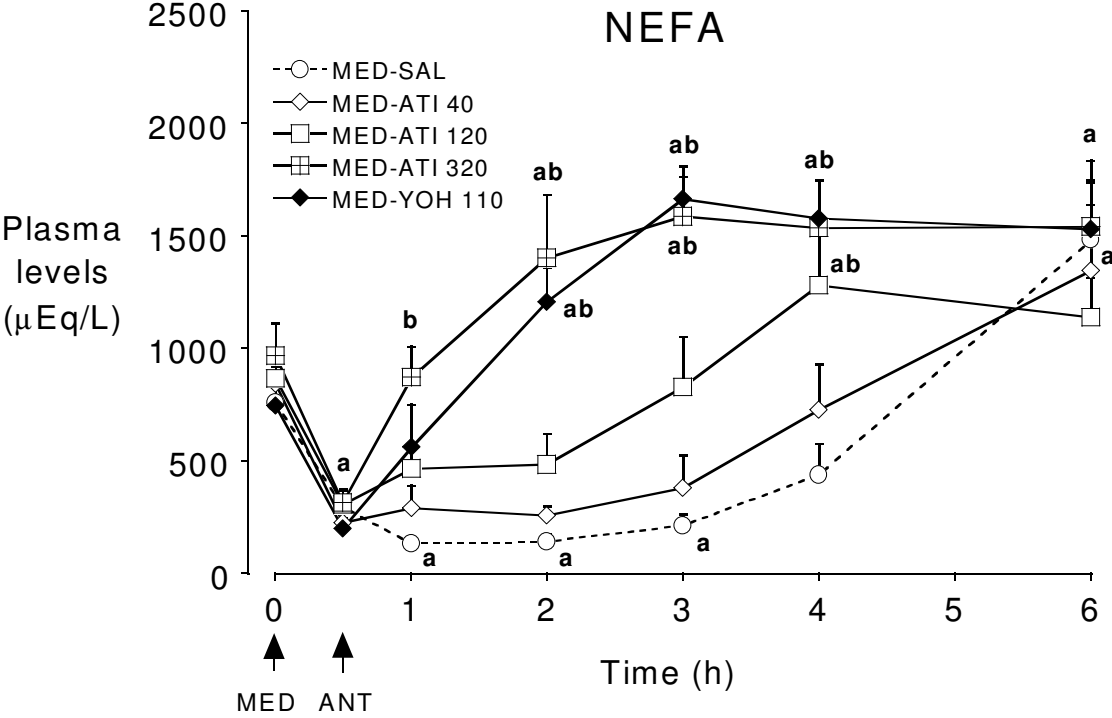
Figure 9. Effects of atipamezole (ATI 40, 120, 320 µg/kg, IM), yohimbine (YOH 110 µg/kg, IM) and saline (SAL, IM) on nonesterified fatty acid (NEFA) plasma levels, 30 min following medetomidine (MED 20 µg/kg, IM) administration in dogs. Arrows show the time of MED and antagonist (ANT) injections. Superscript 'a': significantly different from the initial, 0 h value. Superscript 'b': significantly different from the MED-SAL group at that time-point ($p < 0.05$). Each point and vertical bar represent the mean and SEM (n = 5).

Discussion

In this study, we examined the hormonal and metabolic effects of low, medium and high doses of atipamezole, and a single dose of yohimbine in antagonising medetomidine-induced sedation. Medetomidine (20 µg/kg, IM) suppressed sympathoneural and adrenomedullary activities (assessed by the decrease of plasma NE and EPI levels), insulin release and lipolysis, and increased plasma glucose levels. These effects of medetomidine were similar to those in our previous study [60]. Both atipamezole and yohimbine were able to antagonise these effects. Because the antagonistic effects of atipamezole on plasma NE, glucose, insulin and NEFA were dose-related, we accepted our first hypothesis in these cases. On the other hand, all doses of atipamezole similarly antagonised the suppression of EPI release, consequently we refused the first hypothesis for the plasma EPI levels. Even the smallest dose of atipamezole (40 µg/kg,IM) effectively antagonised the suppression of adrenomedullary activity and prevented the further increase of plasma glucose, but the effect of this dose was rather weak on NE, insulin release and lipolysis. In other words, the small dose of atipamezole could antagonise the hyperglycaemic effect of medetomidine, but the insulin levels continued to be low in this group. These data supports the theory that α_2-adrenergic agents act on the glucogenolysis in the liver irrespective of insulin. Our previous study also suggested this possibility [60]. The medium (120 µg/kg, IM) and high (320 µg/kg, IM) doses of atipamezole effectively antagonised all examined effects of medetomidine. The antagonistic effects of the medium dose were only moderate on plasma NE, glucose, insulin and NEFA, but those of the large dose were always complete.

The potency of yohimbine (110 µg/kg, IM) in antagonising the sedative effect of medetomidine was similar to the medium and high dose of atipamezole, but the onset of

this effect of yohimbine was longer. The antagonistic effects of yohimbine on plasma NE, insulin and NEFA levels were also delayed at 1 h when compared to the high dose of atipamezole. These data suggests that the absorption of yohimbine after IM injection was slower than that of atipamezole. Yohimbine effected plasma EPI, glucose and NEFA levels similarly to the high dose atipamezole but more potently increased NE and insulin levels. In this point yohimbine itself (400 µg/kg, IV) was reported to increase plasma NE, insulin and NEFA levels in dogs [61]. Similarly, a large dose of atipamezole (100 mg, IV) increased plasma NE and EPI levels, while did not affect cortisol and glucose levels in humans [62]. The differences between the actions of atipamezole and yohimbine in the present study may result from the differences in their elimination from the plasma. When both medetomidine and atipamezole are administered, their elimination half-lives are similar, about 1 h in the canine plasma [63]. On the other hand, yohimbine has much longer elimination half-life, about 16 hs in rats [64] and 13 hs in humans [65]. Consequently, yohimbine might have relatively over-antagonised the actions of medetomidine, after the agonist was already eliminated. In the present experiment, only yohimbine increased the cortisol levels at 1 h. Therefore, the increase in cortisol levels after yohimbine treatment is possibly not related to actions on α_2-adrenoceptors. For the reasons above, we refused our second hypothesis that the antagonistic actions of atipamezole and yohimbine were similar.

Glucagon levels did not change in the present experiment similarly to our previous study after medetomidine and xylazine treatments [60]. Therefore, α_2-adrenergic agents may not influence glucagon release in dogs. The plasma lactate levels did not change also in the present study, indicating that the applied dose of medetomidine did not alter anaerobe glycolysis.

We believe that the properties of medetomidine to attenuate the catecholamine and cortisol responses during and after surgery are desirable and their complete antagonism is normally not indicated. From a pharmacokinetic point of view, yohimbine is not a well-fitted antagonist for medetomidine because of its prolonged actions. Based on these findings, when medetomidine-induced sedation is antagonised in dogs, we recommend using atipamezole IM, from 2 to 6 folds the dose of medetomidine, unless otherwise indicated.

Chapter 4

Stress-related neurohormonal and metabolic effects of butorphanol, fentanyl and ketamine in dogs

Butorphanol has become popular in veterinary anaesthesiology as a broad-spectrum painkiller before during and after surgery [66,67]. It has partial agonistic effect on the μ (mu) [68], full agonistic effect on the κ (kappa) opioid receptors [69] and produces relatively few side effects in small animals [67]. Fentanyl is also used in veterinary anaesthesiology for its strong painkilling effect during the intraoperative period [70]. Fentanyl is a full agonist on the μ but also has weak agonistic effect on the δ (delta) and κ opioid receptors [69,71]. Fentanyl is stronger painkiller than butorphanol but it may produce severe respiratory depression and bradycardia [31,37].

Ketamine is an injectable dissociative anaesthetic agent normally used in combination with sedatives to provide balanced anaesthesia in humans [26] and animals [38,72]. The effects of ketamine can not be explained by a single mechanism. The anaesthetic effect of ketamine is attributed to its antagonistic effect on N-methyl-D-aspartate (NMDA) receptors [73] but ketamine also binds to other receptor populations like dopamine [74], opioid, σ (sigma) and cholinergic receptors [75]. Ketamine has considerable analgesic effects also probably attributed to its NMDA receptor antagonistic and partially to its opioid agonistic effects [75]. The psychotomimetic

adverse effects of ketamine may be mediated by cholinergic, κ opioid and σ receptors [75].

Opioids and ketamine also have endocrine and metabolic effects [24,25]. Because analgesics act on similar receptor systems than the physiological stress-response there are certain analogy between the endocrine effects of analgesics and stress-response. The purpose of anaesthesia is to induce optimal painless condition and attenuate stress-response in the majority of cases for veterinary anaesthesia. However, critically ill patients sometimes need sympathetic support and suppressing their otherwise insufficient endocrine stress-response by some analgesics can be disadvantageous [26]. Proper management of the endocrine status is crucial for the surgical patient, therefore examination of the endocrine effects of different analgesics and anaesthetics are in the focus of many recent research projects. For this purpose we compared the stress related neurohormonal and metabolic effects of opioid drugs (butorphanol and fentanyl) and ketamine in healthy adult dogs without surgical intervention.

Materials and Methods

Animals

The study protocol was approved by the Animal Research Committee of Tottori University. Five beagle dogs weighting from 8 to 14 kg and ageing from 2 to 6 years participated in this study. Three female and two male dogs were housed in our laboratory. All dogs fed standard dry dog food and were in good body condition. After physical, haematological and serum biochemical examinations, each animal was found clinically healthy. All dogs received broad-spectrum wormer (Drontal plus, Bayer, Tokyo, Japan) treatment one month before the experiment.

Study design

Each dog was assigned to each of the following treatments in randomised order at one-week intervals: butorphanol tartrate (Stadol 1 mg/mL, Bristol Myers Squibb, Tokyo, Japan) (0.1 mg/kg, IM), fentanyl citrate (Fentanest 0.05 mg/mL, Sankyo, Tokyo, Japan) (10 μg/kg, IM) or ketamine hydrochloride (Ketalar 50 mg/mL, Sankyo, Tokyo, Japan) (10 mg/kg, IM).

Study protocol

One day before the experiment, a 16-gauge central venous (CV) catheter was introduced into the jugular vein under local anaesthesia (Xylocaine 2%, Fujisawa Pharmaceutical, Osaka, Japan). After fixing the catheter, the dogs were placed into individual cages to rest overnight. Food was withheld for 12 hours and water for 2 hours before the experiment. Each dog was injected intramuscularly with butorphanol, fentanyl or ketamine between 9 and 10 o'clock in the morning. Blood samples were taken from the CV catheter at 0 (initial value), 15, 30 minutes, then 1, 1.5, 2, 2.5, 3, 4, 5 and 6 hours following the injection. Additionally, the heart rate was assessed by

auscultation over the chest-wall and the rectal body temperature was measured by a digital thermometer after every blood sampling.

Sample processing

Two point five mL of blood was collected in EDTA tubes. One mL of blood was withdrawn before each sampling and the catheter was flushed with 0.5 mL of heparinized physiological saline solution afterwards. The samples were immediately placed among ice and centrifuged within 15 minutes. Complete blood count (K-4500, Sysmex, Hiroshima, Japan) was performed before centrifuging. The plasma was separated and preserved on –80 ^{o}C as single aliquot. About 1 mL of plasma could be collected with this method. Catecholamines (norepinephrine, epinephrine), cortisol, insulin, glucose, and nonesterified fatty acid (NEFA) levels were determined in each sample, following the order they are mentioned here. All samples were defrosted and refrozen again during each measurement (five times altogether). Every measurement was performed only once. Three hundred μL of plasma was used to determine catecholamines and 200 μL was used for all other measurements. Catecholamines were extracted on activated alumina and measured by a high-performance liquid chromatography (LaChrom, Hitachi, Tokyo, Japan), equipped with an electrochemical detector (Coulochem II, ESA, Chelmsford, Massachusetts, USA)[40]. Cortisol was determined by a single (I-AE16, Eiken Chemical, Tokyo, Japan) and insulin by a double antibody radioimmunoassay technique (I-AJ16, Eiken Chemical, Tokyo, Japan). Glucose and NEFA were measured by use of a spectrophotometer (Auto Sipper Photometer U-1080, Hitachi, Tokyo, Japan). The intra and inter-assay variations were less than 10 % for all of the measurements. The limit of detection was 20 pg/mL for catecholamines, 1 μg/dL for cortisol, and 2.5 μU/mL for insulin.

