# Diuretic Effects of $\alpha_2$-Adrenoceptor Agonists and Their Antagonism by Antagonists in Dogs

（イヌにおける$\alpha_2$-アドレナリン受容体作動薬の利尿作用
および遮断薬によるその拮抗効果）

The United Graduate School of Veterinary
Science, Yamaguchi University

Md. Hasanuzzaman TALUKDER

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# Contents

# General Introduction

The $\alpha_2$-adrenoceptors are the transmembrane G protein coupled receptors that act pre or post- and extrasynaptically in different tissues [1]. The heterogeneity in $\alpha_2$-adrenoceptor subtype, density and location in animals and humans has led to considerable differences in drug doses and overall effects of $\alpha_2$-agonists in various species [2]. Recent advances made on the basis of radioligand binding studies on $\alpha_2$-adrenoceptor pharmacology suggest that several agents acting on this receptor population also bind with strong affinity to nonadrenergic imidazoline preferring receptor sites [3, 4, 5]. Medetomidine is one such agonist drug that makes it potentially superior for use particularly by developing an anti-arrhythmic property, mediated by imidazoline receptor associated with vagal tone stimulation [3]. It has garnered the attention of many small animal practitioners in recent years [2]. In contrast, xylazine is a potent $\alpha_2$-adrenoceptor agonist, clonidine analogue, and non-narcotic old drug having no affinity to imidazoline receptor. Medetomidine has selectivity ratio of 1620/1 ($\alpha_2/\alpha_1$), which is approximately 10-folds higher than that of xylazine (160/1) [1-4]. In spite of these differences, both medetomidine and xylazine are used similarly for their ability to produce reliable sedation, analgesia and muscle relaxation in many species [3]. Although both drugs are mainly used for sedative and analgesic purposes but have considerable pharmacodynamic effects at the recommended doses [2-4].

Both medeomidine and xylazine are known to induce a diuresis in several species [5-9] whereas the diuretic effect of xylazine in dogs is still unknown. In addition, the diuretic effect

of medetomidine was reported in combination with other anesthetic agents and therefore it deserves the merit to study its unique diuretic and hormonal actions in healthy dogs. Moreover, the pharmacodynamics of medetomidine and xylazine suggest that these drugs may possess some benefits as diuretic agents accompanied with sedation in healthy animals.

Regarding the hormonal influence of arginine vasopressin (AVP) in the mechanism of diuresis induced by either medetomidine or xylazine still remained an unresolved issue. Although, it claimed that AVP plays a partial role to induce diuresis mediated by $\alpha_2$-adrenoceptor agonists [6-12]. It is reported that $\alpha_2$-adrenoceptor and/ or imidazoline receptor agonists induce atrial natriuretic peptide (ANP) release which cause diuresis and natriuresis in rats and mice [13-18]. The influences of medetomidine and xylazine on ANP release in dogs are unknown. However, there are no reports that compared the role of AVP and ANP simultaneously in same species, to the author's best knowledge. Furthermore, there are no enough data on the associated changes in urine specific gravity, pH, creatinine, osmolality and electrolytes, during diuresis induced by medetomidine and xylazine in dogs. In addition, time- and dose-dependent data on the diuretic effects of medetomidine is still insufficient in dogs.

On the other hand, the $\alpha_2$-adrenoceptor antagonists, atipamezole and yohimbine have been shown to reverse a variety of clinicophysiological effects produced by $\alpha_2$-adrenoceptor agonists [1-4, 19-27]. The $\alpha_2/\alpha_1$ selectivity of atipamezole and yohimbine are 8526/1 and 40/1, respectively [1-4, 19-26]. Atipamezole is a potent and highly specific antagonist of centrally and peripherally located $\alpha_2$-adrenoceptors compared with yohimbine [25]. The affinities of atipamezole and yohimbine are similar at the $\alpha_{2A}$-, $\alpha_{2B}$- and $\alpha_{2C}$-adrenoceptors but differ by approximately 100-folds at the $\alpha_{2D}$-adrenoceptors [19-26]. In addition,

yohimbine affects serotonergic, cholinergic, dopaminergic and GABA receptor-related mechanisms [26], whereas atipamezole lacks these receptor activities [25]. Furthermore, atipamezole has a similar structure to imidazoline, whereas yohimbine has no imidazoline receptor affinity [1-5, 25-27]. These differences between atipamezole and yohimbine may influence on the antagonistic effects on the actions induced by medetomidine or xylazine. Although a number of previous studies described the diuretic effects of medetomidine or xylazine in several animal species but there is a few report mentioning the antagonistic effect with their specific and selective antagonistic agents either atipamezole or yohimbine. Basically, there is no published report on the antagonistic effects of atipamezole and yohimbine on either medetomidine or xylazine induced diuresis as well as hormonal variables in dogs.

In chapter 1, the study was aimed to investigate and compare the effects of medetomidine and xylazine on diuretic and hormonal variables; urine volume, pH, specific gravity and creatinine and osmolality and sodium, potassium, chloride electrolytes and AVP in both urine and plasma, and plasma ANP level in healthy dogs.

In chapter 2, the study aimed to investigate and compare the antagonistic effects of thee different doses of either atipamezole or yohimbine on the diuresis induced by medetomidine in healthy dogs. The variables examined were urine volume, specific gravity, creatinine values, and osmolality, electrolytes and AVP values in both urine and plasma, and plasma ANP. Since, there are no published reports on the antagonistic effects of atipamezole and yohimbine against medetomidine-induced diuresis in dogs as to author's knowledge. In addition, there is no report that either atipamezole or yohimbine reverses the inhibition of AVP and the release of ANP induced by medetomidine in dogs.

In chapter 3, the study was aimed to investigate and compare the antagonistic effects of thee different doses of either atipamezole or yohimbine on the diuresis induced by xylazine in healthy dogs. The variables examined were urine volume, specific gravity, creatinine values, and osmolality, electrolytes and AVP values in both urine and plasma, and plasma ANP. Since to author's best knowledge, there are no published reports on the antagonistic effects of atipamezole and yohimbine against xylazine-induced diuresis in dogs. In addition, there are no available data about the effects of atipamezole or yohimbine on the AVP and ANP changes after xylazine administrations in dogs. It is hypothesized that the results of these studies may be best interpreted against this background.

# Chapter 1

## Diuretic effects of medetomidine compared with xylazine in healthy dogs

# Introduction

Medetomidine is a potent $\alpha_2$-adrenoceptor agonist, and has a selectivity ratio of 1620/1 ($\alpha_2$ /$\alpha_1$), which is approximately 10-folds higher than that of xylazine (160/1) [1-4, 21, 22]. It has a very low affinity for $\alpha_1$-adrenoceptors, and interacts with central imidazoline receptors, in contrast to xylazine. This makes it potentially superior for use in small animals, particularly by developing an anti-arrhythmic property, mediated by imidazoline receptor associated with vagal tone stimulation [4]. The imidazoline $\alpha_2$-adrenoceptor agonists may act via G-protein coupled mechanisms [1]. Inspite of these differences, both medetomidine and xylazine are used similarly for their ability to produce reliable sedation, analgesia and muscle relaxation in many species [3]. On the other hand, both medetomidine and xylazine are known to induce diuresis associated with changes in urine specific gravity, pH, creatinine, osmolality, sodium, potassium and chloride ions in urine and plasma in several species including dogs [5-9]. In regard to the mechanism of diuresis induced by medetomidine and xylazine, it has been claimed that it was in part due to the $\alpha_2$-adrenoceptor mediated inhibition of AVP release in blood [6-12]. In addition, it has been reported that intravenous (IV) injections of clonidine and moxonidine evoked a dose-dependent diuresis, natriuresis, and an increase of ANP in rats [13]. Imidazolines may also directly act on imidazoline receptors and/or $\alpha_2$-adrenoceptors located in the cortex and outer medulla of kidney in rats [14]. Medetomidine has been reported to markedly induce ANP release in rats [15]. However, there is no report that either medetomidine or xylazine stimulates ANP release in dogs. The circulating ANP acts on the kidney to cause diuresis and natriuresis by exerting direct actions on renal proximal tubules and inner medullary duct cells and by inhibiting the release of renin and AVP and also synthesis and secretion of aldosterone in mice [16-18, 28). To our best knowledge, factors

which are involved in the diuresis other than AVP are not still elucidated in dogs. In addition, time- and dose-dependent data on the diuretic effects of medetomidine and xylazine are still insufficient in dogs. This diuretic effect may limit the use of medetomidine and xylazine in animals with urinary tract obstruction, dehydration or hypovolemia. The purpose of our study was to investigate and compare the effects of medetomidine and xylazine on diuretic and hormonal variables; urine volume, pH, specific gravity and creatinine and osmolality and sodium, potassium, chloride electrolytes and AVP in both urine and plasma, and plasma ANP level in healthy dogs.

## Materials and methods

### Animals

Five adult male healthy dogs of which two beagles and thee mixed-breeds, with a mean age of 6.2 (standard deviation 2.7) years old and mean weight of 10.44 (2.01) kg were used. All the dogs were raised at the laboratory providing animal management facilities and fed a standard commercial dry canine food. Routine hematologic examination was done before the experiment; all values were within normal physiologic ranges. The study protocols were approved by the Animal Research Committee of Tottori University, Tottori, Japan.

### Experimental protocols

The experiment consists of 11 treatment groups in which each dog was given an intramuscular (IM) injection of physiological saline solution (2.0 mL/head) as the control. Other 10 treatments comprised IM injection of 5, 10, 20, 40 or 80 μg/kg of medetomidine hydrochloride (0.1% solution, Domitor®, Meiji Seika, Tokyo, Japan), or 0.25, 0.5, 1, 2, or 4 mg/kg of xylazine hydrochloride (2% solution, Celactal®, Bayer , Tokyo, Japan). The groups will be referred to as control, MED-5, -10, -20, -40 and -80 and XYL-0.25, -0.5, -1, -2 and -4.

Five dogs were assigned to each of the 11 treatment groups in a randomized design. There was at least one week interval between treatments in the same dog. Food was withheld for 12 h prior to drug injection. The dogs had not been accessed to food and water during the experiment. After sample collection of 8 h, food and water were provided once, and again fasted for 12 h to collect the sample at 24 h in the next day. We did not measure the volume of urine voided by the dogs after urinary catheter removal and before placement in the following day, because it was observed that urine volume returns to baseline within 6 to 8 h after injection of medetomidine or xylazine in dogs during the trial experiment. The experiments were performed in a room with air temperature at 25 °C.

**Sample collection**

A 6- or 8-Fr Silicon balloon catheter (All Silicon Foley Catheter, Cliny Medical Corp, Tokyo, Japan) was inserted prior to 1 h of the experiment to empty the bladder and for subsequent urine sampling. The catheter was withdrawn after sampling at 8 h. On the next day at 22 h, the catheter was again inserted and the bladder was made empty. Subsequently, 24 h urine sample was collected. Urine and blood samples were taken at the following 11 times: prior to injection of the agent (Pre), 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24 h after injection. Blood samples (5.5 mL) were collected from the jugular vein by means of 21-gauze needle with a 6 mL disposable syringe, at same time points urine samples were collected. An aliquot of 4.0 mL from each sample was mixed with ethylene diamine tetraacetic acid and aprotinin (Trasylol®, Bayer, Leverksen, Germany) for AVP and ANP measurements, and the remaining 1.5 mL was mixed with heparin for osmolality measurement. The blood samples were immediately centrifuged at 2000 × g at 4 °C for 15 min, and the plasma was separated and kept at -40 °C for analysis. Urine samples were centrifuged at 2000 × g for 5 min, and then

the supernatant was collected and stored at -40 °C until analyzed.

## Analytical methods

Urine volume was measured at each time point by a measuring cylinder after collection from the urine bag. Urine specific gravity and pH were measured by a refractor photometer (Erma®, Tokyo, Japan) and pH meter (pH meter F-52®, Horiba Corp, California, USA), respectively. Urine creatinine concentrations were measured by creatinine assay kit (Wako Pure Chemical Industries Corp, Osaka, Japan) with Jaffe method using spectrophotometer. In both urine and plasma, osmolality and electrolytes were measured by using a vapor pressure osmometer (VAPRO®, Wescor, Utah, USA) and Na-K-Cl ion-concentrations auto analyzer (DRI-CHEM800V®, Tokyo, Japan), respectively. Plasma AVP was extracted following the procedure for solid phase column extraction (Sep-Pak® Cartridges, Waters, Ireland). For the extraction, Sep-Pak C18 cartridges were attached with plastic 10 mL syringe and kept in a test tube rack. Each was washed with 10 mL of 100 % methanol and then washed two times with 10 mL of ultra pure water. Then, plasma sample (0.5 mL) and 1 mL of 0.1 M hydrochloric acid were mixed and poured into each syringe. After dropping out the solution, the syringes were washed with 10 mL of 4 % acetic acid, and all water was taken out by using the plunger. Then, AVP was collected in tubes after putting 1 mL of 100 % methanol into the syringe. Using nitrogen gas with solvent evaporation apparatus, all the AVP solution was desiccated and stored at -40 °C until analyzed. Buffered solution (0.5 mL) was added in to the desiccated AVP tubes, and the tubes were shaked for 15 min using shaking apparatus before measurement. Urine and plasma AVP concentrations were measured by a double antibody radioimmunoassay (RIA) technique with the use of commercially available AVP kit (Mitsubishi Chemical, Tokyo, Japan). The intra-assay coefficients of variation (CVs)

were 10 % and the limits of detection and quantification were 0.063 to 8.0 pg/tube. ANP was also assayed by a double antibody RIA kit (HANP kit®, Eiken Chemical Company, Tokyo, Japan). The intra-assay CV was 15 %. The detection and quantification limits were 10 and 1280 pg/mL, respectively.

### Data evaluation

All data obtained were analyzed together with Prism statistical software (version 4; Graph Pad Software, San Diego, California, USA). One-way analysis of variance for repeated measures was used to examine the time effect within each group and the group effect at each time point. When a significant difference was found, the Tukey test was used to compare the means. The area under the curve (AUC) was calculated for each biochemical variable. The AUC was measured by calculating the sum of the trapezoids formed by the data points. The AUC data were plotted against dose of medetomidine or xylazine, and simple linear regression analysis was applied. When a significant difference was found, the effect of the drug on the plasma level of the examined biochemical was claimed to be dose-related. Mean values are presented with standard error in parenthesis. The level of significance in all tests was set at $P < 0.05$.