Statistical analysis

All data were logarithmically transformed because of the inequality of variances among the data and the often deviation from normal distribution. These logarithmic data were analysed by one-way ANOVA for repeated measures (StatView v.5.0, Abacus Concepts, Berkeley, California, USA) to examine time effect within each group, and one-way ANOVA was used for treatment effect at each time-point. When ANOVA was significant, Fisher's PLSD test was used to compare the means.

Areas under the concentration-time curves (AUC) from 0 to 6 h were calculated by using the original and not the logarithmically transformed data. The sum of the trapezoids formed by the data and the baseline (a straight line fitted on the initial value and parallel to the x-axis) served as AUC. This calculation method provided AUC values, which were less sensitive to variations of baseline and more sensitive to changes across time. The relation between different variables was examined by simple linear regression of the AUC data within each group. The test-wise significance level was set at $p < 0.05$ in every statistical test used in this study.

Results

Sedation data

All dogs become mildly sedated after butorphanol and fentanyl treatments. The animals were calmly sitting or lying in their cages, no excitement or muscle tremor occurred. Two (in five) dogs defecated within 30 min following both treatments. It was not possible to distinguish by physical examination weather the dog received butorphanol or fentanyl treatment. Although, the sedative effect of fentanyl was shorter in duration (75 ± 9 min, mean ± SEM) than that of butorphanol (173 ± 32 min). On the other hand, the dogs suffered from severe spasm, muscle tremor and salivation after ketamine treatment. The animals lay down at 6 ± 2 min and 3 (in five) dogs defecated within 30 min after ketamine injection. Complete loss of consciousness did not occur in this group but the mental state was severely altered. The recovery from sedation was complete at 113 ± 27 min after ketamine injection.

Hormonal and metabolic variables

Plasma levels of norepinephrine significantly elevated from the initial (0 h) value at 30 and 60 min after ketamine injection (Figure 10) but did not change significantly in the other groups. Only one dog had increased norepinephrine plasma level (1.2 ng/mL) at 15 min after fentanyl injection, therefore the mean value non-significantly increased in this group. There were no significant differences in norepinephrine levels among the groups at any time-point. Plasma levels of epinephrine significantly increased in every group at 15 and 30 min (Figure 11). The mean epinephrine value was the highest after fentanyl treatment but the individual variability was also high. One dog reacted with extremely high epinephrine levels (6.4 and 2.3 ng/mL) at 15 min following fentanyl and butorphanol injections, respectively. Epinephrine levels tended to be higher in the fentanyl and butorphanol groups when compared to ketamine, but it was not significant.

The epinephrine AUC values (from 0 to 6 h) significantly correlated to the norepinephrine AUC in the butorphanol group (r = 0.91) and had a non-significant tendency (r = 0.8) in the fentanyl group but did not correlate in the ketamine group (Figure 12).

Cortisol levels significantly increased in every treatment group (Figure 10) but this response was delayed after butorphanol (at 1, 1.5 and 2 h) than fentanyl and ketamine (at 15, 30 and 60 min) treatments. Cortisol levels were significantly lower in the butorphanol group than in the other groups at 15 min, and higher than in the ketamine group at 2 h. Cortisol AUC values did not correlate to the norepinephrine or epinephrine AUC values in any of the treatment groups.

Plasma levels of glucose (Figure 11) significantly elevated after fentanyl (at 15, 30, 60 and 90 min) and ketamine (at 30 min) but not after butorphanol treatments. Glucose levels had tendency to increase in the butorphanol group also but it was not significant because of the high variability of the data. Overall, glucose levels in the fentanyl group tended to be higher than the other groups but there were no significant differences among the groups at any time. Interestingly, the glucose AUC values highly significantly correlated to the epinephrine AUC in the butorphanol and fentanyl but not in the ketamine groups (Figure 13). Similar, significant correlation was observed between norepinephrine and glucose AUC in the butorphanol group (r = 0.91) and non-significant tendency in the fentanyl group (r = 0.68) but no correlation was found in the ketamine group (data is not shown). Additionally, cortisol and glucose AUC had a tendency to positively correlate in the ketamine group (r = 0.79) but not in the other groups (data is not shown).

Plasma levels of insulin and NEFA did not change significantly in this experiment (data is not shown). However, there were tendencies for positive correlation between

insulin and glucose AUC in the fentanyl (r = 0.77) and ketamine groups (r = 0.79). The NEFA AUC significantly positively correlated to norepinephrine (r = 0.95) and epinephrine (r = 0.95) in the butorphanol group, and similar non-significant tendencies were present in the fentanyl group (Figure 14). There were tendencies in the ketamine group for negative correlation between insulin and NEFA AUC (r = 0.75) and positive correlation between norepinephrine and NEFA AUC (r = 0.86) (data is not shown).

Heart rate and body temperature

Ketamine treatment significantly increased hearth rate at 15, 30 and 60 min (Figure 15) but butorphanol and fentanyl did not affect that. Even when the epinephrine levels were very high after fentanyl and butorphanol treatments the hearth rates were only slightly elevated from the pre-treatment levels. The rectal body temperature significantly decreased after butorphanol and fentanyl treatments (Figure 15). This effect of fentanyl was significantly stronger than that of butorphanol. Body temperature did not change significantly in the ketamine group, when compared to the pre-treatment values but had a tendency to increase over baseline at 30 min and decrease below baseline later on.
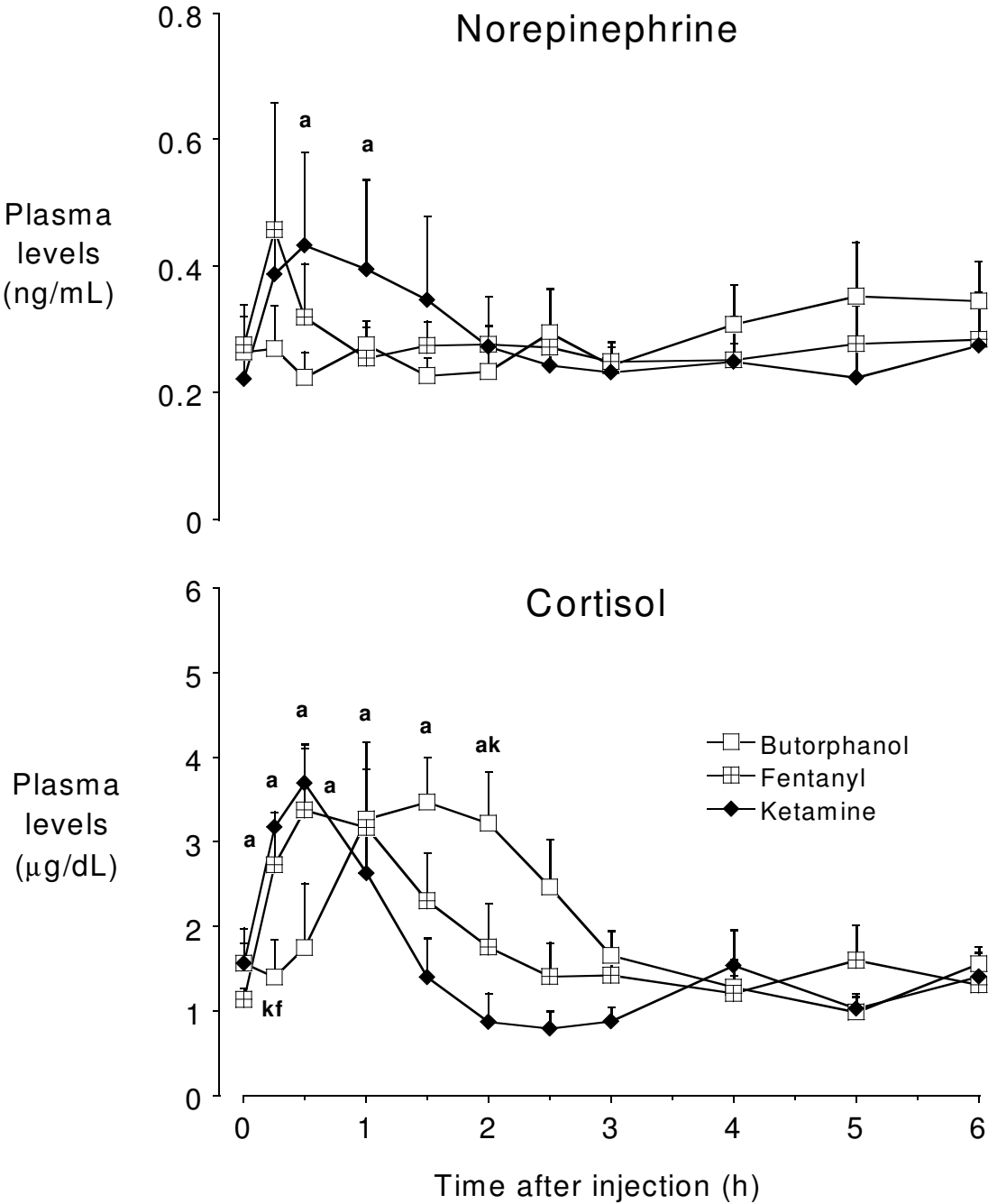
Figure 10. Norepinephrine and cortisol plasma levels of dogs injected with butorphanol (0.1 mg/kg, IM) fentanyl (10 μg/kg, IM) or ketamine (10 mg/kg, IM) respectively. Each point and vertical bar represent the mean and SEM (n = 5). Superscript 'a': significantly different from the initial, 0 h value. Superscripts 'f' and 'k': significantly different from the fentanyl and ketamine groups respectively at that time-point ($p < 0.05$).
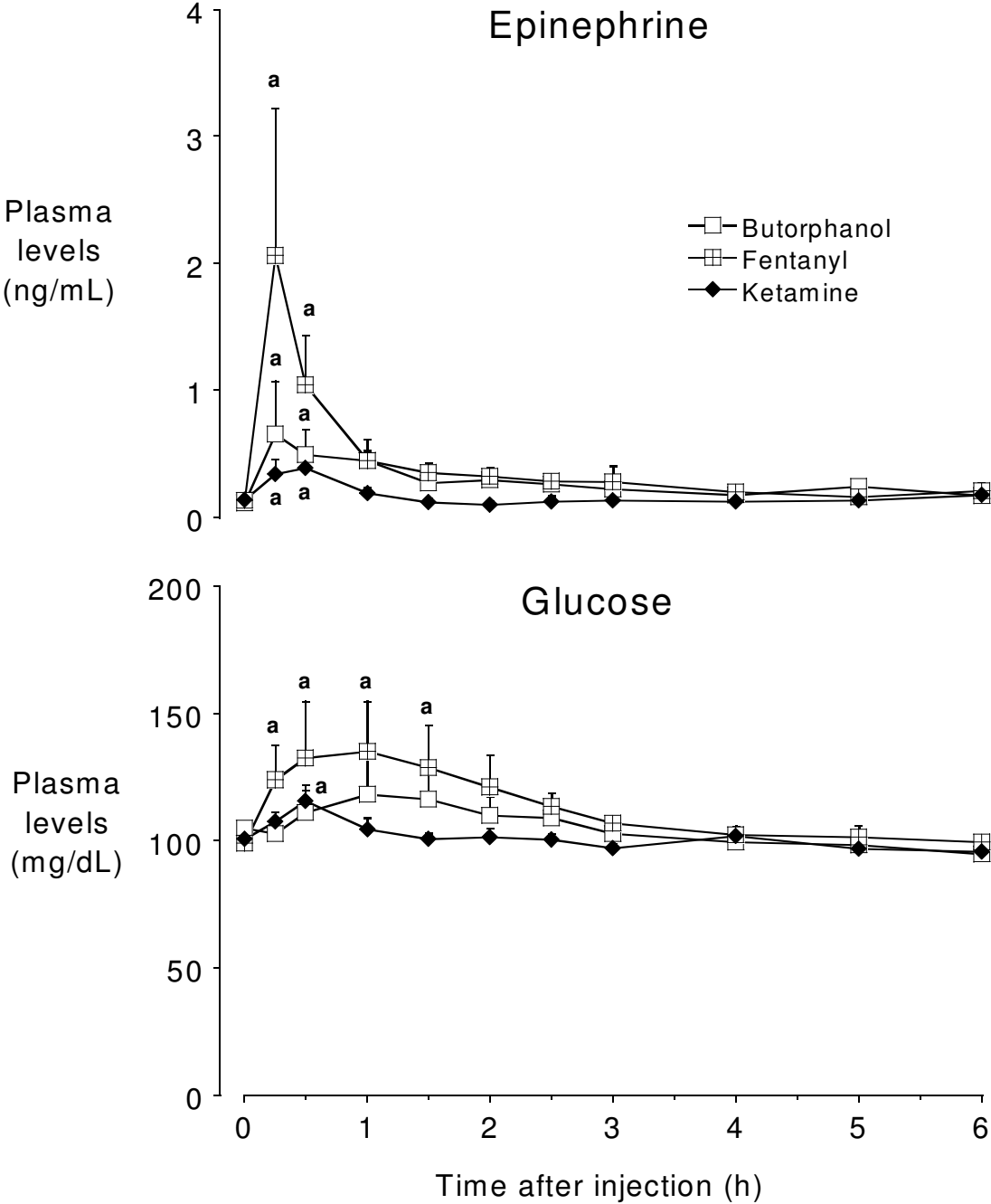
Figure 11. Epinephrine and glucose plasma levels of dogs injected with butorphanol (0.1 mg/kg, IM) fentanyl (10 μg/kg, IM) or ketamine (10 mg/kg, IM) respectively. Each point and vertical bar represent the mean and SEM (n = 5). Superscript 'a': significantly different from the initial, 0 h value ($p < 0.05$).
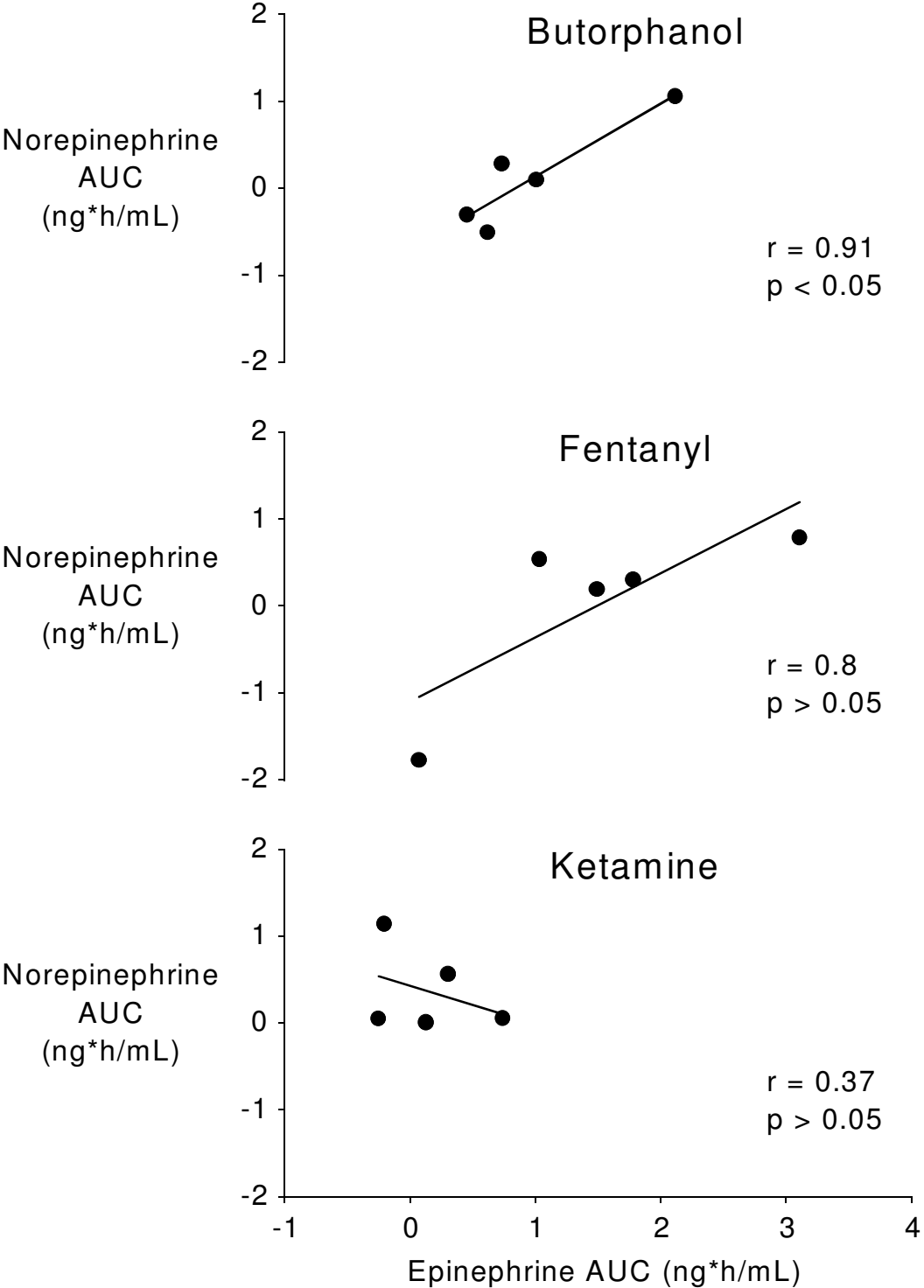
Figure 12. Scatterplots showing correlation between area under curve (AUC, 0-6 h) for plasma epinephrine and norepinephrine levels of dogs injected with butorphanol (0.1 mg/kg, IM) fentanyl (10 μg/kg, IM) or ketamine (10 mg/kg, IM) respectively. The epinephrine and norepinephrine AUC values significantly correlated after butorphanol, tended to correlate after fentanyl but did not correlate after ketamine treatments according to linear regression analysis ($p < 0.05$, n = 5).
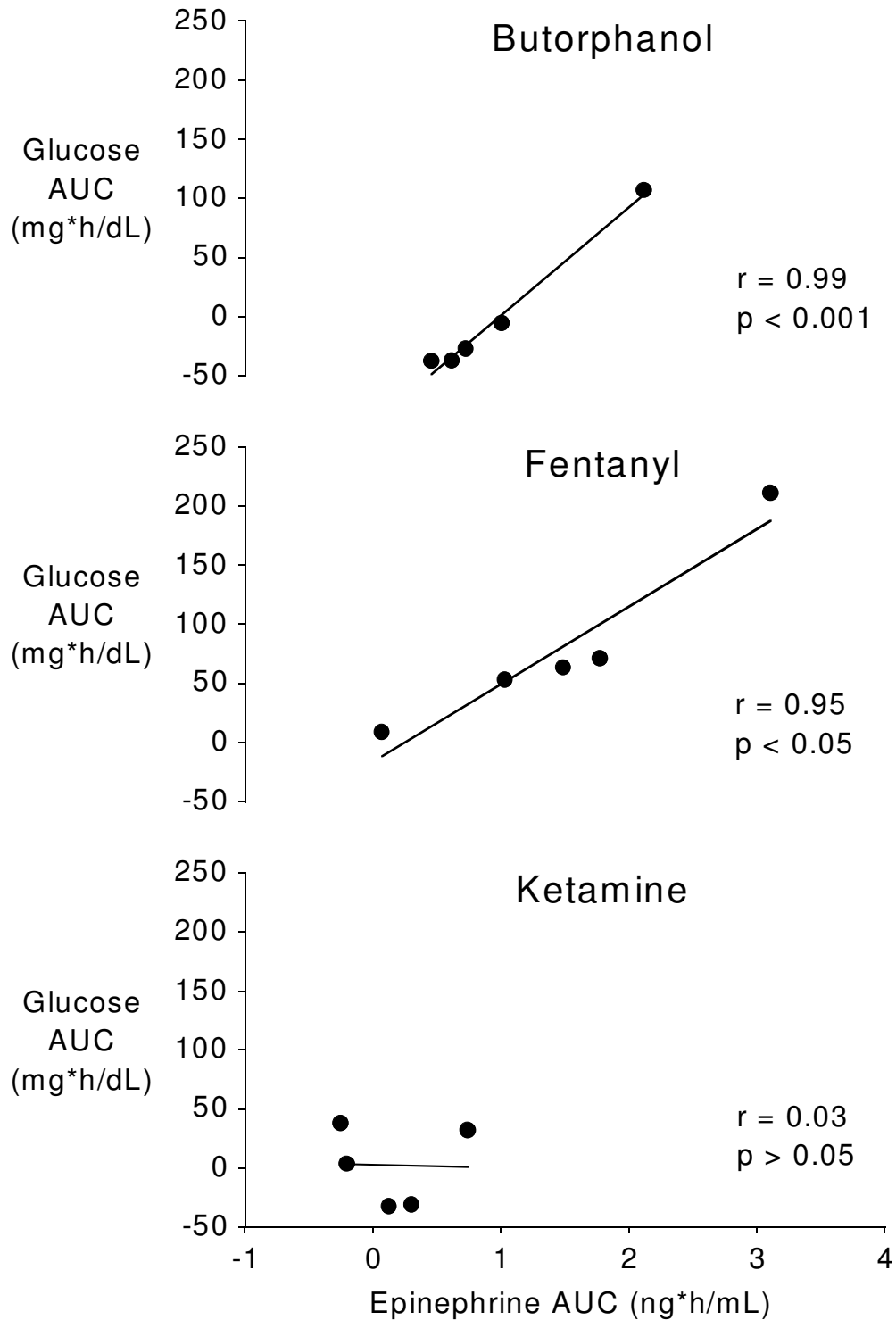
Figure 13. Scatterplots showing correlation between area under curve (AUC, 0-6 h) for plasma epinephrine and glucose levels of dogs injected with butorphanol (0.1 mg/kg, IM) fentanyl (10 μg/kg, IM) or ketamine (10 mg/kg, IM) respectively. The epinephrine and glucose AUC values significantly correlated after butorphanol and fentanyl but not after ketamine treatments according to linear regression analysis ($p < 0.05$, n = 5).
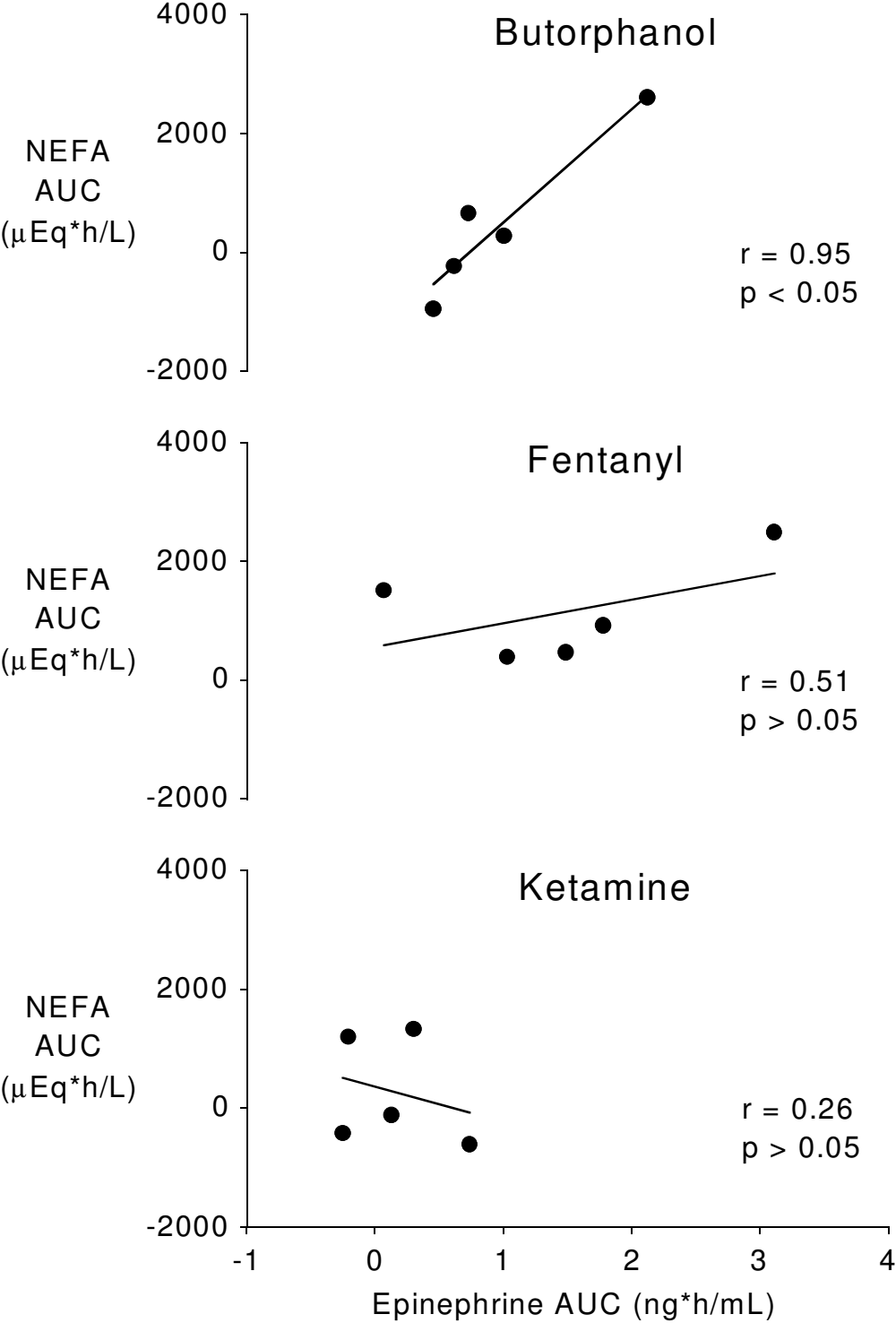
Figure 14. Scatterplots showing correlation between area under curve (AUC, 0-6 h) for plasma epinephrine and nonesterified fatty acid (NEFA) levels of dogs injected with butorphanol (0.1 mg/kg, IM) fentanyl (10 μg/kg, IM) or ketamine (10 mg/kg, IM) respectively. The epinephrine and NEFA AUC values significantly correlated after butorphanol, tended to correlate after fentanyl but did not correlate after ketamine treatments according to linear regression analysis ($p < 0.05$, n = 5).
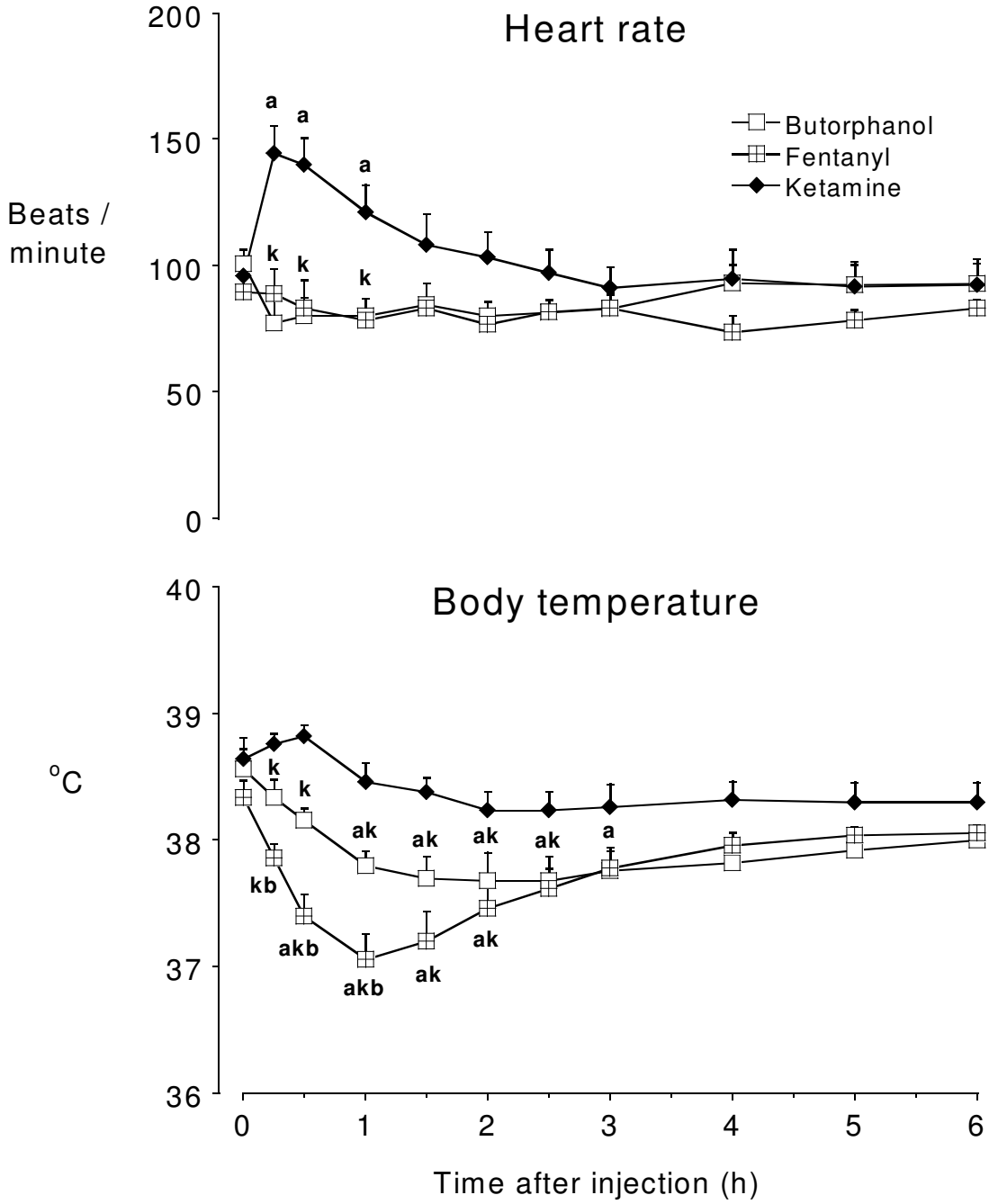
Figure 15. Heart rate and body temperature plasma levels of dogs injected with butorphanol (0.1 mg/kg, IM) fentanyl (10 µg/kg, IM) or ketamine (10 mg/kg, IM) respectively. Each point and vertical bar represent the mean and SEM (n = 5). Superscript 'a': significantly different from the initial, 0 h value ($p < 0.05$). Superscripts 'b' and 'k': significantly different from the butorphanol and ketamine groups respectively at that time-point ($p < 0.05$).