## Results

For all the variables, there were no significant differences between groups at baseline (Pre; before injection of the agents). Diuretic effect was found in all the tested doses of both medetomidine and xylazine compared to the control. The actual diuretic effects persisted up to 4 h after medication (Figure 1A, 1B). All the doses of both medetomidine and xylazine produced significant diuresis during 1 to 3 h compared to the control. Compared with the baseline value, the peak diuretic responses of MED-80 and XYL-4 were 11.56 (1.33) mL/kg

and 15.68 (1.92) mL/kg at 2 h, respectively (Figure 1A, 1B). The linear regression of the total urine volume from 1 to 3 h (Figure 1C, 1D), was significant in both XYL ($P<0.001$) and MED groups ($P<0.05$), indicating that both medetomidine and xylazine caused diuresis in a dose-dependent manner. The dose-dependency of diuretic effect was lower in medetomidine compared to xylazine groups. Similar results were observed with the linear regression of the total urine volume data from 0 to 4 h, 0 to 6 h and 0 to 8 h. However, the time-related diuretic response somewhat differed between medetomidine and xylazine; the peak diuresis occurred at 2 h in XYL- 1, -2 and -4 groups, at 1 h in XYL-0.25 and -0.5 groups, and at 2 h in MED-10, -20, -40 and -80 groups but at 3 h in MED-5 group.

Urine specific gravity increased gradually during 8 h in the control. In both MED and XYL groups, urine specific gravity significantly decreased in a dose-dependent manner compared with the base line value (Figure 2A, 2B). The lowest specific gravity was found during 1 to 3 h in either MED or XYL similarly. In the XYL groups, the lowest mean urine specific gravity was found at 1 h in XYL-0.25 and -0.5, and at 2 h in XYL-1, -2, and -4. In MED groups, the lowest specific gravity was observed at 2 h in MED-5, -10 and -20, and at 3 h in MED-40 and -80. These decreases in urine specific gravity were in correspondence with the increase in urine volume in both MED and XYL groups.

Urine pH decreased significantly in both MED- and XYL- treated groups during 1 to 5 h after injection of the agents and then gradually returned to baseline values (Figure 2C, 2D). The lowest value of urine pH was observed at 5 h in MED-80 and XYL-4 groups. Thereafter, the urine pH in both drug groups increased over the value in the control group during 6 to 8 h. Higher doses of XYL and MED delayed the return from the decreased urine pH to baseline.

Urine creatinine concentrations were decreased significantly in all treated groups. The

lowest mean concentration of urine creatinine was found at 3 h in MED-80 and XYL-4 groups (Figure 2E, 2F). The slope of the recovery phases indicated that xylazine decreased the urine creatinine concentrations in a dose-dependent manner. The higher doses of medetomidine delayed the return from the decreased creatinine concentrations to baseline.

Urine osmolality decreased significantly in both groups compared to baseline value during 1 to 5 h. The lowest mean value of urine osmolality was observed during 2 to 3 h in higher doses of either MED or XYL (Figure 3A, 3B). Xylazine decreased the urine osmolality in a dose-dependent manner. Plasma osmolality in both MED and XYL treated groups significantly increased during 2 to 5 h compared with the pre-value (Figure 3C, 3D). The decreases in urine osmolality in both MED and XYL groups were likely related with the increase in plasma osmolality due to diuresis. Medetomidine increased plasma osmolality in a dose-dependent manner. Higher doses of medetomidine and xylazine delayed the return to baseline from the increased plasma osmolality.

Compared with the baseline value, mean concentrations of urine AVP were significantly lower at 1 to 4 h in MED-20, -40 and -80 groups, at 1 to 3 h in XYL-1 and -2 groups, and at 4 h in XYL-4 group (Figure 4A, 4B). Higher doses of medetomidine decreased the concentrations of urine AVP with greater potency than xylazine. In both MED and XYL groups, return to baseline AVP concentration was delayed in a dose-dependent manner. Thereafter, the AVP concentrations increased over the pre-value from 5 to 8 h in both MED and XYL groups (Figure 4A, 4B). In the XYL groups, urine AVP concentrations were decreased dose-dependently. Also, return to baseline from the decreased AVP concentrations delayed in a dose-dependent manner. The slopes of the recovery phase indicated that medetomidine decreased the urine AVP concentrations in a dose-dependent manner. The

actual amounts of the excreted urine AVP were significantly lower in MED-40 and MED-80 groups compared to control group (Figure 4C). In contrast, there was no significant difference in the total amount of urine AVP excretion from 1 to 3 h (Figure 4D) between XYL and control groups. The actual amounts of the excreted AVP from 1 to 3 h were decreased dose-dependently in the MED groups, but also decreased in the higher doses of XYL groups.

Plasma AVP concentrations were decreased significantly from 0.5 to 2 h in the MED groups, and decreased from 0.5 to 1 h in the XYL groups compared with their baseline values (Figure 5A, 5B). However, plasma AVP increased over the baseline value from 5 to 8 h in both groups. Medetomidine dose-dependently suppressed the return to baseline of the decreased plasma AVP. The AUC data of plasma AVP revealed that MED-40 and MED-80 decreased significantly AVP release from 0.5 to 2h, whereas xylazine did not significantly decrease (Figure 5C, 5D). The linear regression of the AUC data of plasma AVP from 0.5 to 2 h was significant ($P<0.05$) in the MED groups but not in the XYL groups, indicating that medetomidine in contrast to xylazine suppressed plasma AVP in a dose-dependent manner at the early phase after administration.

Plasma ANP concentrations increased significantly at 0.5 h in MED-40 and XYL-2 groups after injection (Figure 6A, 6B). However, higher doses of MED stimulated ANP release with greater potency than XYL groups. The return to baseline from the increased ANP concentrations delayed dose-dependently in the MED groups. The AUC data (0 - 8 h) of plasma ANP revealed that MED-20, -40 and -80 increased significantly ($P<0.05$) ANP release, whereas xylazine did not significantly increase. The linear regression of the AUC data of plasma ANP from 0 to 8 h was significant ($P<0.05$) in the MED groups but not in the

XYL groups (Figure 6C, 6D), indicating that medetomidine in contrast to xylazine induced ANP release in a dose-dependent manner.

Compared with the baseline value, the mean concentrations of urine sodium, potassium and chloride decreased significantly in both MED and XYL groups. The lowest mean concentrations of these urine electrolytes were found from 1 to 4 h in both MED (Figure 7A, 7C, 7E) and XYL (Figure 7B, 7D, 7F) groups. Higher doses of medetomidine markedly decreased the concentrations of urine sodium, potassium and chloride, and return to baseline of the reduced concentrations of urine electrolytes were delayed in a dose-dependent manner. Xylazine decreased the urine sodium, potassium and chloride concentrations in a dose-dependent manner. Total amounts of excreted urine sodium, potassium and chloride did not significantly change during 1 to 4 h in both MED and XYL groups compared to the control. On the other hand, the mean concentrations of plasma sodium, potassium and chloride increased significantly during 2 to 5 h from baseline value of higher doses in both MED (Figure 8A, 8C, 8E) and XYL (Figure 8B, 8D, 8F) groups.

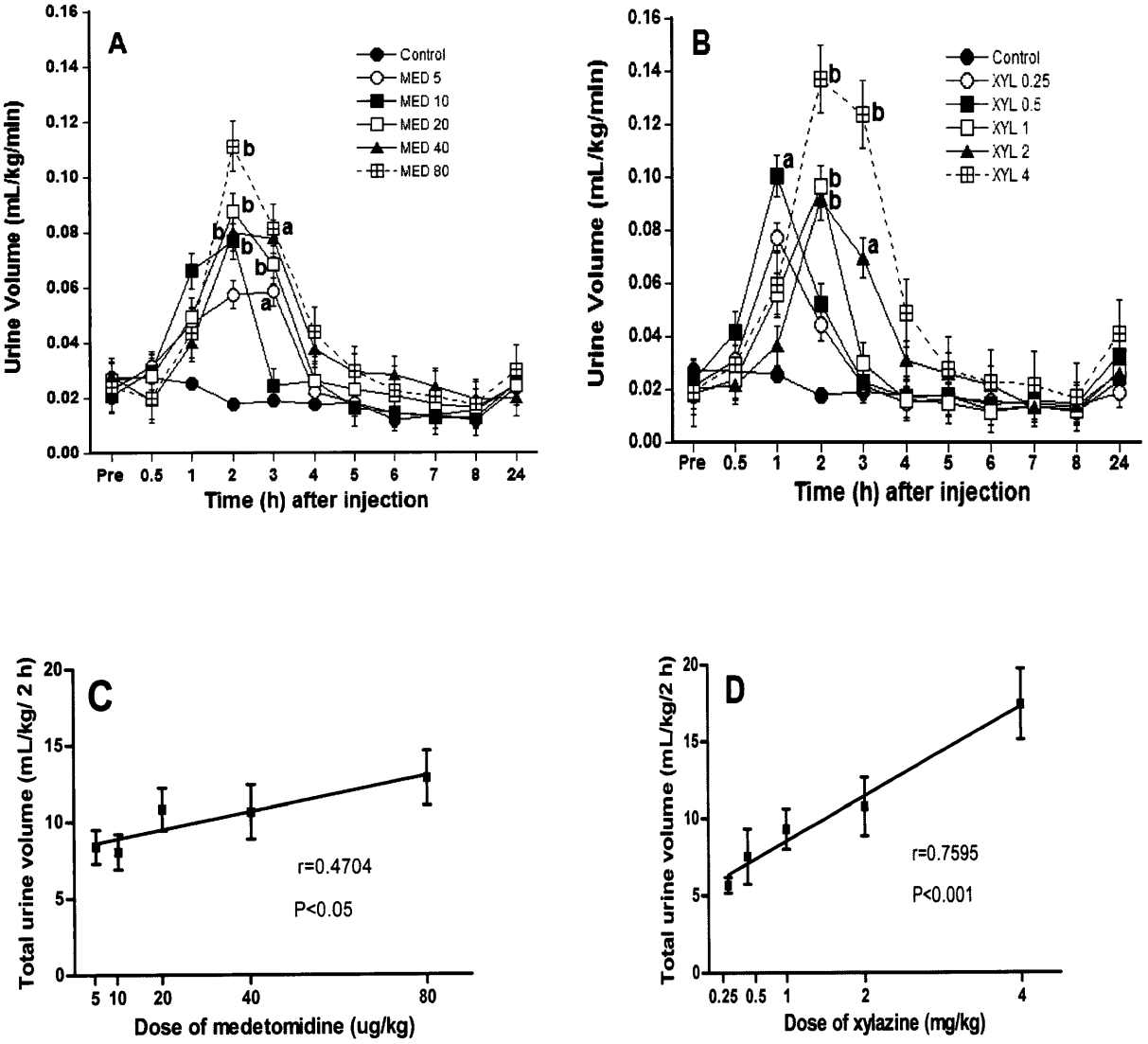


Figure 1. Urine volume following the administration of medetomidine (MED μg/kg, A) and xylazine (XYL mg/kg, B) in dogs. Each point and vertical bar represent the mean and standard error (n=5). Simple linear regression of total urine volume during 1 to 3 h following the administration of medetomidine (MED μg/kg, C) and xylazine (XYL mg/kg, D). Significantly different from the pre-value (a: $P<0.05$, b: $P<0.01$).

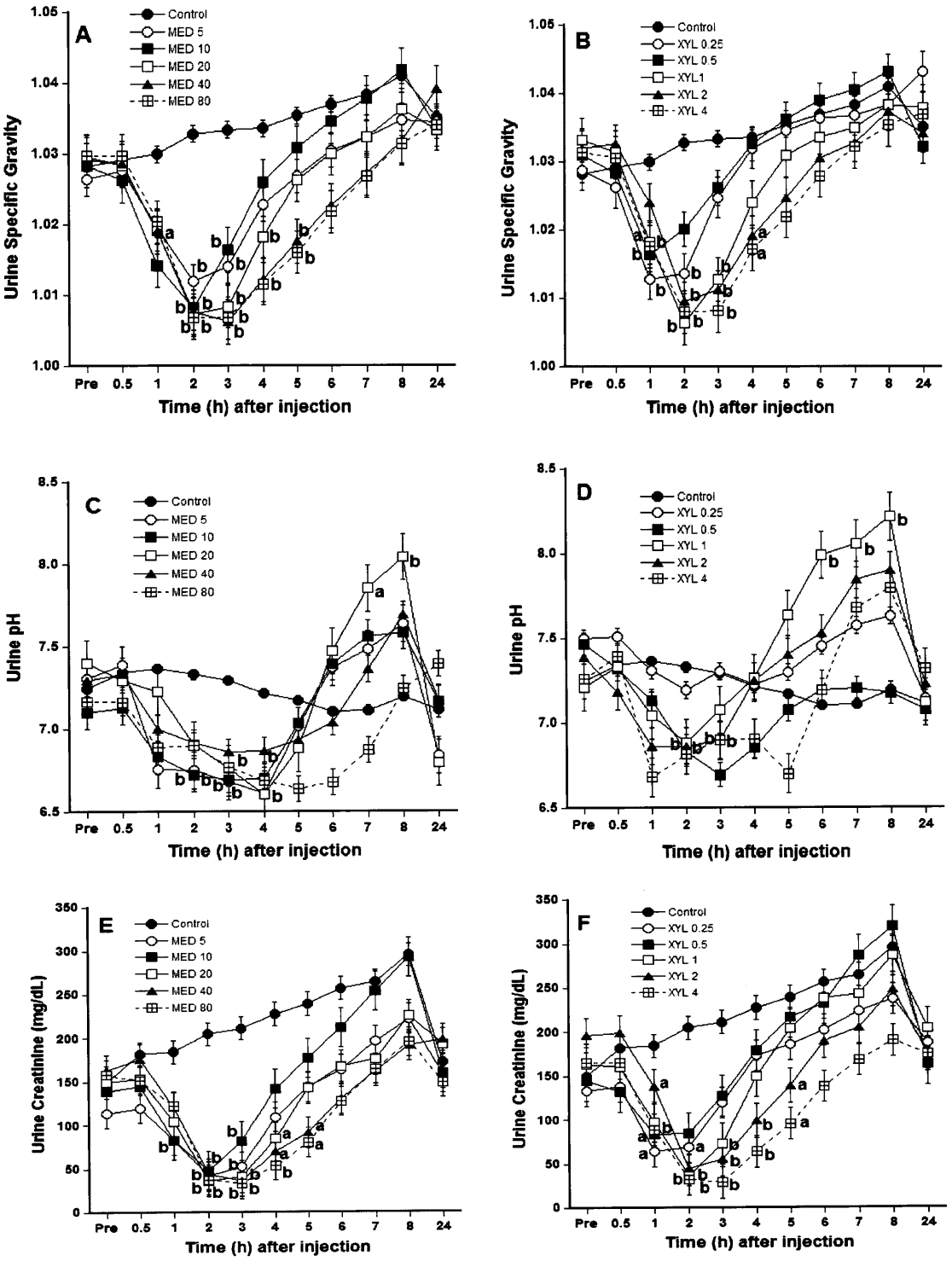


Figure 2. Urine specific gravity (MED μg/kg, A; XYL mg/kg, B), pH (MED μg/kg, C; XYL mg/kg, D) and creatinine concentration (MED, E; XYL, F) following the administration of medetomidine and xylazine in dogs. Meanings of points, bars, and "a" or "b" as for Figure 1.