Discussion

Ketamine treatment increased the norepinephrine and epinephrine levels. The effect that ketamine blocks the reuptake and therefore increase the plasma levels of catecholamines is well known [25,76].

Norepinephrine levels did not change significantly after butorphanol and fentanyl treatments but both treatments increased the epinephrine levels possibly through action on central μ opioid receptors [77,78]. Fentanyl increased the epinephrine levels with a (non-significantly) greater potency than butorphanol possibly because fentanyl is full agonist and butorphanol is only partial agonist on the μ opioid receptors. Norepinephrine AUC data correlated with the epinephrine AUC in the butorphanol and showed a tendency for correlation in the fentanyl group but not in the ketamine group. This result suggests differences between the catecholamine increasing effects of opiates and ketamine. The variances among the epinephrine data in the butorphanol and fentanyl groups were much higher when compared to the ketamine group.

Cortisol plasma levels increased in every group with similar potency. The variances among the cortisol data were moderate and similar in each group. Cortisol AUC data did not correlate with the epinephrine AUC in any groups suggesting that their releases may be mediated through different pathways. We can not explain why the increase in cortisol levels delayed after butorphanol treatment.

The hyperglycaemic effect of morphine is known to be mediated centrally, through the release of epinephrine [79]. Our results are in accordance of this theory. The correlation between the AUC data of epinephrine and glucose suggest that epinephrine should be the key mediator for the increase of plasma glucose after butorphanol and fentanyl but not after ketamine treatments.

The mean insulin and NEFA levels did not change significantly in any of the groups. Although in some cases when the epinephrine levels were very high in the butorphanol and fentanyl groups, insulin and NEFA levels seemed to increase also. The correlation of the AUC data between epinephrine and NEFA was significant in the butorphanol group, showed a tendency in the fentanyl group but no correlation was observed in the ketamine group. These results suggest that epinephrine may have important role in increasing lipolysis after opioid treatments.

Epinephrine seems to be the key mediator for the increase in plasma glucose and NEFA levels after butorphanol and fentanyl but not after ketamine treatments. According to the literature N-methyl-D-aspartate (NMDA) itself caused increase in plasma levels of ACTH, corticosterone, glucose and insulin and did not change the catecholamine levels after IV injection in rats [80]. The anaesthetic effects of ketamine are associated with its antagonistic action on the NMDA receptors but comparing the results of the present experiment to the hormonal effects of NMDA we suspect that the changes in cortisol, glucose and insulin levels are probably not mediated by NMDA receptors after ketamine treatment.

Chapter 5

Stress-related neurohormonal and metabolic effects of medetomidine combined with butorphanol, fentanyl or ketamine in dogs

Alpha-2 adrenoceptor agonists are often used in combination with opioids to produce sedation, analgesia and balanced anaesthesia for different species [27-30]. Such a combination is advantageous because the sedative and analgesic effects are more than additive between these drugs [31-33]. This can be explained with the synergistic interaction in antinociception between the α_2-adrenoceptors and the μ opioid receptors in the spinal cord [34,81]. Especially the medetomidine-butorphanol combinations became widely used in the canine practice because of their few side effects [35,36]. In contrast, fentanyl, a potent μ opioid receptor agonist, is rarely used in combination with medetomidine because of the profound respiratory depression it may cause [37]. Medetomidine is also often combined with ketamine, a dissociative anaesthetic agent, to provide balanced anaesthesia in dogs [38] and cats [39].

In previous studies [60,82] and in the chapter 4 we examined the stress-related neurohormonal and metabolic effects of medetomidine, butorphanol, fentanyl and ketamine as a sole agent, and concluded that medetomidine attenuated the sympathoneural, sympathoadrenal and adrenocortical activities and the other three drugs

enhanced them. All of the four drugs significantly increased the plasma levels of glucose in those studies except for butorphanol. In the present study we examined the stress-related neurohormonal effect of medetomidine in combination with butorphanol, fentanyl or ketamine in beagle dogs.

Materials and Methods

Animals

The study protocol was approved by the Animal Research Committee of Tottori University. Five beagle dogs from both sexes, weighting from 9 to 14 kg and ageing from 1 to 5 years, participated in this study. The dogs were housed in our laboratory. All dogs fed standard dry dog food and were in good body condition. After physical, haematological and serum biochemical examinations, each animal was found clinically healthy.

Study design

There were four treatment groups: medetomidine hydrochloride (1 mg/mL, Domitor, Meiji Seika Kaisha, Tokyo, Japan) (20 µg/kg) was combined with either physiological saline (0.1 mL/kg, MED-SAL), butorphanol tartrate (Stadol 1 mg/mL, Bristol Myers Squibb, Tokyo, Japan) (0.1 mg/kg, MED-BUT), fentanyl citrate (Fentanest 0.05 mg/mL, Sankyo, Tokyo, Japan) (10 µg/kg, MED-FEN) or ketamine hydrochloride (Ketalar 50 mg/mL, Sankyo, Tokyo, Japan) (10 mg/kg, MED-KET), respectively. These mixtures were intramuscularly administered from a single syringe. Each dog was assigned to each of the treatments in randomised order at one-week intervals.

Study protocol

The study protocol and the sample processing were similar to previously described in the chapter 4. Shortly, one day before the experiment a central venous (CV) catheter was introduced into the jugular vein. After fixing the catheter, the dogs were placed into individual cages to rest overnight. Food was withheld for 12 hours and water for 2 hours before the experiment. Each dog was injected intramuscularly with one of the treatments. Blood samples were taken from the CV catheter at 0 (initial value), 15, 30 minutes, then 1, 1.5, 2, 2.5, 3, 4, 5 and 6 hours following the injection.

Sample processing

Two point five mL of blood was collected as samples in EDTA tubes. The samples were immediately placed among ice and centrifuged within 15 minutes. Complete blood count (K-4500, Sysmex, Hiroshima, Japan) was performed before centrifuging. The plasma was separated and preserved on –80 ^{o}C as single aliquot. Catecholamines (norepinephrine, epinephrine), cortisol, insulin, glucose, and nonesterified fatty acid (NEFA) levels were determined in each sample. Catecholamines were extracted on activated alumina and measured by a high-performance liquid chromatography (LaChrom, Hitachi, Tokyo, Japan), equipped with an electrochemical detector (Coulochem II, ESA, Chelmsford, Massachusetts, USA)[40]. Cortisol was determined by a single (I-AE16, Eiken Chemical, Tokyo, Japan) and insulin by a double antibody radioimmunoassay technique (I-AJ16, Eiken Chemical, Tokyo, Japan). Glucose and NEFA were measured by use of a spectrophotometer (Auto Sipper Photometer U-1080, Hitachi, Tokyo, Japan). The intra and inter-assay variations were less than 10 % for all of the measurements. The limit of detection was 20 pg/mL for catecholamines, 1 μg/dL for cortisol, and 2.5 μU/mL for insulin.

Statistical analysis

The catecholamine, cortisol, glucose and insulin data were analysed by one-way ANOVA for repeated measures (StatView v.5.0, Abacus Concepts, Berkeley, California, USA) to examine time effect within each group, and one-way ANOVA was used for treatment effect at each time-point. When ANOVA was significant, Tukey's test was used to compare the means. Only the NEFA data were logarithmically transformed because of the inequality of variances among the data and the deviation from normal distribution. These logarithmic NEFA data were subjected to ANOVA and Tukey's test similarly to described above.

Areas under the concentration-time curves (AUC) from 0 to 6 h were calculated by using the original and not the logarithmically transformed data for all variables. The sum of the trapezoids formed by the data and the x-axis served as AUC. The AUC data were compared by one-way ANOVA among the groups.

Additionally the sedation data were also analysed by one-way ANOVA and Tukey's test across the groups. The test-wise significance level was set at $p < 0.05$ in every statistical test used in this study.

Results

Sedation data

The dogs became recumbent 24 ± 7 min (Mean ± SEM) after MED-SAL injection and the first signs of arousal were observed at 161 ± 11 min in this group. These findings are different than after intramuscular injection of the same medetomidine dose as undiluted Domitor in our previous studies, where the time to recumbency was about 10 min and the arousal time about 100 min following the injections [60,82]. The depth of sedation also seemed to be more superficial than in those studies when undiluted Domitor was injected. On the other hand, the time to recumbency was 8 ± 1 min in the MED-BUT, 17 ± 4 min in the MED-FEN and 6 ± 1 min in the MED-KET groups. Dogs in the MED-KET group lay down significantly faster than in the MED-SAL group. The first signs of arousal were detected at 132 ± 9 min in the MED-BUT, 110 ± 12 min in the MED-FEN and 75 ± 9 min in the MED-KET groups. The arousal time was significantly shorter in the MED-FEN and MED-KET than in the MED-SAL groups. The dogs became deeply sedated in the MED-BUT and MED-KET groups but the sedative effect of the MED-FEN combination was lighter and more unreliable.

Hormonal and metabolic variables

Plasma levels of norepinephrine significantly decreased in every group at 15 and 30 min after injections comparing to the initial values (Figure 16). There were no significant differences among the groups at these time-points. The norepinephrine levels remained significantly lower than the initial values until 2.5 h in the MED-BUT and MED-FEN, and until 3 h in the MED-SAL groups. On the other hand norepinephrine levels in the MED-KET group gradually returned to the baseline from 1 h and become significantly higher than that of the MED-SAL group at 1, 1.5, 2, 2.5 and 5 h. The

norepinephrine AUC values were also significantly higher in the MED-KET than in the MED-SAL group.

The epinephrine plasma levels significantly decreased below the initial values at 15, 30, and 60 min in every group (Figure 16). The epinephrine levels remained significantly lower than the initial values until 1.5 h in the MED-BUT, and until 2.5 h in the MED-FEN and MED-SAL groups. The epinephrine levels were significantly higher in the MED-KET group than in the MED-SAL group at 2 and 2.5 h. There were no significant differences among the epinephrine AUC values.

There were no significant differences among the cortisol levels in this experiment (data not shown).

The glucose plasma levels started to increase from 1 h and became significantly elevated from the initial value at 2.5 and 3 h in the MED-SAL group (Figure 17). Interestingly, glucose plasma levels had a tendency to increase from 30 min and significantly elevated from the initial values at 2 and 3 h in our previous studies when the same medetomidine dose was injected as undiluted Domitor. Glucose levels tended to increase in the MED-BUT (at 2 and 2.5 h) and the MED-FEN groups (at 2.5 and 3 h) but it was not significant. On the other hand, glucose levels in the MED-KET group significantly increased at 1.5 h comparing to the initial value then returned to the baseline. Glucose levels were significantly lower in the MED-BUT (at 3 h), MED-FEN (at 3 h) and MED-KET groups (at 2.5, 3 and 4 h) when compared to the MED-SAL group at that time-point. Additionally, the glucose AUC was significantly lower in each balance anaesthesia group than in the MED-SAL group.

Plasma levels of insulin significantly decreased below the initial values in the MED-BUT, MED-FEN and MED-SAL groups at 0.5, 1, 1.5 and 2 h than returned to the baseline. On the other hand, insulin levels in the MED-KET group decreased only at 0.5

and 1 h significantly, comparing to the initial value and significantly elevated over the MED-SAL group at 1.5 h. The insulin AUC values were not different among the groups.

Plasma levels of NEFA significantly decreased below the initial values from 0.5 h to 2 h in every group except for the MED-FEN group where NEFA levels decreased from 1 to 2 h. The NEFA levels significantly increased comparing to the initial value at 5 and 6 h in the MED-KET group. The NEFA AUC values were not different among the groups.
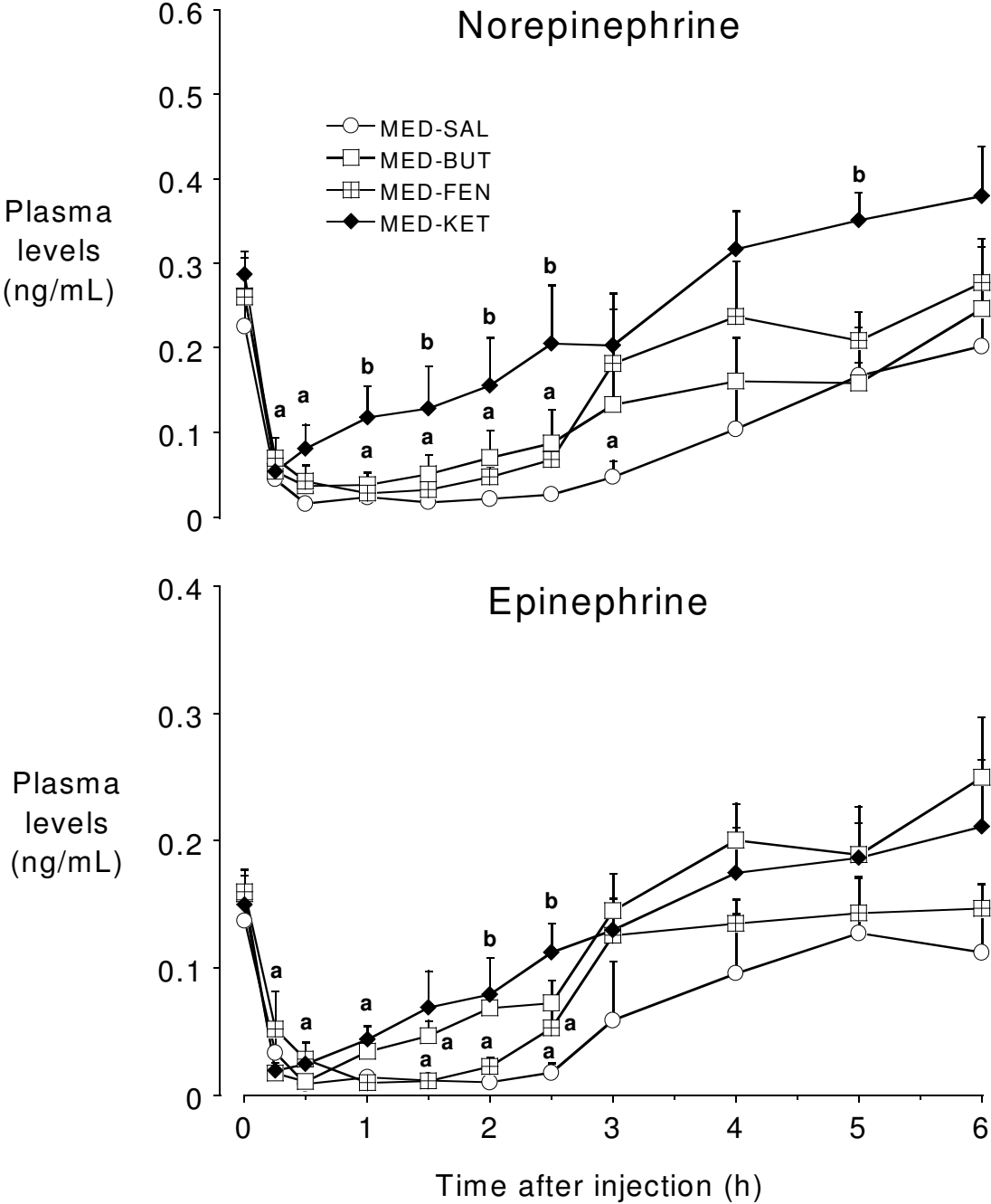
Figure 16. Norepinephrine and epinephrine plasma levels of dogs injected intramuscularly with medetomidine (20 μg/kg) in combination with either saline (0.1 mL/kg, MED-SAL), butorphanol (0.1 mg/kg, MED-BUT) fentanyl (10 μg/kg, MED-FEN) or ketamine (10 mg/kg, MED-KET) respectively. Each point and vertical bar represent the mean and SEM (n = 5). Superscript 'a': significantly different from the initial, 0 h value. Superscript 'b': significantly different from the MED-SAL group at that time-point ($p <$ 0.05).