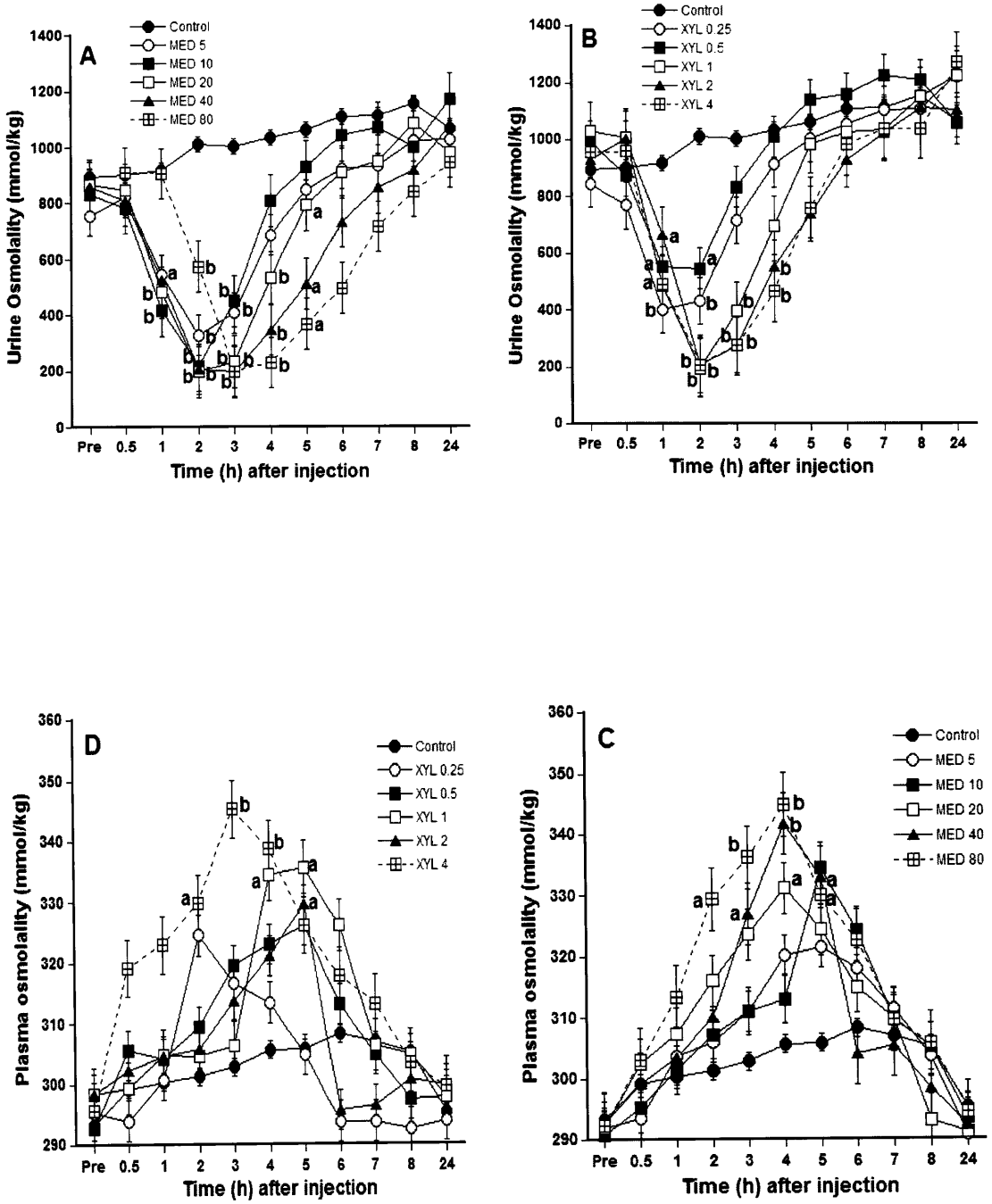


Figure 3. Urine osmolality (MED μg/kg, A; XYL mg/kg, B) and plasma osmolality (MED μg/kg, C; XYL mg/kg, D) following the administration of medetomidine and xylazine in dogs. Meanings of points, bars, and "a" or "b" as for Figure 1.

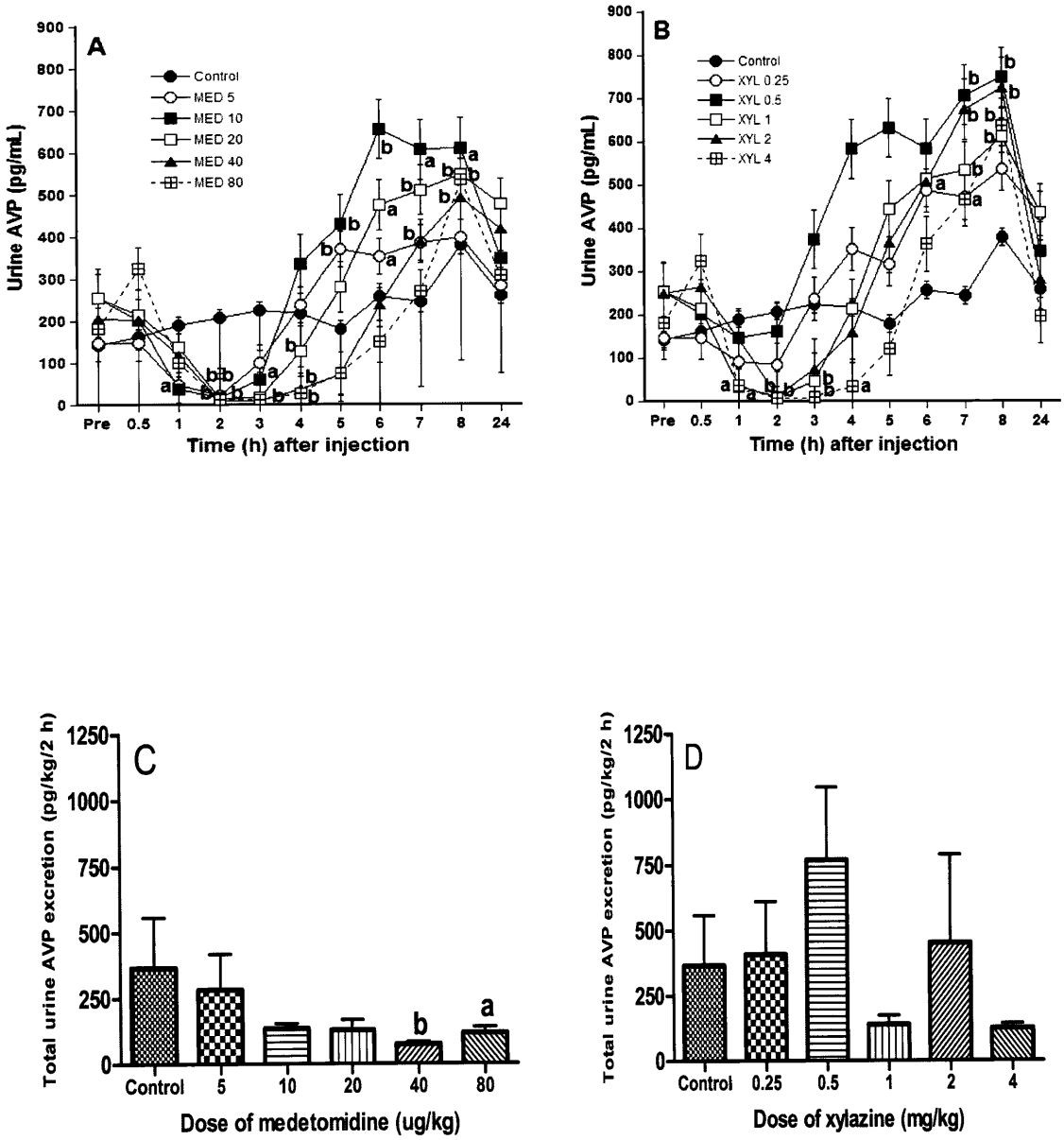


Figure 4. Urine AVP concentration following the administration of medetomidine (MED μg/kg, A) and xylazine (XYL mg/kg, B) in dogs. Total urine AVP excretion during 1 to 3 h following the administration of medetomidine (MED μg/kg, C) and xylazine (XYL mg/kg, D) in dogs. Meanings of points, bars, and "a" or "b" as for Figure 1.

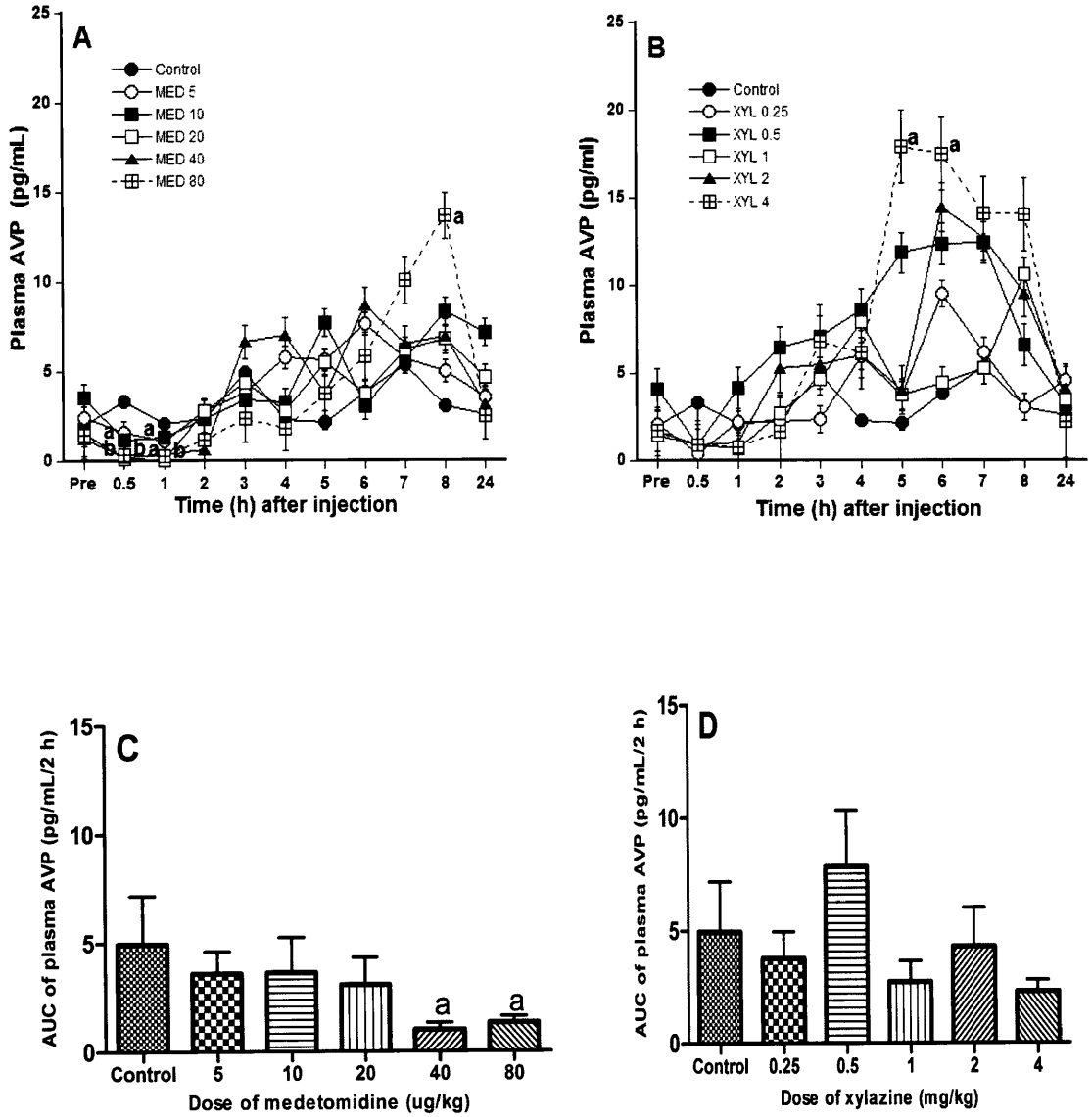


Figure 5. Plasma AVP concentration following the administration of medetomidine (MED μg/kg, A) and xylazine (XYL mg/kg, B) in dogs. The AUC data of plasma AVP during 0.5 to 2 h after administration of medetomidine (MED μg/kg, C) and xylazine (XYL mg/kg, D) in dogs. Meanings of points, bars, and "a" or "b" as for Figure 1.

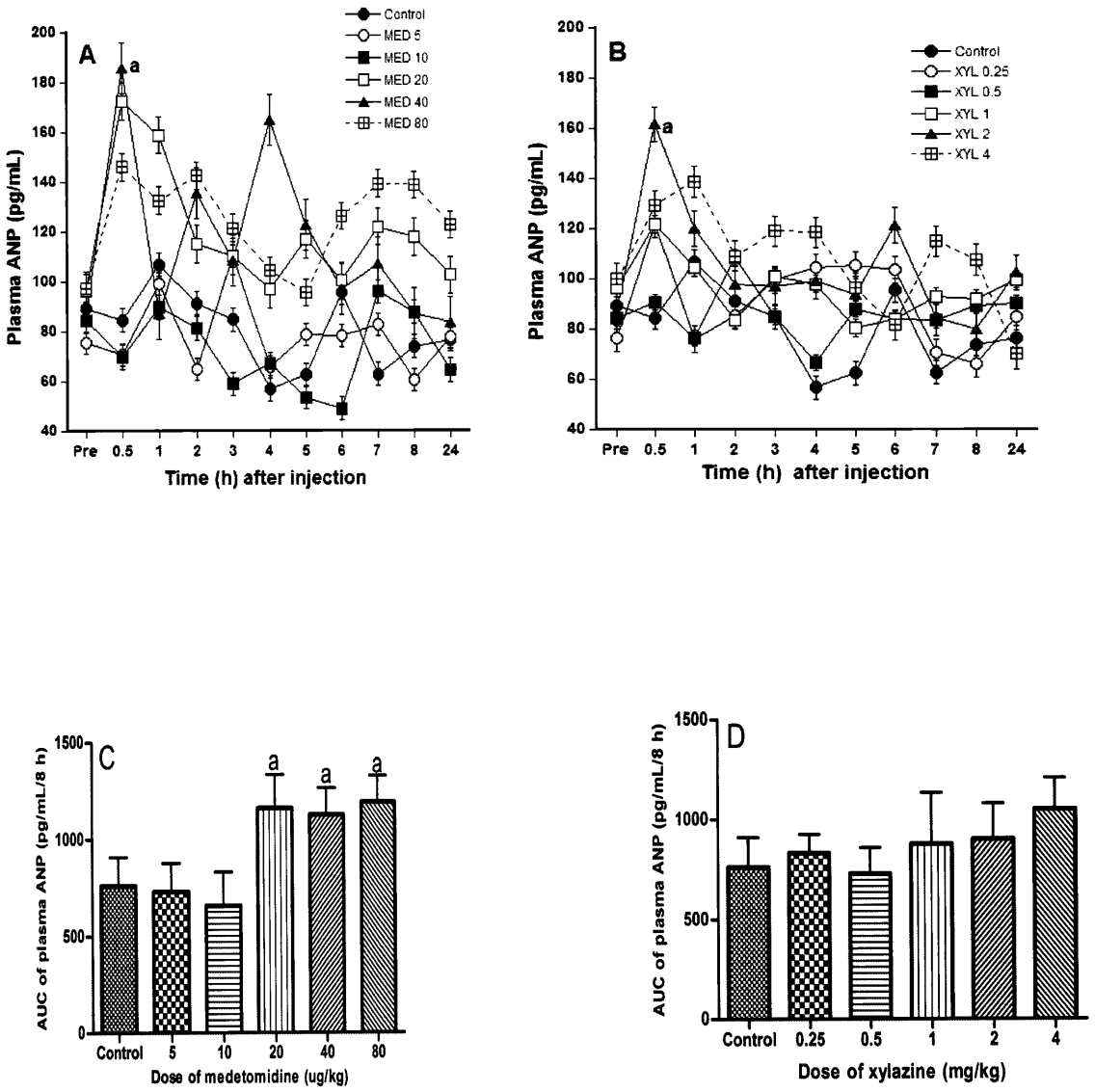


Figure 6. Plasma atrial natriuretic peptide (ANP) concentration following the administration of medetomidine (MED μg/kg, A) and xylazine (XYL mg/kg, B), and the AUC data of plasma ANP from 0 to 8 h after administration of medetomidine (MED μg/kg, C) and xylazine (XYL mg/kg, D) in dogs. Meanings of points, bars, and "a" or "b" as for Figure 1.

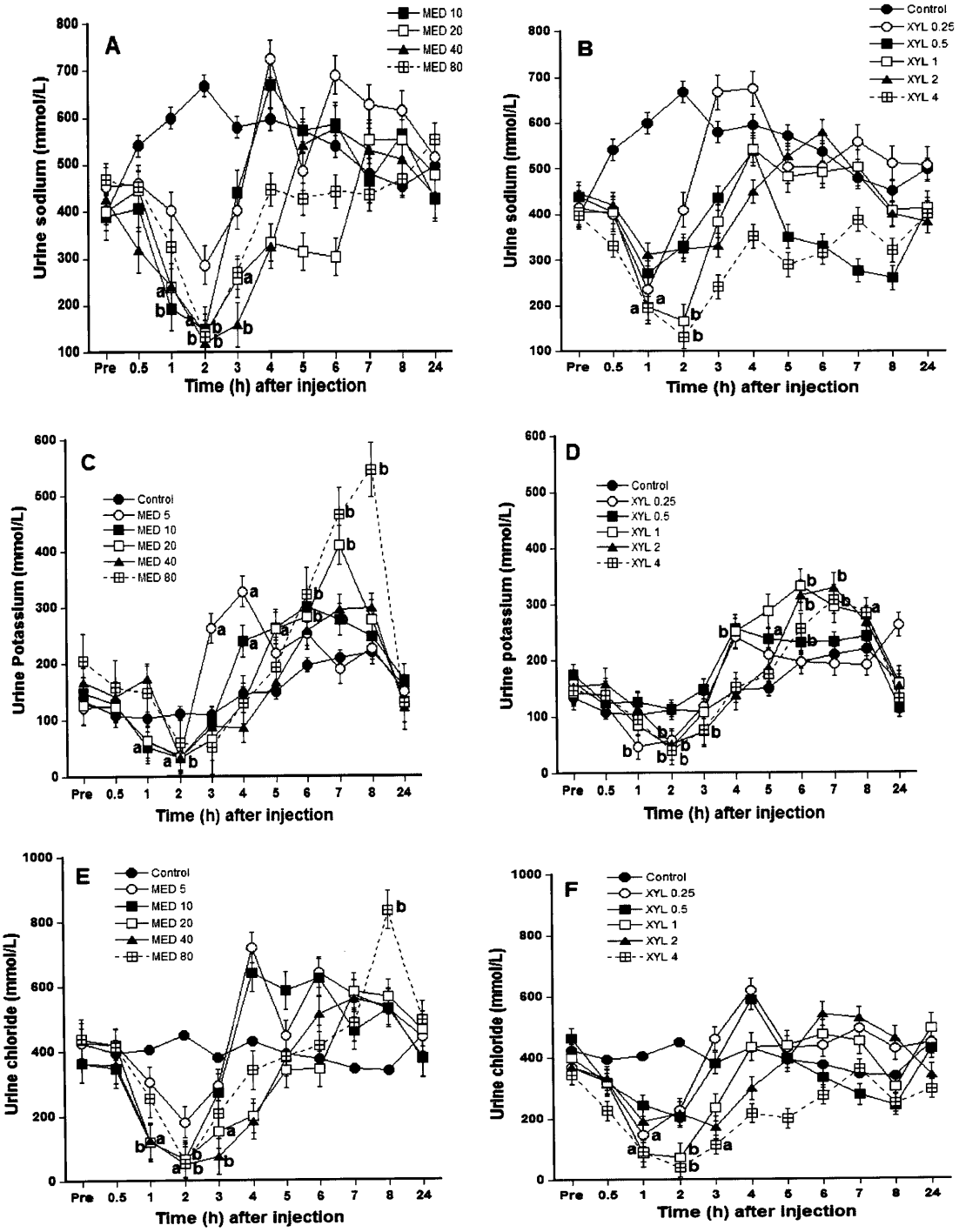


Figure 7. Urine electrolytes concentrations following the administration of medetomidine (MED μg/kg, A: sodium, C: potassium, E: chloride) and xylazine (XYL mg/kg, B: sodium, D: potassium, F: chloride) in dogs. Meanings of points, bars, and "a" or "b" as for Figure 1.

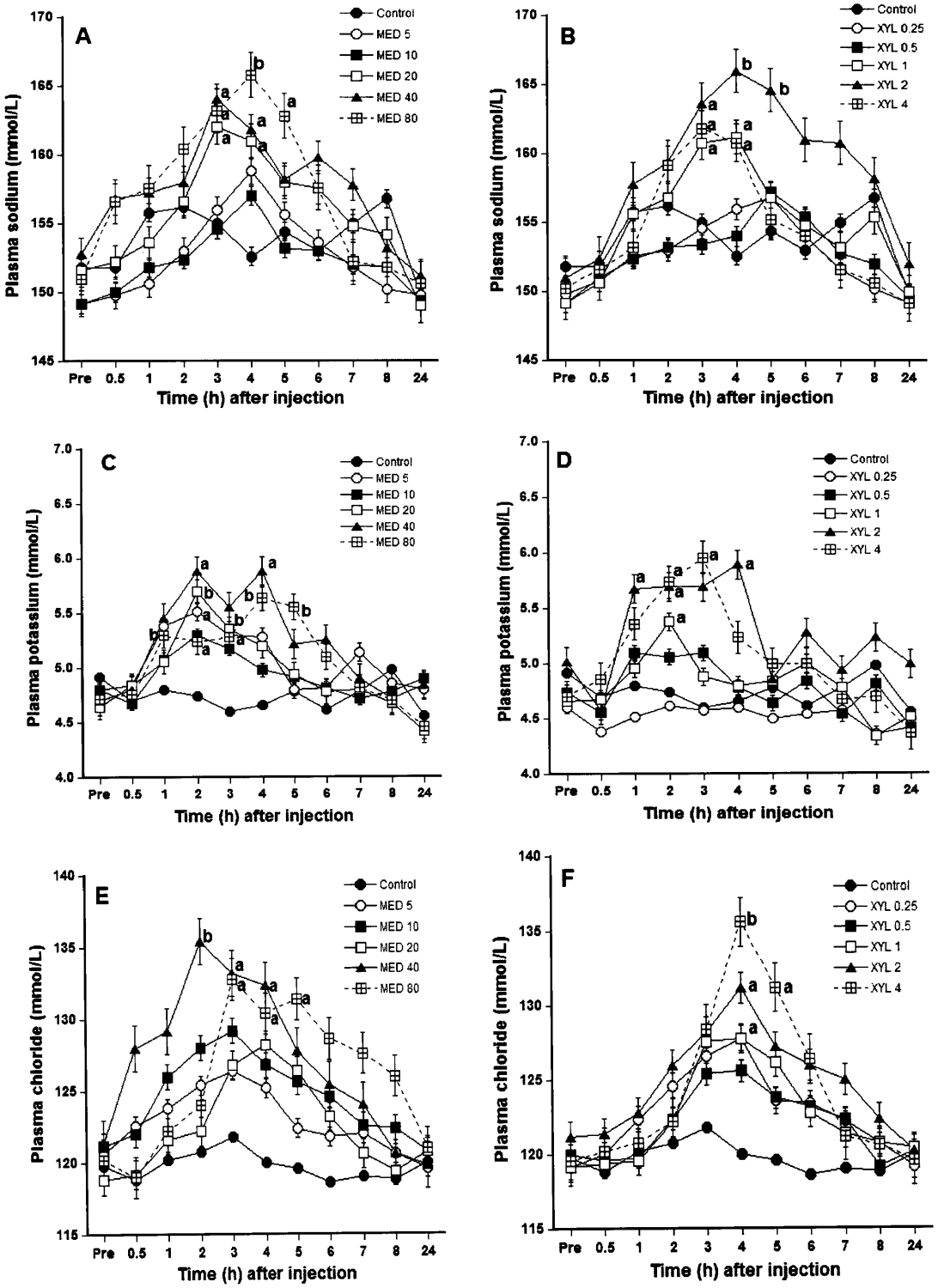


Figure 8. Plasma electrolytes concentrations following the administration of medetomidine (MED μg/kg, A: sodium, C: potassium, E: chloride) and xylazine (XYL mg/kg, B: sodium, D: potassium, F: chloride) in dogs. Meanings of points, bars, and "a" or "b" as for Figure 1.

## Discussion

The present study demonstrated that IM administrations of medetomidine and xylazine have a profound diuretic effect in healthy dogs up to approximately 4 h. Access to food and water after the sample collection of 8 h would not largely influence diuresis at 24 h, because it was observed that the urine volume returned to pre-value in all groups within 6 to 8 h after injection of either medetomidine or xylazine. The dose-dependent diuretic effect was more pronounced in xylazine compared with medetomidine at the tested doses. Profound diuretic effects induced by these two drugs in dogs of our study were in agreements with previous reports in dogs [5, 6] and goats [7] that were administered medetomidine, and those in cattle [8], horses [9] and rats [11] that was administered xylazine. Other $\alpha_2$-adrenoceptor agonists such as clonidine [17, 18], moxonidine [28], BHT-933 [10] in rats, and rilmenidine [29] and guanabenz [30] in dogs, have been also shown to produce a diuretic response in anesthetized or conscious conditions. In dogs, previous studies have reported that intravenous (IV) administrations of 10 and 20 μg/kg medetomidine alone, and 20 and 40 μg/kg medetomidine combined with isoflurane, produced diuretic effects [5, 6]. However, it has been reported that an IM administration of 80 μg/kg medetomidine alone did not significantly change in urine volume in dogs [6]. Our results revealed that an IM administration of 80 μg/kg medetomidine significantly increased urine volume, which is disagreement with a previous report [6]. This difference might be due to the use of a combination of medetomidine with isoflurane in a previous report [6]. The present results revealed that the diuretic response between medetomidine and xylazine was apparently different. To our best knowledge, this is the first report outlining the dose-dependent diuretic effect of medetomidine and xylazine in dogs. Although the diuretic effects of medetomidine and xylazine have been reported in several

species as mentioned with specific references earlier in this text, there were no reports as to the comparison of these two drugs in the same animal species. The present study revealed that both medetomidine and xylazine induced a profound diuretic effect in dogs. This study further revealed that xylazine induced highly significant dose-dependent diuresis, whereas the dose-dependency by medetomidine was lower. This difference in their diuretic response may be due to the different receptor selectivity and specificity between medetomidine and xylazine.