Figure 17. Glucose and insulin plasma levels of dogs injected intramuscularly with medetomidine (20 μg/kg) in combination with either saline (0.1 mL/kg, MED-SAL), butorphanol (0.1 mg/kg, MED-BUT) fentanyl (10 μg/kg, MED-FEN) or ketamine (10 mg/kg, MED-KET) respectively. Each point and vertical bar represent the mean and SEM (n = 5). Superscript 'a': significantly different from the initial, 0 h value. Superscript 'b': significantly different from the MED-SAL group at that time-point ($p < 0.05$).
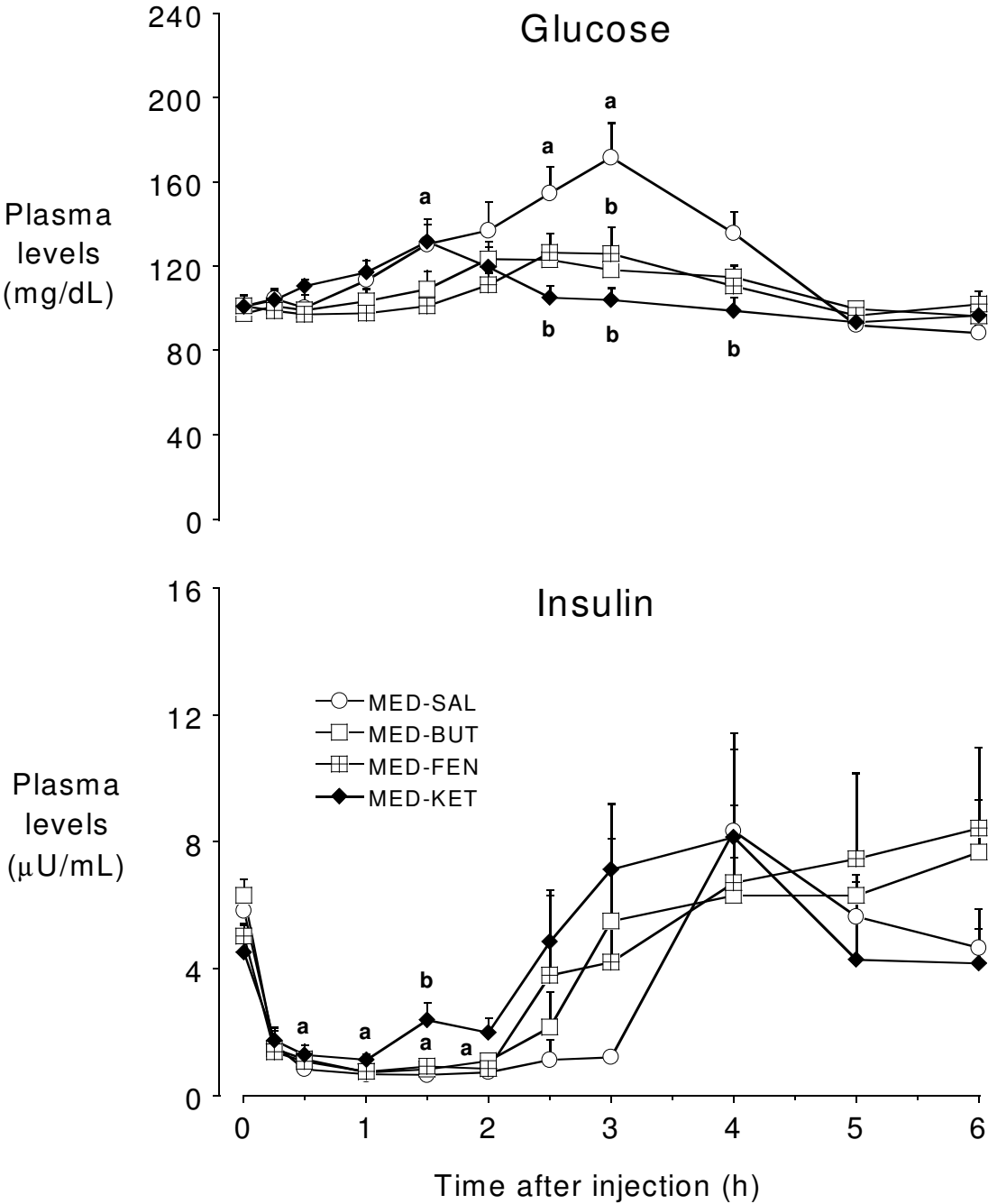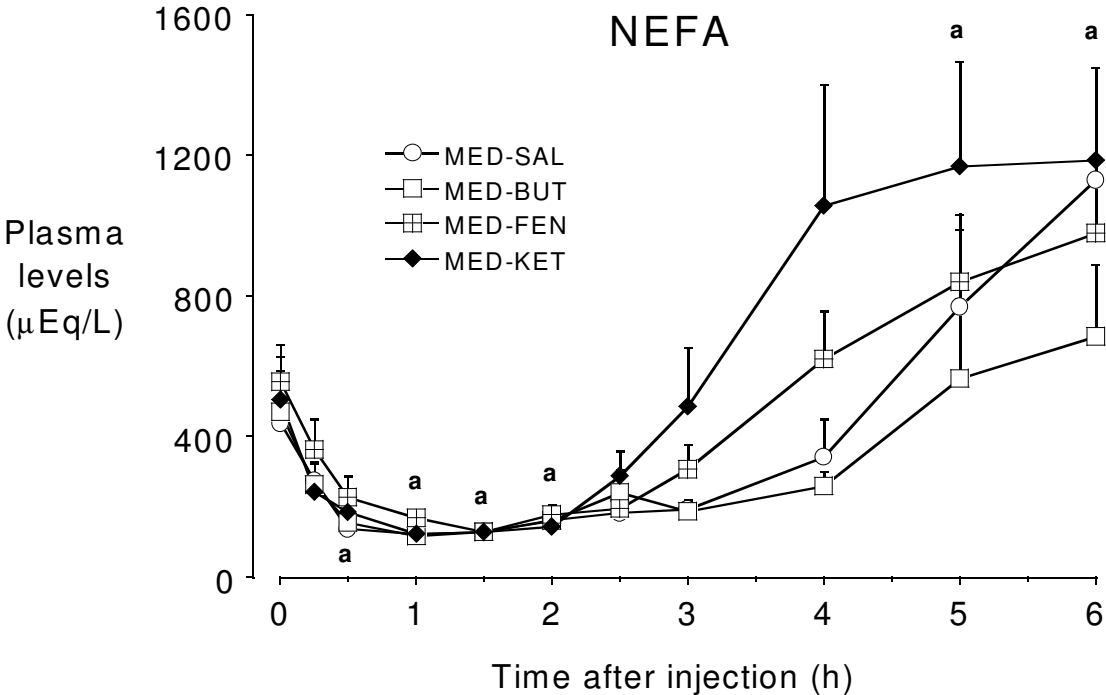
Figure 18. Nonesterified fatty acid (NEFA) plasma levels of dogs injected intramuscularly with medetomidine (20 μg/kg) in combination with either saline (0.1 mL/kg, MED-SAL), butorphanol (0.1 mg/kg, MED-BUT) fentanyl (10 μg/kg, MED-FEN) or ketamine (10 mg/kg, MED-KET) respectively. Each point and vertical bar represent the mean and SEM (n = 5). Superscript 'a': significantly different from the initial, 0 h value ($p < 0.05$).

Discussion

In the present study we examined the neurohormonal and metabolic effects of three different balanced anaesthesia protocols (MED-BUT, MED-FEN and MED-KET) and compared them to the effects of medetomidine as a control group (MED-SAL). All combinations were administered intramuscularly from a single syringe. The speed and completeness of absorption from the intramuscular site may influence the effects of a treatment. Diffusion of molecules through a semipermeable membrane (as during absorption after an intramuscular injection) is effected by their concentration. For this reason physiological saline solution was mixed with medetomidine in the control group (MED-SAL) in order to reach similar medetomidine concentration as in the balance anaesthesia groups. The volume of saline solution used in the MED-SAL group (0.1 mL/kg) was similar to the volume of butorphanol (0.1 mL/kg) but smaller than the volumes of fentanyl and ketamine (0.2 mL/kg) used in the balance anaesthesia groups. In this respect the original medetomidine solution (Domitor inj.) was diluted 6 times in the MED-SAL and MED-BUT groups but 11 times in the MED-FEN and MED-KET groups. Therefore, the MED-SAL group used in this experiment is not a perfectly fitted control for MED-FEN and MED-KET treatments but it is still a better approach than using the original medetomidine solution (Domitor inj.).

Other factors like chemical and pharmacokinetic interactions between medicines may also influence the absorption, distribution and elimination of drugs [83]. All of these issues must be taken into account when evaluating the effect of balanced anaesthesia treatments. Medetomidine can be mixed with butorphanol, fentanyl or ketamine in a single syringe without any adverse chemical reaction [28,33]. Every drug used in this study absorbs fast and complete when injected intramuscularly as a sole agent [26,84-

86]. However, pharmacokinetic interactions are supposed to exist between the drugs used in this study.

Dogs in the MED-SAL group became recumbent about 2.5 times later and started to wake up 1.6 times later than after the same dose of medetomidine injection in our previous studies [60,82]. The level of sedation seemed to be lighter in the MED-SAL group then in those experiments. The increase in the plasma level of glucose also shifted to the right in the MED-SAL group when compared to the previous experiments. The delay in the onset of medetomidine effect may be attributed to the delayed absorption from the intramuscular site because the injected medetomidine concentration was lower. Because medetomidine is metabolised rapidly in the liver slower absorption may be associated with lower peak plasma levels and a loss of effect. According to the authors knowledge this is the first study to report the loss of medetomidine effect when injected IM in a diluted solution. Whether this effect is similar when medetomidine is mixed in the same syringe with butorphanol, ketamine or midazolam and injected IM needs to be further researched.

Sympathoneural and sympathoadrenal activities, as assessed by the norepinephrine and epinephrine plasma levels, decreased after every treatment. Previously we also found that medetomidine decreased the plasma levels of norepinephrine and epinephrine [60,82]. In the chapter 4 we demonstrated that only ketamine treatment increased the norepinephrine levels, but each of the butorphanol, fentanyl and ketamine treatments increased the epinephrine levels. Consequently the effects of medetomidine dominated in the balance anaesthesia groups and ketamine had significantly stronger potency to increase sympathoneural activity when compared to butorphanol or fentanyl.

In the chapter 4 we observed that fentanyl and ketamine treatments caused significant hyperglycaemia, while butorphanol showed only a tendency for

hyperglycaemia. We also suggested the possibility that the hyperglycaemic response is mediated through different pathways between opioids and ketamine. In the present study, the plasma levels of glucose similarly increased in the opioid combined balance anaesthesia groups but increased faster in the MED-KET group. Interestingly, the overall glucose levels were lower in every balance anaesthesia groups than in the MED-SAL group. These results can not be explained with the present knowledge about α_2-adrenergic agents. Medetomidine is commonly used in veterinary anesthesia to reduce perioperative stress response. From this point of view hyperglycemia may be considered as an adverse reaction of medetomidine, therefore the balance anesthesia combinations examined in this study may be advantageous over single medetomidine treatment.

The plasma levels of insulin were very similar in every treatment group except for MED-KET where there was a slight but significant increase at 1.5 h. Interestingly, the glucose levels peaked at same time in the same treatment group. There is a possibility that ketamine potentiated the blood circulation because of its sympathomimetic effect, consequently the hepatic blood flow might increase and medetomidine might be metabolised faster than after sole medetomidine injection. Therefore the decreasing medetomidine concentration might not suppress the insulin levels with sufficient potency and insulin levels increased in response to hyperglycaemia. Similar pharmacokinetic interaction has already been reported between medetomidine and atipamezole in dogs [63].

There were no meaningful differences in NEFA levels among the groups.

In conclusion, delayed onset and loss of sedative effect of medetomidine were observed in this study when injected as a 6 times diluted Domitor solution. The neurohormonal and metabolic effects of medetomidine were predominant in the balance anaesthesia protocols examined in this study. The norepinephrine levels were less

depressed in the MED-KET group probably because of the potency of ketamine to increase sympathoneural activity. On the other hand, balanced anaesthesia with butorphanol, fentanyl and ketamine provided lower plasma glucose levels than medetomidine alone.

Chapter 6

General discussion

The α_2-adrenoceptor agonists are well known to inhibit the sympathetic outflow in the central nervous system through their actions on α_2-adrenoceptors, hence decreasing the level of circulating catecholamines [2,11]. Our results in chapter 2 support this theory because the plasma catecholamine levels decreased after an injection of either medetomidine or xylazine. There are several data demonstrating the inhibition of catecholamine release at blood levels associated with the usage of α_2-agonists. For example, Benson *et al* [19] found that medetomidine administered pre-operatively reduced the catecholamine levels in both ovariohysterectomised and non-operated dogs under isoflurane anaesthesia. Talke *et al* [41] reported that dexmedetomidine administered to human patients postoperatively using a computer-controlled infusion technique decreased the plasma levels of catecholamines during infusion. Benson *et al* [9] also reported that xylazine injected after onychectomy reduced plasma concentrations of catecholamines in cats. In addition, Flacke *et al* [42] reported that treatment with clonidine reduced the catecholamine level in dogs anaesthetised with fentanyl-enflurane-nitrous oxide. However, there were no reports to compare the effects of medetomidine and xylazine at different dosages on the plasma catecholamine levels in dogs under basal conditions. In the present study, both medetomidine and xylazine suppressed norepinephrine release dose-dependently with similar potency (Figure 1). On the other hand, medetomidine dose-dependently suppressed the epinephrine secretion. Xylazine also tended to inhibit epinephrine release dose-dependently (Figure

2). However, the potency of medetomidine in reducing the plasma epinephrine levels was greater than that of xylazine.

The plasma cortisol levels are influenced by both the peripheral site at the adrenal cortex, and the central site through the release of corticotrophin-releasing factor (CRF) and adrenocorticotrophic hormone (ACTH) in the brain. The effects of α_2-agonists on the plasma cortisol level have been assessed under different experimental conditions in a variety of species. Maze *et al* [43] found that an intramuscular injection of 80 μg/kg dexmedetomidine reduced the basal cortisol level and the cortisol release to ACTH stimulation at 3 h post-injection in dogs, and concluded that only high dosages of dexmedetomidine inhibit adrenal steroidogenesis. In humans, it was reported that 0.45 mg clonidine administered orally for 3 days reduced plasma cortisol level [44], but 0.1 and 0.2 mg clonidine for 4 days did not affect it under basal conditions [45]. Haas *et al* [46] reported that an intraperitoneal administration of 1 mg/kg clonidine significantly decreased the hypothalamic CRF-like immunoreactivity in rats. Furthermore, Taylor *et al* [47] reported in ponies that an intravenous anaesthesia using detomidine, ketamine and guaiphenesin decreased the cortisol level, but did not significantly change the ACTH concentration. On the other hand, there are several reports with respect to the influence of α_2-agonists on the cortisol release associated with surgical stimulation. Premedication with medetomidine was reported to reduce or delay the increase of plasma cortisol levels induced by ovariohysterectomy in dogs [19,48]. Sedation with xylazine diminished the increase in the plasma cortisol level after intradermal testing in dogs [10]. In addition, clonidine prevented the elevation of cortisol level during laparoscopy in humans [49]. These findings indicate that treatments with α_2-agonists such as medetomidine, xylazine or clonidine, show inhibitory effect on the release of cortisol. However, whether it is due to the α_2-adrenoceptor-mediated specific action,

other receptor-mediated actions, or the result of non-specific effects by providing sedation and analgesia which reduce stress response, is unknown. In this respect, recent studies have evidenced that imidazoline receptors but not α_2-adrenoceptors may a play role in the direct inhibition of cortisol release in the adrenal cortex. An *in vitro* study revealed that the imidazoline α_2-adrenergic agents, medetomidine, detomidine and atipamezole, all suppressed the release of cortisol from porcine adrenocortical cells [50]. As medetomidine and detomidine are selective α_2-agonists, and atipamezole is a selective α_2-antagonist, this effect is unrelated to their actions on α_2-adrenoceptors. Maze *et al* found [43] that the selective α_2-agonist, dexmedetomidine (*d*-medetomidine) and its clinically ineffective enantiomer, *l*-medetomidine were equally effective in blocking the ACTH-stimulated corticosterone secretion in adrenocortical cells of rats. This finding also supports that imidazoline receptors may also be responsible for inhibition of adrenal steroidogenesis.