In our study, the decreases in urine specific gravity, urine osmolality and urine creatinine concentrations were almost simultaneous with the increase of urine volume in both MED and XYL groups. These indicated that both medetomidine and xylazine produced diuretic effects with the decrease re-absorption in the narrow tube of the kidney. Urine pH decreased in both MED and XYL groups in this study. In addition, higher doses of both drugs tended to delay recovery from the lowered urine pH. Presumably, the decrease in urine pH observed in this study may be due to arterial hypercapnea [31]. The expected response of the kidney to acute hypercapnea is to enhance renal tubular reabsorption of bicarbonate slightly [31], which may be in part reflected as a decrease in urine pH observed in our results. However, as the kidney may not respond rapidly to acute hypercapnea, other organic acids might partially affect a decrease in urine pH in our study. The higher pH values at the late hours of this study may be attributable to a decrease in renal tubular hydrogen ion secretion or a decrease in bicarbonate re-absorption.

The decreases in urine osmolality observed after administrations of MED or XYL in our experiment were in agreement with the previous results given medetomidine in dogs [5, 6] and xylazine in rats [11]. In this study, both MED and XYL significantly increased plasma osmolality in a dose-dependent manner, suggesting that the increased production of diluted

urine due to renal excretion of water caused more concentrated serum such as the elevated concentrations of plasma electrolytes observed in this study.

Our study revealed that urine AVP concentrations significantly decreased in higher doses of the MED and the XYL groups in the early phase of injection, and subsequently AVP concentration increased at the latter phase in both groups. Importantly, total amounts of urine AVP excretion during 1 to 3 h decreased significantly and dose-dependently in higher doses of medetomidine. On the other hand, plasma AVP levels decreased significantly during 0.5 to 2 h in the MED but not in the XYL groups after injection of these agents. The AUC data of plasma AVP revealed that MED-40 and MED-80 significantly decreased AVP release from 0.5 to 2 h, whereas xylazine did not. In addition, the linear regression of the normalized AUC data of plasma AVP from 0.5 to 2 h was significant in the MED groups but not in the XYL groups, indicating that medetomidine in contrast to xylazine suppresses plasma AVP release in a dose-dependent manner at the early phase after administration. Based on these findings, it was confirmed that AVP alone was not responsible for a profound diuresis induced by MED and XYL in our experiment. In the XYL groups, although urine volume was highly dose-dependent during this time at the tested doses, total amounts of urine AVP excretion and AUC of plasma AVP during 1 to 3 h did not significantly decrease and the linear regression of AUC did not show a dose-dependency. In contrast, total amounts of urine AVP excretion during same time decreased significantly and also plasma AVP values decreased significantly in the MED group. So definitely, there are different mechanisms between medetomidine- and xylazine-induced diuresis. It is indicated that medetomidine apparently inhibits AVP release compared with xylazine. Although the precise mechanism of the difference between medetomidine and xylazine on AVP release is unknown, it may be in part due to their differences on receptor selectivity and actions mediated via central nervous system (CNS),

since $\alpha_2$-adrenoceptor selectivity of medetomidine is approximately 10-folds greater than xylazine, and also medetomidine has central imidazoline receptor affinity [1-4]. Earlier studies have shown that, imidazoline $\alpha_2$-adrenoceptor agonists such as moxonidine, clonidine and its analogue ST-91, mediate their action via both $\alpha_2$-adrenoceptors and imidazoline receptors [13, 14, 18].

The diuretic effects of $\alpha_2$-adrenoceptor agonists have been reported to involve their actions on AVP and the renin-angiotensin system [32]. The $\alpha_2$-adrenoceptor agonists were reported to inhibit the secretion of AVP from the pituitary gland in dogs anesthetized with sodium pentobarbital-clonidine [33] and in rats anesthetized with ketamine-xylazine [11, 34]. A previous study in dogs [6] has reported that the IV injections of 20 and 40 μg/kg medetomidine under isoflurane anesthesia induced the diuresis and the decrease of plasma AVP concentration, whereas an IM injection of 80 μg/kg medetomidine increased plasma AVP concentration that differs with our findings. This difference might be due to the combined effect of isoflurane and medetomidine in a previous report [6]. We believe that the authenticity of our results is much higher as the diuretic effects of medetomidine as single active ingredient. In addition, a previous study has reported that plasma AVP concentration increased in dogs associated with surgery and anesthesia with acepromazine IM, but decreased to baseline within half an hour and diuresis occurred in presence of high plasma AVP [35]. Many evidences indicate that the activation of renal $\alpha_2$-adrenoceptors is predominant mechanism by which selective $\alpha_2$- agonists produce diuretic responses in rats [10-12, 34].

The $\alpha_2$-adrenoceptor agonists may produce diuretic response by inhibiting the CNS secretion and/or renal tubular actions of AVP [11, 34]. Furthermore, sedation with medetomidine and xylazine can also impact the renin-angiotensin system directly or

indirectly. The in vitro experiments have demonstrated a decrease in renin production directly via specific renal $\alpha_2$-adrenoceptors in the isolated-perfused rat kidney [32]. However, the renin-angiotensin system may also be affected indirectly by $\alpha_2$-agonist-induced hypertension [3, 11]. In regard to the central mechanisms, both medetomidine and xylazine may activate $\alpha_2$-adrenoceptors in the paraventricular nucleus of the hypothalamus contributing to the diuresis in dogs. In addition to a direct renal action, it is possible that a portion of the diuretic response elicited by the IV infusion of xylazine in ketamine-anesthetized rats is partially mediated by a pathway involving $\alpha_2$-adrenoceptors located in the CNS [34]. More specifically, the increase in urine production induced by both medetomidine and xylazine may at least in part, result from a central action of these drugs to inhibit the secretion of AVP. Such a mechanism would be consistent with the results of a number of studies showing that activation of central adrenergic receptors, in particular the $\alpha_2$-adrenoceptor subtype, inhibits the release of AVP in conscious or anesthetized animals [11, 32-34]. At the cellular level, the $\alpha_2$-adrenoceptor agonists such as clonidine and BHT-933 can inhibit AVP-stimulated cAMP formation in rats and rabbits [10, 12, 36-38]. Furthermore, an intrarenal infusion of clonidine in rats has been shown to produce a rapid redistribution of aquaporin-2 (AQP2) away from the luminal membrane of the medullary collecting duct to the cytosol and the reduction in AQP2 mRNA, suggesting that the $\alpha_2$-adrenoceptors regulate water excretion at least in part by effects on AQP2 [39].

Xylazine and medetomidine have been reported to induce an increase in serum glucose by suppressing insulin release and stimulating glucagon release [5, 6, 8, 9, 40]. We did not measure the plasma glucose in this study, because it has been reported in our laboratory [41]. Osmotic diuresis attributable to glucosuria is unlikely to be an appreciable factor in the diuretic effect of these drugs, because plasma glucose level did not change the tubular

maximum for glucose reabsorption [5]. Therefore, osmotic diuresis does not appear to be a cause in medetomidine- or xylazine-induced diuresis in dogs.

In this study, plasma ANP level reached the peak within half an hour of injection in dogs stimulated by medetomidine and xylazine. According to the authors best knowledge, this is the first report that medetomidine and xylazine stimulate plasma ANP release in dogs. Since higher doses of $\alpha_2$-agonists dominate hypertension due to peripheral post-synaptic adrenoceptors causing vasoconstriction, which results in a baroreceptor-mediated reflex bradycardia [3, 4]; this mechanism may be involved in ANP release by medetomidine and xylazine in our study. In addition, two partial $\alpha_2$-adrenoceptor agonists, clonidine and ST-91, potentially stimulate the release of ANP by activation of heart $\alpha_2$-adrenoceptors and/or imidazoline receptors, suggesting that this may account for the elevated plasma ANP and subsequent diuresis observed in vivo after administration of clonidine and its analogues [18]. As revealed in our study, the significant release of ANP in response to higher doses of medetomidine in contrast to xylazine may be due to interaction of medetomidine with imidazoline receptors. Previous findings [13-16] and our results indicate that the peripheral actions of medetomidine are probably mediated by both $\alpha_2$-adrenoceptors and imidazoline receptors, and may involve direct stimulation of ANP release that may account for the increase in plasma ANP levels after medetomidine injections in this study. Therefore, plasma ANP might partially influence the diuretic effects in our study.

The present study demonstrated that both MED and XYL significantly decreased urine sodium, potassium and chloride concentrations during profound diuresis, but actual amounts of these electrolytes excreted in the urine did not significantly change compared with control, indicating that the diluted urine was excreted after MED or XYL administration. In fact, plasma concentrations of sodium, potassium and chloride were increased dose-dependently

in both MED and XYL groups, indicating that the urinal excretion of these electrolytes did not significantly change during profound diuresis of this experiment. It has been reported that medetomidine [5, 6] and xylazine [11] may interfere with AVP-mediated tubular re-absorption of sodium, because dehydration occurred after administration of these agents, as supported by the increased plasma osmolality in our experiment. Potassium is reabsorbed in the proximal tubule of the nephon, and it undergoes resorption by intercalated cells in the connecting segments and cortical ducts. Finally, re-absorption or secretion of the potassium occurs in the medullary collecting duct [42]. As the urine flow rate in the distal tubules has an important influence on urine potassium secretion, it is possible that the tubular increased flow rate was partially responsible for the increased potassium ions in blood plasma after MED or XYL administrations. A temporal increase of plasma chloride ions may be attributable to direct effects of MED and XYL on renal tubular function. However, other indirect factors such as an initial decrease in cardiac output and renal blood flow, or the strong acid-base regulatory effect associated with chloride, must be considered. Medetomidine- and xylazine-induced changes in electrolytes might be important in hypokalemic or hypochloremic dogs. In support of these premise, it has been demonstrated that an intra-renal artery infusion of low doses of clonidine selectively increased water but not electrolyte excretion [43]. Based on previous findings, therefore, the present study suggests that the diuretic response of medetomidine and xylazine may be mediated via activation of complex peripheral and CNS $\alpha_2$-adrenoceptor systems.

In conclusion, both medetomidine and xylazine had profound diuretic effects in healthy dogs. The dose-dependent diuretic response of xylazine was more profound than that of medetomidine at our tested doses. This is our first outlining report of dose-dependent diuretic action of these agents in dogs. From our results of both urine and plasma AVP, AVP alone

may not be responsible for the dose-dependent diuretic effects of both medetomidine and xylazine. The present study has also demonstrated for the first time that medetomidine stimulate ANP release with greater potency in comparison with xylazine, which partially may influence diuresis. Medetomidine and xylazine may have differences in the mechanism of the diuresis, because the diuresis is the net products of multiple hemodynamic, neural, hormonal and local factors in kidney, and there are definitely differences between medetomidine and xylazine in the selectivity and specificity on $\alpha_2$-adrenoceptors and/or imidazoline receptors. Both drugs can be used as effective diuretic agents accompanied by sedation.

# Chapter 2

## Antagonistic effects of atipamezole and yohimbine on medetomidine-induced diuresis in healthy dogs

# Introduction

The $\alpha_2$-adrenoceptors are the transmembrane G protein coupled receptors that act pre- or post- and extrasynaptically in different tissues [1]. Pharmacological subtypes ($\alpha_{2A}$, $\alpha_{2B}$, $\alpha_{2C}$, $\alpha_{2D}$) of the $\alpha_2$-adrenergic receptors have been identified based on their ligand affinitiy [19]. Some ligands have an imidazole or imidazoline ring which enables them to bind to non-adrenergic imidazole-prefering receptors, as well as to the $\alpha_2$-adrenoceptors [20]. Medetomidine (4(5)-[1, 2, 3-dimethylphenyl]-imidazole) is the prototype of the novel selective $\alpha_2$-adrenoceptor agonists having imidazoline receptor affinity [21]. The $\alpha_2/\alpha_1$ receptor binding selectivity of medetomidine is 1620, whereas that of xylazine, detomidine or clonidine is 160, 260 or 220, respectively [2]. Medetomidine is also more lipophilic than xylazine, detomidine or clonidine [22]. It has been widely used for sedation, analgesia, muscle relaxation, immobilization, and reductance of peristalsis during gastro-intestinal surgery or endoscopy in veterinary practice [23, 24, 41]. The $\alpha_2$-adrenoceptor antagonists, atipamezole and yohimbine have been shown to reverse a variety of clinicophysiological effects produced by $\alpha_2$-adrenoceptor agonists [1-4, 19-27]. The $\alpha_2/\alpha_1$ selectivities of atipamezole and yohimbine are 8526/1 and 40/1, respectively [1-4, 19-26]. Atipamezole is a potent and highly specific antagonist of centrally and peripherally located $\alpha_2$-adrenoceptors compared with yohimbine [25]. The affinities of atipamezole and yohimbine are similar at the $\alpha_{2A}$-, $\alpha_{2B}$- and $\alpha_{2C}$-adrenoceptors but differ by approximately 100-folds at the $\alpha_{2D}$-adrenoceptors [1-4]. In addition, yohimbine affects serotonergic, cholinergic, dopaminergic and GABA receptor-related mechanisms [26], whereas atipamezole lacks these receptor activities [25]. Furthermore, atipamezole has a similar structure to imidazoline, whereas yohimbine has no imidazoline receptor affinity [1-5, 25-27]. These differences between atipamezole and yohimbine may influence on the antagonistic effects of medetomidine-

induced actions. Medetomidine is known to induce diuresis in several species including dogs [5-7]. Recently, we have found that medetomidine has a dose-dependent diuretic effect associated with changes in urine specific gravity, pH, creatinine values, and osmolality, sodium, potassium and chloride ions in both urine and plasma in healthy dogs [44]. In that study, it has been also demonstrated that medetomidine decreases dose-dependently the urine AVP excretion and suppresses plasma AVP, and also increases plasma ANP dose-dependently at the early phase after administration. Previous reports and our recent study have suggested that the decrease in plasma AVP played a partial role in the profound diuretic effect of medetomidine though its action on $\alpha_2$-adrenoceptors [5, 6, 12, 44-46]. Medetomidine has been also reported to markedly induce ANP release in normotensive rats [15]. ANP is a potent vasodilator, diuretic and natriuretic hormone, primarily of cardiac origin [28]. In our recent study, we claimed that ANP might partially influence on the medetomidine-induced diuresis in dogs, because it has been reported that ANP exerts a diuretic and natriuretic action on renal proximal tubules and inner medullary duct cells of the kidney in rats [13-16, 28] and mice [47]. The sedative and analgesic actions of medetomidine are accompanied with initial hypertension followed by prolonged hypotension, respiratory acidosis and hypoxemia in several species of animals [23, 24]. It is unknown whether this kind of actions exerted on the diuretic effect due to graded doses of medetomidine. The regulation of water excretion has implications for a number of clinical situations. However, to our best knowledge, there are no published reports on the antagonistic effects of atipamezole and yohimbine against medetomidine-induced diuresis in dogs. In addition, there is no report that either atipamezole or yohimbine reverses the inhibition of AVP and the release of ANP induced by medetomidine in dogs. This study aimed to investigate and compare the antagonistic effects of thee different doses of either atipamezole or yohimbine on the diuresis induced by

medetomidine in healthy dogs. The variables examined were urine volume, specific gravity, pH, creatinine values, and osmolality, electrolytes and AVP values in both urine and plasma, and plasma ANP.

## Materials and methods

### Animals

Five adult male healthy dogs of which thee beagles and two mixed-breeds, with a mean age of 5.8 (standard deviation 2.7) y and mean weight of 11.2 (1.8) kg were used. All the dogs were raised at the laboratory providing animal management facilities and fed a standard commercial dry canine food. Routine hematologic examination was done before the experiment; all values were within normal physiological ranges [48]. The study protocols were approved by the Animal Research Committee of Tottori University, Tottori, Japan.

### Experimental protocols

The experiment consists of 8 treatment groups. Five dogs were assigned to each of the 8 treatment groups in a randomized design at 1 wk intervals in the same dog. Each dog in a group was given an IM injection of 2.0 mL/head physiological saline solution as non-medicated control. The dogs in other groups received a 1st treatment of 20 μg/kg medetomidine hydrochloride (Domitor®, Meiji Seika, Tokyo, Japan) with an IM injection of a 0.1% solution at the beginning of the experiment. This was followed 0.5 h later by a 2nd IM treatment of 0.5 mL/head physiological saline solution, 50, 100, and 300 μg/kg atipamezole hydrochloride (0.5 % solution; Antisedan®, Meiji Seika, Tokyo, Japan); and 50, 100 and 300 μg/kg yohimbine hydrochloride (Sigma Chemical, St. Louis, MO, USA). Yohimbine was dissolved in distilled water at the concentration of 0.5 mg/mL. The groups will be referred to as Saline, MED, MED-ATI 50, MED-ATI 100, MED-ATI 300, MED-YOH 50, MED-YOH 100 and MED-YOH 300. As the $\alpha_2$-adrenoceptor agonists have been

often used for an IM injection [49], this route was preferred in our study. The quadriceps muscle was used for injection site. The dogs were fasted for 12 h prior to experiment. After sample collection at 8 h, feeding was done once, and again fasted for 12 h to collect the sample at 24 h in the next day. The experiments were performed in a room with air temperature at 25°C.

### Sample collection

A 6- or 8-Fr Silicon balloon catheter (All Silicon Foley Catheter, Cliny Medical Corp, Tokyo, Japan) was inserted prior to 1 h of the experiment to empty the bladder and for subsequent urine sampling. The catheter was withdrawn after sampling at 8 h. On next day at 22 h, the catheter was again inserted and the bladder was made empty. Subsequently, urine sample was collected at 24 h. Urine and blood samples were taken for the following 11 times: prior to injection of the agent (0), 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24 h after injection of medetomidine. Blood samples (5.5 mL) were collected from the jugular vein by means of a 21-gauze needle with a 6 mL disposable syringe, at same time points urine samples were collected. An aliquot of 4.0 mL from each sample was mixed with ethylene diamine tetraacetic acid and aprotinin (Trasylol®, Bayer, Leverksen, Germany) for AVP and ANP measurements, and the remaining 1.5 mL was mixed with heparin for osmolality measurement. The blood samples were immediately centrifuged at 2000 × g at 4 °C for 15 min, and the plasma was separated and kept at -40 °C for analysis. Urine samples were centrifuged at 2000 × g for 5 min, and then the supernatant was collected and stored at -40 °C until analyzed.

### Analytical methods

Urine volume was measured at each time point by a measuring cylinder after collection from the urine bag. Urine specific gravity and pH were measured by a refractor photometer

(Erma®, Tokyo, Japan) and pH meter (pH meter F-52®, Horiba Corp, California, USA), respectively. Urine creatinine concentrations were measured by creatinine assay kit (Wako Pure Chemical Industries Corp, Osaka, Japan) with Jaffe method using spectrophotometer. In both urine and plasma, osmolality and electrolytes were measured by using vapor pressure osmometer (VAPRO®, Wescor, Utah, USA) and Na-K-Cl ion-concentrations auto analyzer (DRI-CHEM800V®, Tokyo, Japan), respectively. Plasma AVP was extracted following the standard protocol for solid phase column extraction (Sep-Pak® Cartridges, Waters, Ireland). Urine and plasma AVP concentrations were measured by a double antibody radioimmunoassay (RIA) technique with the use of commercially available AVP kit (Mitsubishi Chemical, Tokyo, Japan). The intra-assay coefficients of variation (CVs) were 10 % and the limits of detection and quantification were 0.063 to 8.0 pg/tube. ANP was also assayed by a double antibody RIA kit (HANP kit®, Eiken Chemical Company, Tokyo, Japan). The intra-assay CV was 15 %. The detection and quantification limits were 10 and 1280 pg/mL, respectively.

**Data evaluation**

All data obtained were analyzed together with Prism statistical software (version 4; Graph Pad Software, San Diego, California, USA). One-way analysis of variance for repeated measures was used to examine the time effect within each group and the group effect at each time point. When a significant difference was found, the Tukey test was used to compare the means. The area under the curve (AUC) was calculated for each biochemical variable. The AUC was measured by calculating the sum of the trapezoids formed by the data points. The AUC data were plotted against the doses of atipamezole or yohimbine, and simple linear regression analysis was applied. When a significant difference was found, the effect of the drug on the plasma level of the examined biochemical was claimed to be dose-

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

## Results

For all the variables, there were no significant differences between groups at baseline (0 h). No significant changes of urine volume and other biochemical and hormonal variables were observed in the Saline group. Medetomidine increased significantly urine production at 1 and 2 h and the diuretic effects persisted approximately up to 4 h after injection (Figure 9A, 9B). While, comparing with the peak means value of urine volume at 2 h in the MED group, it was recognized that ATI 100 and 300, YOH 50, 100 and 300 groups significantly inhibited medetomidine-induced diuresis (Figure 9A, 9B). Moreover, the linear regression of the total urine volume from 0.5 to 4 h was highly significant ($P<0.001$) in the MED-ATI groups but not in the MED-YOH groups, indicating that atipamezole inhibited medetomidine-induced diuresis dose-dependently in contrast to yohimbine at tested doses (Figure 9C, 9D). Similar results were observed with the linear regression of the total urine volume data from 0.5 to 2 h, 0.5 to 3 h, 0.5 to 6 h, and 0.5 to 8 h.

Medetomidine decreased urine specific gravity significantly during 0.5 to 4 h. MED-ATI 50 and MED-YOH 50 groups decreased mean urine specific gravity significantly compared with their respective baseline values (Figure 10A, 10B). However, ATI 100 and 300, and YOH 100 and 300 did not significantly decrease mean specific gravity. These decreases in urine specific gravity were in correspondence with the decrease in urine volume in both MED-ATI and MED-YOH groups.

Mean urine pH decreased significantly only in the MED and MED-ATI and MED-YOH 100 groups during 1 to 4 h after injection of the agents and then gradually returned to

baseline values. Thereafter, the urine pH in all groups increased over the value in the Saline group during 6 to 8 h.

Urine creatinine concentration was increased gradually during 8 h in the Saline group, whereas it was decreased significantly in the MED, MED-ATI 50 and MED-YOH 50 groups during 2 to 3 h (Figure 10C, 10D). The lowest mean concentration of urine creatinine was found at 2 h in the MED-ATI 50 and MED-YOH 50 groups. Atipamezole dose-dependently reduced the decrease of urine creatinine concentrations induced by medetomidine. The higher doses of yohimbine prevented the decrease of creatinine concentrations.

Mean urine osmolality in both MED-ATI and MED-YOH groups decreased significantly and similarly during 1 to 3 h compared to the baseline value (Figure 11A, 11B). Higher doses of both atipamezole and yohimbine reduced the decrease of mean urine osmolality induced by MED. On the other hand, mean plasma osmolality in the MED group significantly increased during 2 to 4 h compared to the baseline value (Figure 11C, 11D). Treatments with all doses of yohimbine prevented the increase of plasma osmolality induced by MED.

Actual amounts of urine AVP excretion from 0.5 to 4 h lowered in the MED group than the Saline group (Figure 12A, 12B). There were no significant differences in actual amounts of urine AVP excretion between MED and MED-ATI groups (Figure 12A). In the MED-YOH groups, yohimbine dose-dependently reversed the MED-induced decrease in urine AVP excretion (Figure 12B). The linear regression of the total urine AVP excretion during 0.5 to 4 h was significant ($r=0.5809$; $P<0.05$) in the MED-YOH groups but not in the MED-ATI groups ($r=0.2026$; $P>0.05$), indicating that the MED-ATI groups did not dose-dependently increase the actual amounts of excreted urine AVP in contrast to the MED-YOH groups. Similar results were obtained with linear regression of the total urine AVP excretion from 0.5 to 2 h, 0.5 to 3 h and 0.5 to 8 h.

The AUC data of plasma AVP from 0.5 to 2 h was significantly higher in both MED-ATI 100 and 300 groups and MED-YOH 100 and 300 groups than in the MED group (Figure 13A, 13B). Similar results in the AUC data of plasma AVP were obtained from 0.5 to 3 h and 0.5 to 4 h. The linear regression of the AUC data of plasma AVP from 0.5 to 2 h was highly significant in the MED-ATI groups ($r=0.6249$; $P<0.01$), and was not in the MED-YOH groups ($r=0.4054$, $P>0.05$). These results showed that both atipamezole and yohimbine reversed the medetomidine-induced decreases in plasma AVP concentration. Similar results were found with the linear regression analysis of the AUC data from 0.5 to 3 h and 0.5 to 4 h.

The AUC data of plasma ANP from 0.5 to 4 h revealed that the MED-ATI 300 group significantly increased ANP release compared with the MED group, while all doses of MED-YOH treatment did not significantly alter the ANP release. The linear regression of the AUC data of plasma ANP from 0.5 to 4 h in the MED-ATI and MED-YOH groups revealed that ATI increased dose-dependently ANP release, but YOH did not (Figure 14A, 14B). Similar results were obtained with the linear regression of AUC data of plasma ANP from 0.5 to 2 h and 0.5 to 3 h.

In the MED group, the mean concentrations of urine sodium, potassium and chloride were lower during 0.5 to 4 h compared with the baseline values and then were increased over baseline values during 5 to 8 h . In the MED-ATI and MED-YOH groups, higher doses of ATI or YOH prevented the decreases in the urine concentrations of sodium, potassium and chloride induced by MED (Table I). Treatments with higher doses of both ATI and YOH did not significantly change urine concentrations of sodium, potassium and chloride during 24 h after administration of MED. Total amounts of excreted urine sodium, potassium and chloride did not significantly change during 1 to 3 h in both MED-ATI and MED-YOH

groups compared to the MED and Saline groups, respectively.

Plasma sodium, potassium and chloride concentrations increased significantly in the MED group compared with the baseline (Table II). Both ATI and YOH treatments prevented the increase of plasma concentrations of sodium, potassium and chloride that were induced by MED.

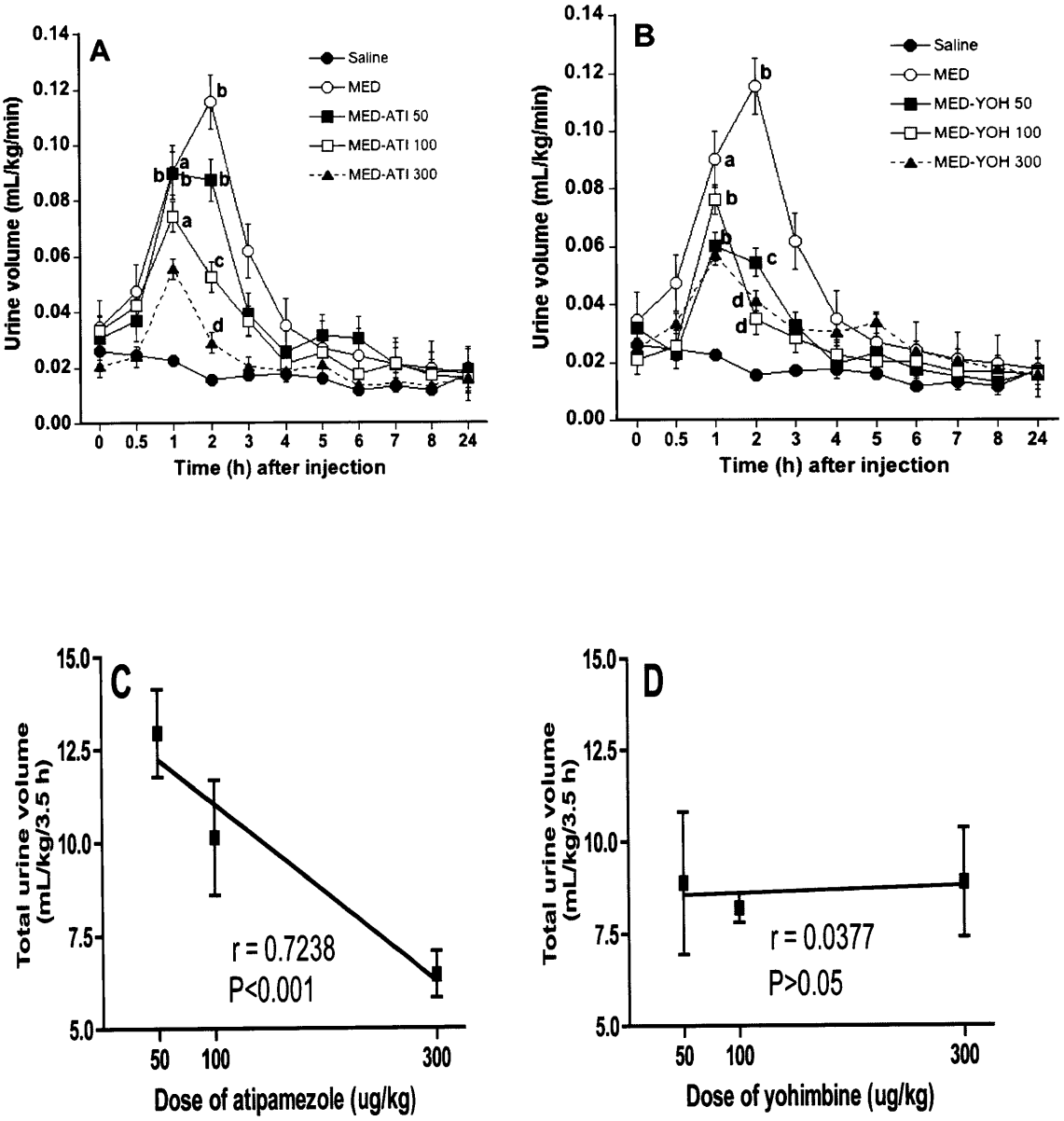


Figure 9. Urine volume (MED-ATI, μg/kg, A; MED-YOH, μg/kg, B) and simple linear regression of total urine volume (MED-ATI, μg/kg, C; MED-YOH, μg/kg, D) during 0.5 to 4 h after the administration of medetomidine followed by atipamezole and yohimbine in dogs. Each point and vertical bar represent the mean and standard error (n=5). Significantly different from the 0 h value (a: $P<0.05$, b: $P<0.01$); significantly different from MED group (c: $P<0.05$, d: $P<0.01$).