In the present study, we examined the plasma cortisol level under basal conditions without surgical stress or ACTH stimulation, and found that both medetomidine and xylazine failed to significantly alter the plasma cortisol levels at the examined dosages. The possible explanation why an imidazoline derivative, medetomidine did not significantly decrease the cortisol level may be that its applied dose was too little to decrease cortisol release [43].

The most important finding of this study was that medetomidine did not increase the plasma glucose level in a dose-related manner in the examined dosages, whereas xylazine increased it dose-dependently (Figure 3). Our finding that the hyperglycaemic effect of medetomidine was not dose-dependent is the first report in dogs. On the other hand, the present study revealed that either medetomidine or xylazine decreased plasma insulin levels in all dosages used (Figure 5). The comparison of the AUC insulin data

also suggested that the potencies of medetomidine and xylazine to suppress insulin release were similar. There are numerous reports as to the effects of α_2-agonists on the blood glucose and insulin levels in different species. Either clonidine or xylazine was found to dose-dependently increase blood glucose in cattle [16,17] and rats [51,52]. It was reported in dogs that an intramuscular injection of 2.2 mg/kg xylazine increased blood glucose and decreased insulin level [12], which were in agreement with the present results. Similar results have also been reported in cats [13] and sheep [18]. Two reports are available on the effect of medetomidine in dogs. Burton *et al* [53] found that 10 and 20 μg/kg medetomidine administered intravenously to beagle dogs tended to elevate the plasma glucose level, but it did not reach the level of significance and remained within the normal physiological range. They observed a peak in glucose level of about 90 mg/dL at 3 h after 20 μg/kg medetomidine treatment. This is in contrast with our results because the glucose level in the MED-20 group of our study significantly elevated at 2 and 3 h post-injection up to the level of 161 ± 8 and 144 ± 14 mg/dL (mean ± SEM) respectively. In that study, they also found that the insulin levels decreased similarly to our results, and that those effects were not different between 10 and 20 μg/kg medetomidine treatment. In addition, Benson *et al* [19] have reported that an intramuscular injection of 15 μg/kg medetomidine induced a decrease in insulin level, but did not significantly alter the glucose level in the non-operated dogs under isoflurane anaesthesia. The insulin levels in their study [19] were similar to those observed in MED-10 and -20 of our study, but in contrast with our results, the plasma glucose level almost did not change 75 min after 15 μg/kg medetomidine injection in their study. It is not clear whether isoflurane anaesthesia in their study might influenced the glucose response. The fact that insulin plasma levels decreased after treatment and the glucose levels did not increase [19] suggests that insulin might not be the only factor

controlling the plasma glucose levels after medetomidine treatment. Our results also suggest that the difference in the hyperglycaemic response between medetomidine and xylazine found in our study can not be explained only by the α_2-adrenoceptor-mediated inhibition of insulin release. There may be other factors to consider.

It is well known that α_2-agonists inhibit the insulin release through their actions on α_2-adrenoceptors in the pancreas β-cells [54,55]. Clonidine at high concentrations has been reported to increase the glucose release from bovine and canine liver slices *in vitro* [16,56]. The subtype of α-adrenoceptors involved in this action seems to be α_1 rather than α_2, because prazosin, a specific α_1-adrenoceptor antagonist blocked this glucose release more effectively than the α_2-antagonist, yohimbine [56]. As both xylazine and clonidine have similar α_2/α_1 selectivity ratios [57], high dosages of xylazine might exert some α_1-adrenoceptor-mediated effect similar to clonidine. This could be one reason for the extreme increasing of plasma glucose levels in XYL-4 and -8 groups of our study.

The central effects of α_2-agonists are also obscure. Xylazine administered intracerebroventricularly (ICV) at a dosage of 0.5 mg did not change the blood glucose level in a cat [13]. Clonidine administered ICV to rats dose-dependently increased the blood glucose level [52], but whether it was central effect or the result of systemic absorption remained unclear. On the other hand, central imidazoline receptors may have a role in the regulation of glucose metabolism, because ICV-injected agmatine, a putative endogenous ligand for imidazoline receptors, exerted anti-hyperglycaemic effect [58]. Medetomidine shows higher α_2/α_1 receptor selectivity than xylazine [57], but because of its imidazoline structure it may also have affinity to bind to the imidazoline receptors [43,50]. A possible action on the putative imidazoline receptors might explain why the glucose levels did not increase further after administration of high dosages of medetomidine in our study.

Other hormones such as growth hormone (GH) and glucagon may also influence the blood glucose level. Xylazine is known to increase the blood level of GH in dogs and cats [5]. However, GH values were not determined in the present study. On the other hand, the plasma glucagon levels did not change significantly in the treatment groups of the present study, indicating that it may not be related to the hyperglycaemic effects of both medetomidine and xylazine. This was in agreement with previous reports that xylazine did not significantly change the plasma glucagon level in dogs [59] and cats [13]. Therefore, further work may be necessary to clarify the differences in the hyperglycaemic effects of medetomidine and xylazine in dogs.

Both medetomidine and xylazine similarly dose-dependently decreased the plasma NEFA levels in this study (Figure 6). According to the authors' knowledge this is the first report available on the effects of either medetomidine or xylazine on NEFA levels in dogs. The suppression of lipolysis in dogs may be mediated by both central and peripheral α_2-adrenoceptors [14,15].

In conclusion, the present study revealed that both medetomidine and xylazine similarly inhibited norepinephrine release and lipolysis dose-dependently. Medetomidine suppressed epinephrine release dose-dependently with greater potency than xylazine. The cortisol and glucagon levels did not change significantly in any treatment group. Both drugs suppressed insulin secretion with similar potency. Furthermore, this study demonstrated that the hyperglycaemic effect of medetomidine, in contrast with xylazine, was not dose-dependent at the tested dosages, and suggested that the effect of medetomidine on glucose metabolism may not be only due to α_2-adrenoceptor mediated actions.

In chapter 3, we examined the hormonal and metabolic effects of low, medium and high doses of atipamezole, and a single dose of yohimbine in antagonising

medetomidine-induced sedation. Medetomidine (20 µg/kg, IM) suppressed sympathoneural and adrenomedullary activities (assessed by the decrease of plasma NE and EPI levels), insulin release and lipolysis, and increased plasma glucose levels. These effects of medetomidine were similar to those in our previous study [60]. Both atipamezole and yohimbine were able to antagonise these effects. Because the antagonistic effects of atipamezole on plasma NE, glucose, insulin and NEFA were dose-related, we accepted our first hypothesis in these cases. On the other hand, all doses of atipamezole similarly antagonised the suppression of EPI release, consequently we refused the first hypothesis for the plasma EPI levels. Even the smallest dose of atipamezole (40 µg/kg, IM) effectively antagonised the suppression of adrenomedullary activity and prevented the further increase of plasma glucose, but the effect of this dose was rather weak on NE, insulin release and lipolysis. In other words, the small dose of atipamezole could antagonise the hyperglycaemic effect of medetomidine, but the insulin levels continued to be low in this group. These data supports the theory that α_2-adrenergic agents act on the glucogenolysis in the liver irrespective of insulin. Our previous study also suggested this possibility [60]. The medium (120 µg/kg, IM) and high (320 µg/kg, IM) doses of atipamezole effectively antagonised all examined effects of medetomidine. The antagonistic effects of the medium dose were only moderate on plasma NE, glucose, insulin and NEFA, but those of the large dose were always complete.

The potency of yohimbine (110 µg/kg, IM) in antagonising the sedative effect of medetomidine was similar to the medium and high dose of atipamezole, but the onset of this effect of yohimbine was longer. The antagonistic effects of yohimbine on plasma NE, insulin and NEFA levels were also delayed at 1 h when compared to the high dose of atipamezole. These data suggests that the absorption of yohimbine after IM injection

was slower than that of atipamezole. Yohimbine effected plasma EPI, glucose and NEFA levels similarly to the high dose atipamezole but more potently increased NE and insulin levels. In this point yohimbine itself (400 µg/kg, IV) was reported to increase plasma NE, insulin and NEFA levels in dogs [61]. Similarly, a large dose of atipamezole (100 mg, IV) increased plasma NE and EPI levels, while did not affect cortisol and glucose levels in humans [62]. The differences between the actions of atipamezole and yohimbine in the present study may result from the differences in their elimination from the plasma. When both medetomidine and atipamezole are administered, their elimination half-lives are similar, about 1 h in the canine plasma [63]. On the other hand, yohimbine has much longer elimination half-life, about 16 hs in rats [64] and 13 hs in humans [65]. Consequently, yohimbine might have relatively over-antagonised the actions of medetomidine, after the agonist was already eliminated. In the present experiment, only yohimbine increased the cortisol levels at 1 h. Therefore, the increase in cortisol levels after yohimbine treatment is possibly not related to actions on α_2-adrenoceptors. For the reasons above, we refused our second hypothesis that the antagonistic actions of atipamezole and yohimbine were similar.

Glucagon levels did not change in the present experiment similarly to our previous study after medetomidine and xylazine treatments [60]. Therefore, α_2-adrenergic agents may not influence glucagon release in dogs. The plasma lactate levels did not change also in the present study, indicating that the applied dose of medetomidine did not alter anaerobe glycolysis.

We believe that the properties of medetomidine to attenuate the catecholamine and cortisol responses during and after surgery are desirable and their complete antagonism is normally not indicated. From a pharmacokinetic point of view, yohimbine is not a well-fitted antagonist for medetomidine because of its prolonged actions. Based on

these findings, when medetomidine-induced sedation is antagonised in dogs, we recommend using atipamezole IM, from 2 to 6 folds the dose of medetomidine, unless otherwise indicated.

In chapter 4 ketamine treatment increased the norepinephrine and epinephrine levels. The effect that ketamine blocks the reuptake and therefore increase the plasma levels of catecholamines is well known [25,76].

Norepinephrine levels did not change significantly after butorphanol and fentanyl treatments but both treatments increased the epinephrine levels possibly through action on central μ opioid receptors [77,78]. Fentanyl increased the epinephrine levels with a (non-significantly) greater potency than butorphanol possibly because fentanyl is full agonist and butorphanol is only partial agonist on the μ opioid receptors. Norepinephrine AUC data correlated with the epinephrine AUC in the butorphanol and showed a tendency for correlation in the fentanyl group but not in the ketamine group. This result suggests differences between the catecholamine increasing effects of opiates and ketamine. The variances among the epinephrine data in the butorphanol and fentanyl groups were much higher when compared to the ketamine group.

Cortisol plasma levels increased in every group with similar potency. The variances among the cortisol data were moderate and similar in each group. Cortisol AUC data did not correlate with the epinephrine AUC in any groups suggesting that their releases may be mediated through different pathways. We can not explain why the increase in cortisol levels delayed after butorphanol treatment.

The hyperglycaemic effect of morphine is known to be mediated centrally, through the release of epinephrine [79]. Our results are in accordance of this theory. The correlation between the AUC data of epinephrine and glucose suggest that epinephrine

should be the key mediator for the increase of plasma glucose after butorphanol and fentanyl but not after ketamine treatments.

The mean insulin and NEFA levels did not change significantly in any of the groups. Although in some cases when the epinephrine levels were very high in the butorphanol and fentanyl groups, insulin and NEFA levels seemed to increase also. The correlation of the AUC data between epinephrine and NEFA was significant in the butorphanol group, showed a tendency in the fentanyl group but no correlation was observed in the ketamine group. These results suggest that epinephrine may have important role in increasing lipolysis after opioid treatments.