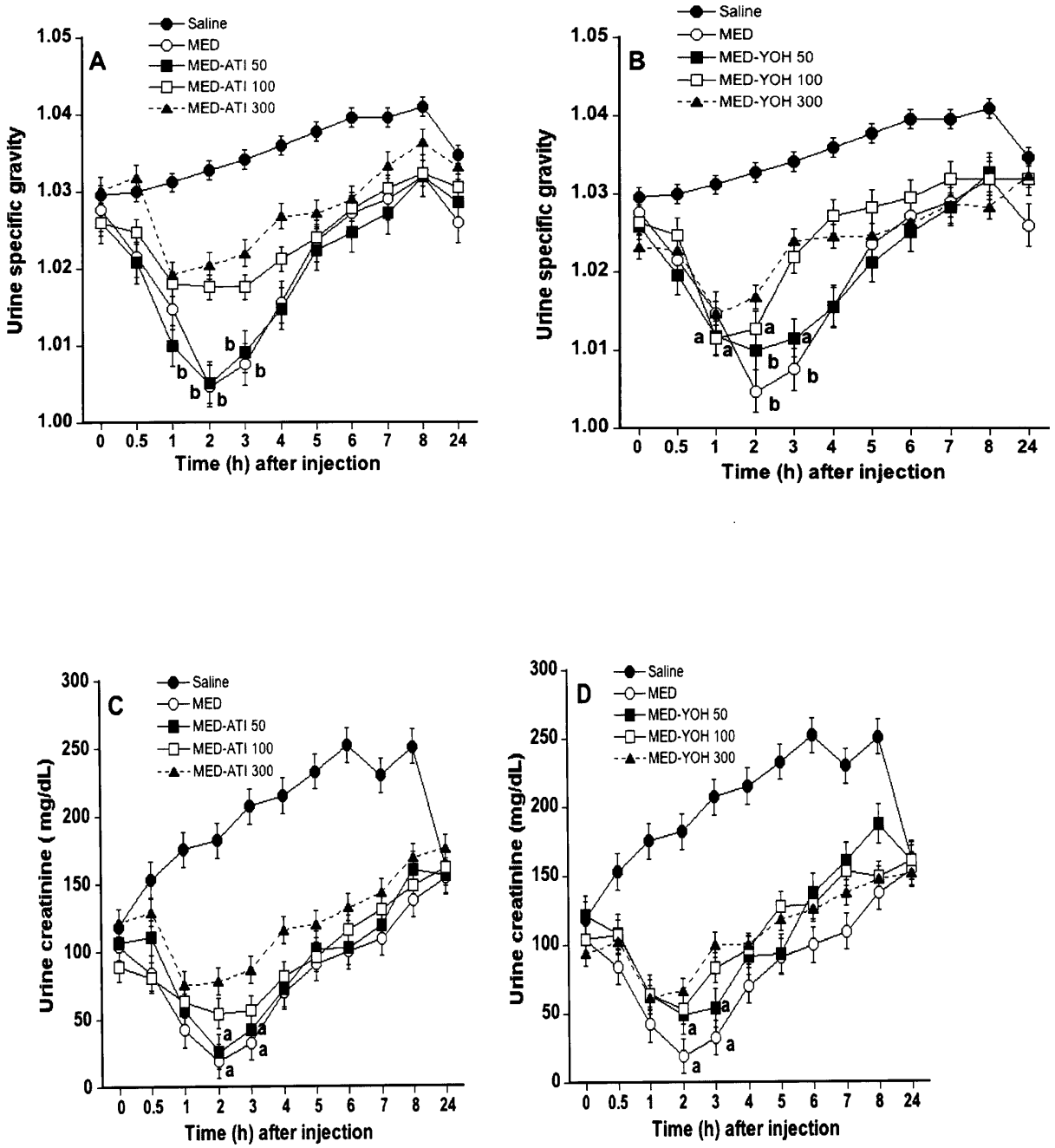


Figure 10. Urine specific gravity (MED-ATI, μg/kg, A; MED-YOH, μg/kg, B) and creatinine concentrations (MED-ATI, μg/kg, C; MED-YOH, μg/kg, D) after the administration of medetomidine followed by atipamezole and yohimbine in dogs. Meanings of points, bars, and "a" or "b" as for Figure 9.

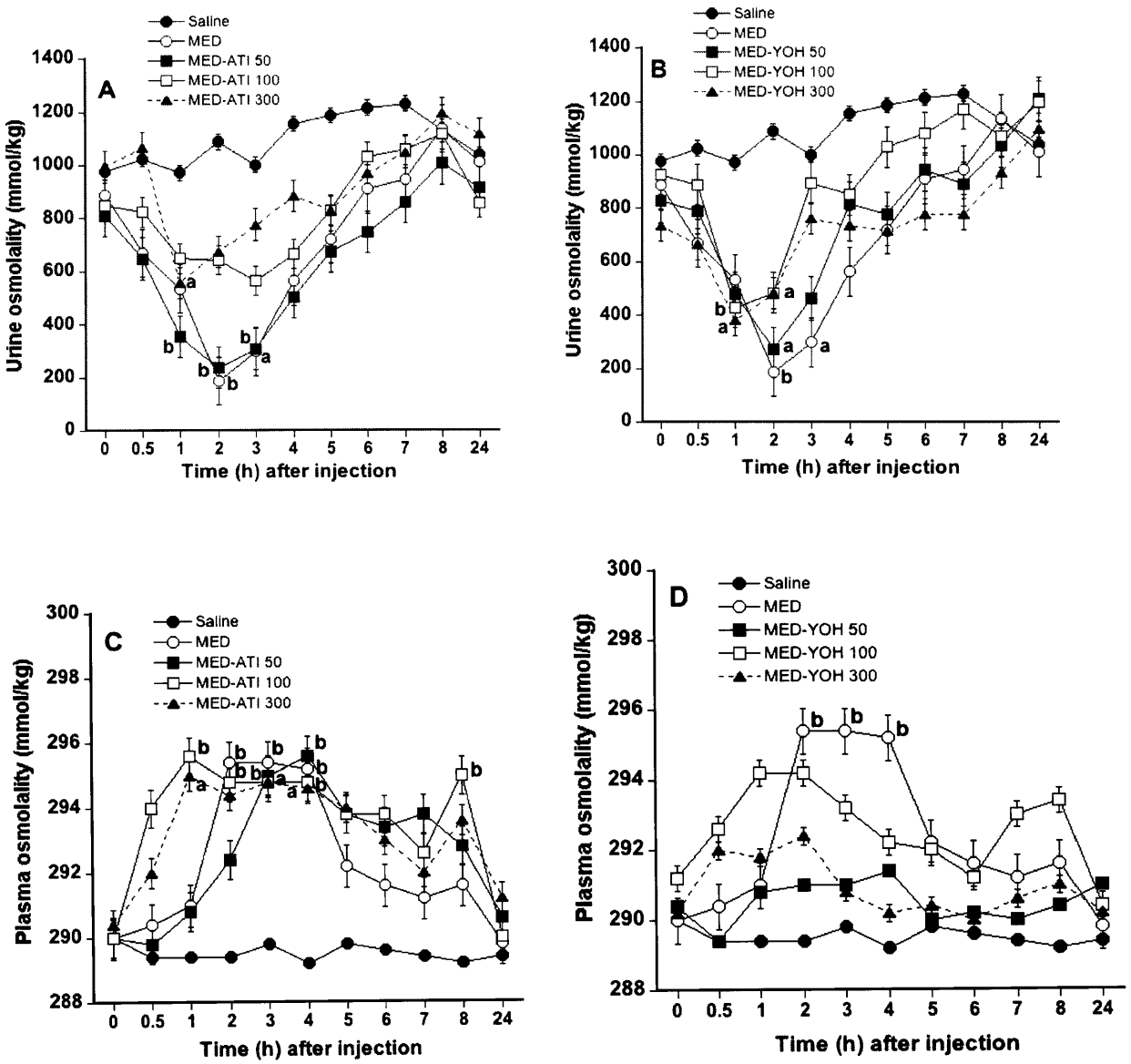


Figure 11. Urine osmolality (MED-ATI, μg/kg, A; MED-YOH μg/kg, B) and plasma osmolality (MED-ATI, μg/kg, C; MED-YOH, μg/kg, D) after the administrations of medetomidine followed by atipamezole and yohimbine in dogs. Meanings of points, bars, and "a" or "b" as for Figure 9.

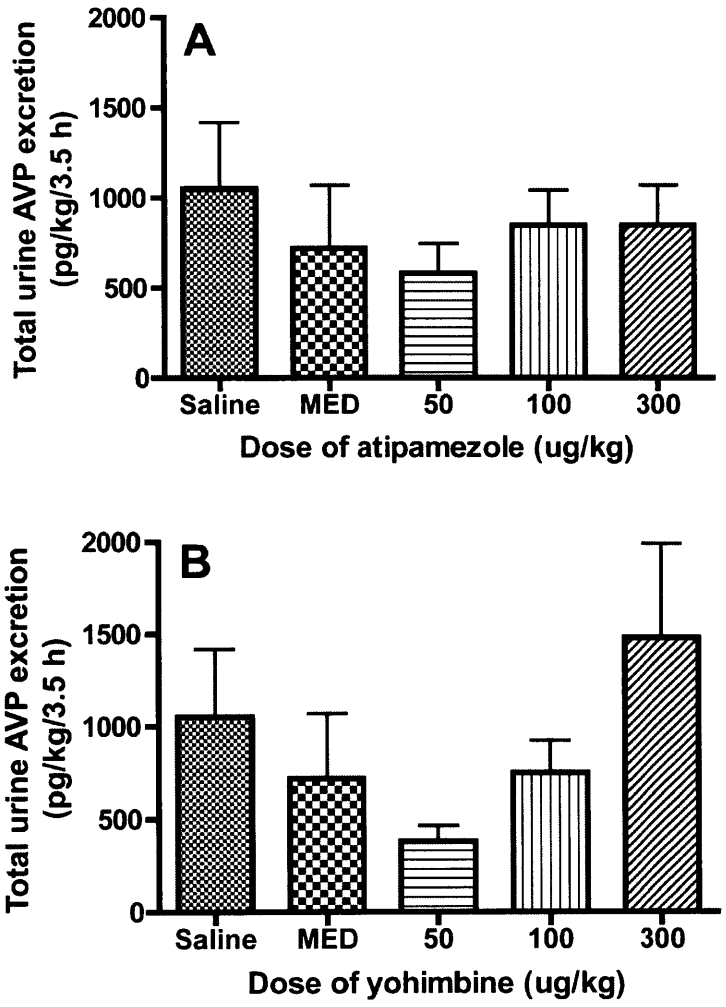


Figure 12. Total urine AVP excretion (MED-ATI, μg/kg, A; MED-YOH, μg/kg, B) during 0.5 to 4 h after the administrations of medetomidine followed by atipamezole and yohimbine in dogs.