Epinephrine seems to be the key mediator for the increase in plasma glucose and NEFA levels after butorphanol and fentanyl but not after ketamine treatments. According to the literature N-methyl-D-aspartate (NMDA) itself caused increase in plasma levels of ACTH, corticosterone, glucose and insulin and did not change the catecholamine levels after IV injection in rats [80]. The anaesthetic effects of ketamine are associated with its antagonistic action on the NMDA receptors but comparing the results of the present experiment to the hormonal effects of NMDA we suspect that the changes in cortisol, glucose and insulin levels are probably not mediated by NMDA receptors after ketamine treatment.

In chapter 5, we examined the neurohormonal and metabolic effects of three different balanced anaesthesia protocols (MED-BUT, MED-FEN and MED-KET) and compared them to the effects of medetomidine as a control group (MED-SAL). All combinations were administered intramuscularly from a single syringe. The speed and completeness of absorption from the intramuscular site may influence the effects of a treatment. Diffusion of molecules through a semipermeable membrane (as during absorption after an intramuscular injection) is effected by their concentration. For this reason

physiological saline solution was mixed with medetomidine in the control group (MED-SAL) in order to reach similar medetomidine concentration as in the balance anaesthesia groups. The volume of saline solution used in the MED-SAL group (0.1 mL/kg) was similar to the volume of butorphanol (0.1 mL/kg) but smaller than the volumes of fentanyl and ketamine (0.2 mL/kg) used in the balance anaesthesia groups. In this respect the original medetomidine solution (Domitor inj.) was diluted 6 times in the MED-SAL and MED-BUT groups but 11 times in the MED-FEN and MED-KET groups. Therefore, the MED-SAL group used in this experiment is not a perfectly fitted control for MED-FEN and MED-KET treatments but it is still a better approach than using the original medetomidine solution (Domitor inj.).

Other factors like chemical and pharmacokinetic interactions between medicines may also influence the absorption, distribution and elimination of drugs [83]. All of these issues must be taken into account when evaluating the effect of balanced anaesthesia treatments. Medetomidine can be mixed with butorphanol, fentanyl or ketamine in a single syringe without any adverse chemical reaction [28,33]. Every drug used in this study absorbs fast and complete when injected intramuscularly as a sole agent [26,84-86]. However, pharmacokinetic interactions are supposed to exist between the drugs used in this study.

Dogs in the MED-SAL group became recumbent about 2.5 times later and started to wake up 1.6 times later than after the same dose of medetomidine injection in our previous studies [60,82]. The level of sedation seemed to be lighter in the MED-SAL group then in those experiments. The increase in the plasma level of glucose also shifted to the right in the MED-SAL group when compared to the previous experiments. The delay in the onset of medetomidine effect may be attributed to the delayed absorption from the intramuscular site because the injected medetomidine concentration was lower.

Because medetomidine is metabolised rapidly in the liver slower absorption may be associated with lower peak plasma levels and a loss of effect. According to the authors knowledge this is the first study to report the loss of medetomidine effect when injected IM in a diluted solution. Whether this effect is similar when medetomidine is mixed in the same syringe with butorphanol, ketamine or midazolam and injected IM needs to be further researched.

Sympathoneural and sympathoadrenal activities, as assessed by the norepinephrine and epinephrine plasma levels, decreased after every treatment. Previously we also found that medetomidine decreased the plasma levels of norepinephrine and epinephrine [60,82]. In the chapter 4 we demonstrated that only ketamine treatment increased the norepinephrine levels, but each of the butorphanol, fentanyl and ketamine treatments increased the epinephrine levels. Consequently the effects of medetomidine dominated in the balance anaesthesia groups and ketamine had significantly stronger potency to increase sympathoneural activity when compared to butorphanol or fentanyl.

In the chapter 4 we observed that fentanyl and ketamine treatments caused significant hyperglycaemia, while butorphanol showed only a tendency for hyperglycaemia. We also suggested the possibility that the hyperglycaemic response is mediated through different pathways between opioids and ketamine. In the present study, the plasma levels of glucose similarly increased in the opioid combined balance anaesthesia groups but increased faster in the MED-KET group. Interestingly, the overall glucose levels were lower in every balance anaesthesia groups than in the MED-SAL group. These results can not be explained with the present knowledge about α_2-adrenergic agents. Medetomidine is commonly used in veterinary anesthesia to reduce perioperative stress response. From this point of view hyperglycemia may be considered

as an adverse reaction of medetomidine, therefore the balance anesthesia combinations examined in this study may be advantageous over single medetomidine treatment.

The plasma levels of insulin were very similar in every treatment group except for MED-KET where there was a slight but significant increase at 1.5 h. Interestingly, the glucose levels peaked at same time in the same treatment group. There is a possibility that ketamine potentiated the blood circulation because of its sympathomimetic effect, consequently the hepatic blood flow might increase and medetomidine might be metabolised faster than after sole medetomidine injection. Therefore the decreasing medetomidine concentration might not suppress the insulin levels with sufficient potency and insulin levels increased in response to hyperglycaemia. Similar pharmacokinetic interaction has already been reported between medetomidine and atipamezole in dogs [63].

There were no meaningful differences in NEFA levels among the groups.

In conclusion, delayed onset and loss of sedative effect of medetomidine were observed in this study when injected as a 6 times diluted Domitor solution. The neurohormonal and metabolic effects of medetomidine were predominant in the balance anaesthesia protocols examined in this study. The norepinephrine levels were less depressed in the MED-KET group probably because of the potency of ketamine to increase sympathoneural activity. On the other hand, balanced anaesthesia with butorphanol, fentanyl and ketamine provided lower plasma glucose levels than medetomidine alone.

Summary

The α_2-adrenoceptor agonists, medetomidine and xylazine, are widely used in veterinary practice as sedative, muscle relaxant and analgesic agents for different species. Although α_2-agonists are multipotent drugs, they should be used carefully, because unexplained and sometimes fatal accidents may be associated with their use in the healthy small animal patient, even without painful intervention. These are usually associated with the cardiovascular side effects of these drugs. However, whether the neuroendocrine and metabolic effects of α_2-agonists are involved in the causes of these accidents are not fully understood.

Medetomidine can reduce stress response to surgery as assessed by the attenuation of plasma catecholamine, adrenocorticotrophic hormone and cortisol levels. Stress is a generalised response of the body to various factors, called stressors. Pain, blood loss, excitement, and underlying pathological conditions may all act as stressors in the surgical patient. The endocrine and metabolic stress response is characterised by the increase of catecholamine, cortisol, glucose, and NEFA blood levels, and the decrease of insulin levels. Adrenoceptors play an important role in the co-ordination of these events therefore α_2-adrenergic agents may interfere to the pathophysiology of stress response before, during and after anaesthesia. That is why there is an increasing interest in using medetomidine as a pre-anaesthetic adjuvant or as a part of balanced anaesthesia in combination with several analgesics. On the other hand, analgesics also have endocrine and metabolic effects. Because analgesics act on similar receptor systems than the physiological stress-response there are certain analogy between the endocrine effects of analgesics and stress-response. Consequently, the examination of the effects of different analgesics on stress-response is also important. The aim of this study was to

examine the stress-related neurohormonal and metabolic effects of α_2-adrenergic agents and their combinations with opioid drugs (butorphanol and fentanyl) and ketamine in healthy adult beagle dogs without surgical intervention.

In chapter 2, the effects of medetomidine (10, 20, 40 and 80 µg/kg, IM) and xylazine (1, 2, 4 and 8 mg/kg, IM) were compared. Both drugs similarly, dose-dependently inhibited norepinephrine release and lipolysis. Medetomidine suppressed epinephrine release dose-dependently with greater potency than xylazine. Xylazine also tended to decrease epinephrine levels dose-dependently. The cortisol and glucagon levels did not change significantly in any treatment group. Both drugs suppressed insulin secretion and increased glucose levels. The hyperglycaemic effect of medetomidine, in contrast with xylazine, was not dose-dependent at the tested dosages. The results suggested that the effect of medetomidine on glucose metabolism might not be due only to α_2-adrenoceptor mediated actions.

In chapter 3, the antagonistic effects of atipamezole (40, 120, and 320 µg/kg, IM) and yohimbine (110 µg/kg, IM) were compared 30 minutes following medetomidine (20 µg/kg, IM). The effects of medetomidine were similar then described in the chapter 2. Both atipamezole and yohimbine antagonised these effects. The reversal effects of atipamezole were dose-dependent, except on epinephrine. Yohimbine caused prolonged increases in plasma norepinephrine and insulin levels comparing to atipamezole, possibly because of its longer elimination half-life. Only yohimbine increased the cortisol levels. Neither glucagon nor lactate levels changed significantly. Based on these findings, when medetomidine-induced sedation is antagonised in dogs, we recommend using atipamezole IM, from 2 to 6 folds the dose of medetomidine, unless otherwise indicated.

In chapter 4, effects of three injectable analgesics butorphanol (0.1 mg/kg, IM) fentanyl (10 µg/kg, IM) and ketamine (10 mg/kg, IM) were compared. Plasma levels of epinephrine and cortisol significantly increased after every treatment. Norepinephrine levels only increased after ketamine treatment and glucose levels increased after fentanyl and ketamine treatments. Changes in cortisol levels were not in correlation with those of the epinephrine levels in any treatment group, but changes in glucose levels significantly correlated to the epinephrine levels after butorphanol and fentanyl but not after ketamine treatments. The NEFA levels also significantly correlated to the epinephrine levels in the butorphanol group and had tendency for correlation in the fentanyl group but not in the ketamine group. Based on these findings, single injections of butorphanol and fentanyl induced hormonal and metabolic changes similar to the physiological stress response but the effects of ketamine were somewhat different. Epinephrine seems to be the key mediator of these changes after butorphanol and fentanyl but not after ketamine treatments. The effects of ketamine can not be explained with its antagonistic effect on N-methyl-D-aspartate (NMDA) receptors. The involvement of other receptor systems in these effects of ketamine is highly probable. The hormonal and metabolic changes observed in this study are undesirable for the stress free management of the patients, therefore butorphanol, fentanyl and ketamine are recommended to use as part of a balanced anaesthesia and not as single treatments.

In chapter 5, the effects of balanced anaesthesia with medetomidine (20 µg/kg, IM) in combination with butorphanol (0.1 mg/kg, IM), fentanyl (10 µg/kg, IM) or ketamine (10 mg/kg, IM) were examined. Norepinephrine, epinephrine, insulin, and NEFA levels significantly decreased in every treatment groups. However, the norepinephrine levels were significantly higher in the medetomidine-ketamine than in the medetomidine-saline groups. Cortisol levels did not change significantly. Plasma glucose levels

significantly increased in every group except for the medetomidine-butorphanol group where only increasing tendency was observed. Interestingly, the glucose levels in the medetomidine-saline group were significantly higher than in the other groups. The neurohormonal and metabolic effects of medetomidine were predominant in the balance anaesthesia protocols examined in this study. The norepinephrine levels were less depressed in the medetomidine-ketamine group probably because of the potency of ketamine to increase sympathoneural activity. On the other hand, balanced anaesthesia with butorphanol, fentanyl and ketamine provided lower plasma glucose levels than medetomidine alone.

In conclusion, α_2-adrenergic agents suppress sympathoneural, sympathoadrenal and adrenocortical activities and lipolysis therefore able to attenuate stress-response from this point of view. On the other hand, they suppress insulin secretion and causes hyperglycaemia, similarly to stress-response itself. Therefore, medetomidine can not be considered as an ideal agent for reducing stress-response to various stimuli. The main different between medetomidine and xylazine was in their hyperglycaemic effect. Xylazine caused significantly dose-dependent hyperglycaemia, whereas medetomidine did not. The α_2-adrenoceptor antagonist yohimbine is not a well-fitted antagonist for medetomidine because it causes prolonged increase in sympathoadrenal activity and insulin secretion. Based on these findings, when medetomidine-induced sedation is antagonised in dogs, we recommend using atipamezole IM, from 2 to 6 folds the dose of medetomidine, unless otherwise indicated. Combining medetomidine to opiates or ketamine may offer advantages in reducing sympathoneural, sympathoadrenal and adrenocortical activities as well as hyperglycaemia.

Acknowledgements

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