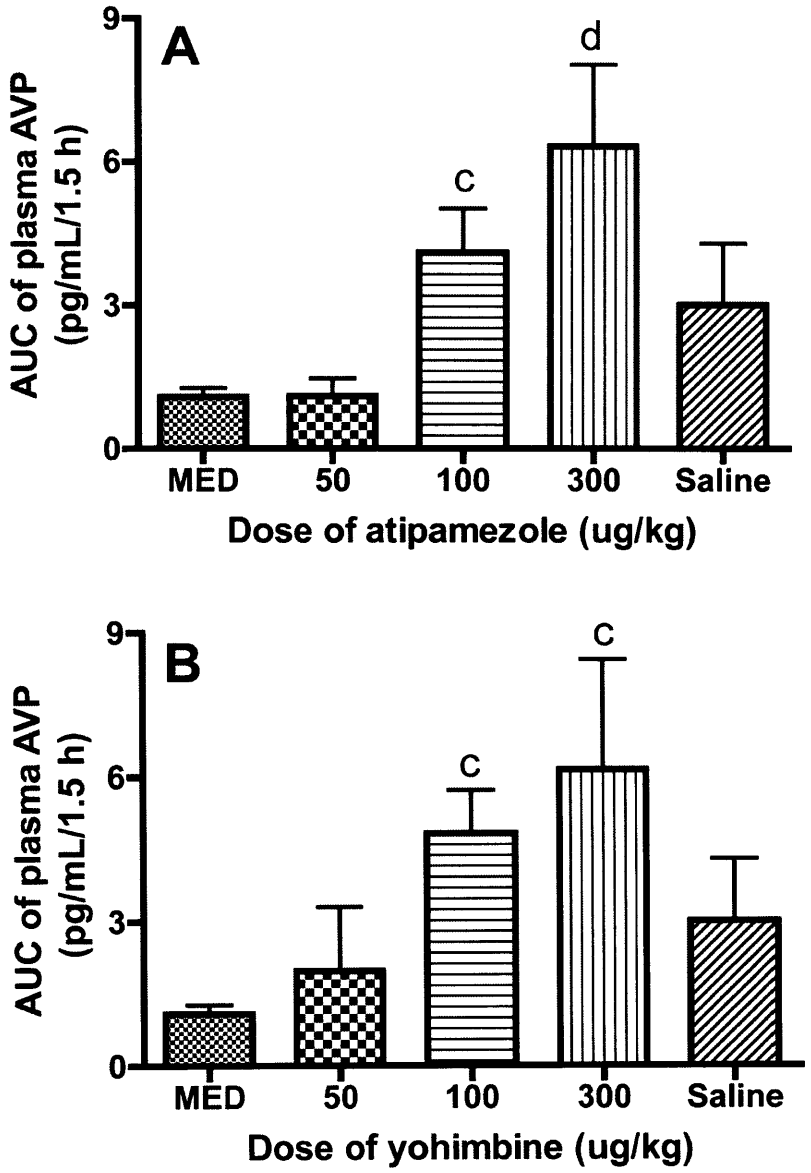


Figure 13. AUC data of plasma AVP during 0.5 to 2 h (MED-ATI, μg/kg, A; MED-YOH, μg/kg, B) after the administrations of medetomidine followed by atipamezole and yohimbine in dogs. Meanings of points, bars, and "c" or "d" as for Figure 9.

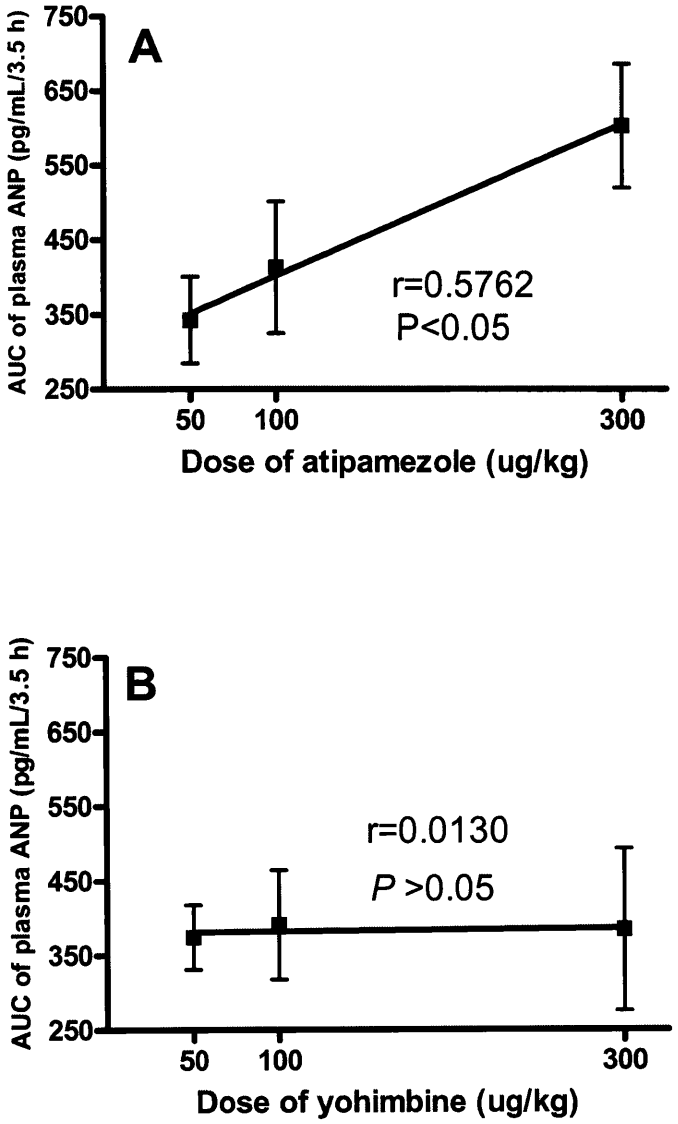


Figure 14. Simple linear regression of AUC data of plasma ANP (MED-ATI, μg/kg, A; MED-YOH, μg/kg, B) during 0.5 to 4 h after the administrations of medetomidine followed by atipamezole and yohimbine in dogs.

Table I. Urine electrolytes concentration after administration of the test agents to 5 dogs

| Variables | Group | Time after administration (h) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.5 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 24 |
| Sodium (mmol/L) | Saline | 272 ± 64 | 296 ± 116 | 334 ± 111 | 350 ± 157 | 396 ± 151 | 405 ± 159 | 413 ± 184 | 420 ± 158 | 417 ± 150 | 403 ± 128 | 233 ± 69 |
| | MED | 245 ± 43 | 200 ± 38 | 101 ± 13 | 76 ± 24 | 143 ± 54 | 285 ± 68 | 500 ± 133 | 545 ± 235 | 557± 136 | 584 ± 158 | 227 ± 53 |
| | ATI50 | 213 ± 43 | 143 ± 41 | 100 ± 23 | 74.4 ± 14 | 157 ± 39 | 276 ± 63 | 479 ± 111 | 394 ± 80 | 457 ± 95 | 448± 101 | 264 ± 96 |
| | ATI100 | 246 ± 64 | 188 ± 47 | 186 ± 54 | 167 ± 63 | 207 ± 56 | 287 ± 48 | 335± 92 | 423 ± 102 | 392 ± 107 | 301± 57 | 215 ± 49 |
| | ATI300 | 260 ± 61 | 226 ± 55 | 206 ± 49 | 143 ± 25 | 281 ± 48 | 310 ± 68 | 328 ± 80 | 363 ± 90 | 371 ± 88 | 358 ± 61 | 234 ± 51 |
| | YOH50 | 205 ± 56 | 201 ± 45 | 126 ± 35 | 108 ± 37 | 238 ± 67 | 330 ± 104 | 367 ± 93 | 421 ± 132 | 429 ± 112 | 427± 138 | 239 ± 47 |
| | YOH100 | 243 ± 76 | 209 ± 53 | 187 ± 60 | 109 ± 22 | 387 ± 83 | 338 ± 81 | 511± 98 | 479 ± 83 | 493 ± 116 | 563± 129 | 235 ± 53 |
| | YOH300 | 218 ± 54 | 208 ± 69 | 189 ± 59 | 128 ± 39 | 274 ± 92 | 279 ± 82 | 307 ± 104 | 311 ± 103 | 317 ± 89 | 326 ± 74 | 216 ± 50 |
| Potassium (mmol/L) | Saline | 190 ± 54 | 145 ± 39 | 139 ± 39 | 127 ± 32 | 124 ± 32 | 165 ± 37 | 179 ± 38 | 239 ± 57 | 2390 ± 51 | 213 ± 59 | 221 ± 80 |
| | MED | 203± 43 | 132 ± 35 | 81 ± 43 | 23 ± 7[a] | 36 ± 13[a] | 102 ± 29 | 292 ± 64 | 340 ± 68 | 386 ± 112 | 379 ± 75 | 254 ± 58 |
| | ATI50 | 147 ± 37 | 120 ± 30 | 35 ± 8[b] | 36 ± 11[b] | 52 ± 13[a] | 162 ± 58 | 388 ± 114 | 336 ± 99 | 437 ± 122[a] | 408 ± 114 | 292 ± 77 |
| | ATI100 | 257 ± 76 | 181 ± 83 | 140 ± 76 | 143 ± 70 | 147 ± 57[a] | 151 ± 31 | 242 ± 76 | 379 ± 93 | 353 ± 90 | 318 ± 62 | 278 ± 88 |
| | ATI300 | 234 ± 65 | 194 ± 60 | 87 ± 22 | 143 ± 31 | 222 ± 51 | 183 ± 48 | 290 ± 97 | 226 ± 61 | 304 ± 83 | 335 ± 79 | 274 ± 43 |
| | YOH50 | 156 ± 42 | 131 ± 40 | 64 ± 27 | 42± 13 | 76 ± 23 | 151 ± 43 | 158 ± 39 | 179 ± 50 | 168 ± 53 | 175 ± 42 | 224 ± 42 |
| | YOH100 | 191 ± 53 | 170 ± 37 | 69 ± 20 | 93 ± 28 | 205 ± 49 | 200 ± 50 | 299 ± 79 | 248 ± 54 | 220 ± 54 | 208 ± 31 | 219 ± 45 |
| | YOH300 | 181 ± 43 | 150 ± 37 | 104 ± 22 | 115 ± 31 | 167 ± 31 | 140 ± 33 | 103 ± 18 | 92 ± 15 | 118 ± 31 | 118 ± 27 | 218 ± 48 |
| Chloride (mmol/L) | Saline | 276 ± 67 | 230 ± 62 | 312 ± 114 | 336 ± 112 | 342 ± 123 | 342 ± 85 | 361 ± 137 | 364 ± 134 | 379 ± 155 | 402 ± 140 | 242 ± 74 |
| | MED | 262 ± 69 | 226 ± 58 | 94 ± 15 | 41 ± 11[a] | 94 ± 38 | 195 ± 54 | 544 ± 156 | 549 ± 153 | 636 ± 198[a] | 502 ± 158 | 235 ± 86 |
| | ATI50 | 260 ± 96 | 182 ± 72 | 58 ± 19 | 41 ± 14[a] | 110 ± 35 | 220 ± 59 | 506 ± 153 | 389 ± 120 | 416 ± 113 | 393 ± 115 | 247 ± 63 |
| | ATI100 | 281 ± 73 | 215 ± 79 | 173 ± 84 | 186 ± 64 | 186 ± 58 | 219 ± 63 | 379 ± 133 | 499 ± 160 | 433 ± 159 | 360 ± 98 | 236 ± 62 |
| | ATI300 | 273 ± 59 | 243 ± 49 | 148 ± 18 | 154 ± 46 | 241 ± 67 | 285 ± 64 | 337 ± 52 | 396 ± 113 | 353 ± 86 | 353 ± 73 | 240 ± 36 |
| | YOH50 | 233 ± 54 | 156 ± 29 | 62 ± 19[b] | 70 ± 25[a] | 196 ± 46 | 205 ± 54 | 232 ± 65 | 256 ± 41 | 310 ± 41 | 340 ± 78 | 270 ± 69 |
| | YOH100 | 280 ± 96 | 233 ± 53 | 103 ± 24 | 162 ± 56 | 358 ± 109 | 339 ± 95 | 434 ± 100 | 374 ± 67 | 362 ± 87 | 398 ± 67 | 246 ± 53 |
| | YOH300 | 241 ± 46 | 154 ± 32 | 70 ± 24 | 177 ± 86 | 233 ± 81 | 227 ± 59 | 230 ± 92 | 244 ± 98 | 256 ± 67 | 265 ± 85 | 253 ± 59 |

MED – medetomidine 20 μg/kg; ATI50 – medetomidine 20 μg/kg + atipamezole 50 μg/kg; ATI100 – medetomidine 20 μg/kg + atipamezole 100 μg/kg; ATI300 – medetomidine 20 μg/kg + atipamezole 300 μg/kg; YOH50 – medetomidine 20 μg/kg + yohimbine 50 μg/kg; YOH100 – medetomidine 20 μg/kg + yohimbine 100 μg/kg; YOH300 – medetomidine 20 μg/kg + yohimbine 300 μg/kg. Each value represent the mean ± SE (n = 5). Significantly different from the initial value (a: $P < 0.05$; b: $P<0.01$).

Table II. Plasma electrolytes concentration after administration of the test agents to 5 dogs

| Variables | Group | Time after administration (h) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.5 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 24 |
| Sodium (mmol/L) | Saline | 151 ± 27 | 151 ± 27 | 151 ± 27 | 151 ± 27 | 151 ± 27 | 151 ± 27 | 151 ± 27 | 151 ± 27 | 151 ± 27 | 151 ± 27 | 151 ± 27 |
| | MED | 152 ± 3 | 152 ± 4 | 154 ± 4 | 157 ± 3 | 160 ± 4[a] | 160 ± 3[a] | 158 ± 4 | 158 ± 3 | 155± 3 | 154 ± 3 | 152 ± 2 |
| | ATI50 | 152 ± 2 | 152 ± 1 | 156 ± 5 | 156 ± 2 | 155 ± 4 | 159 ± 1[a] | 156 ± 2 | 155 ± 1 | 155 ± 3 | 157± 5 | 152 ± 1 |
| | ATI100 | 152 ± 27 | 152 ± 27 | 152 ± 27 | 153 ± 27 | 154 ± 27 | 154 ± 27 | 153± 27 | 153 ± 28 | 153 ± 28 | 152± 28 | 151 ± 28 |
| | ATI300 | 151± 27 | 151± 27 | 153± 28 | 152± 27 | 152± 27 | 153± 27 | 153± 28 | 153± 28 | 154± 28 | 152± 27 | 151± 27 |
| | YOH50 | 152 ± 2 | 152 ± 2 | 155 ± 5 | 157 ± 1 | 156 ± 2 | 156 ± 2 | 155 ± 1 | 156 ± 3 | 155 ± 3 | 155 ± 4 | 152 ± 1 |
| | YOH100 | 151 ± 27 | 151 ± 27 | 152 ± 27 | 153 ± 27 | 154 ± 27 | 154 ± 27 | 153± 27 | 152 ± 27 | 153 ± 27 | 152± 27 | 151 ± 28 |
| | YOH300 | 151± 27 | 152± 27 | 153± 28 | 153± 27 | 152± 27 | 153± 27 | 152± 27 | 152± 27 | 152 ± 28 | 151± 27 | 152± 27 |
| Potassium (mmol/L) | Saline | 4.3 ± 0.8 | 4.3 ± 0.7 | 4.4 ± 0.8 | 4.3 ± 0.8 | 4.2 ± 0.7 | 4.3 ± 0.8 | 4.1 ± 0.7 | 4.0 ± 0.7 | 4.0 ± 0.7 | 4.0 ± 0.7 | 4.1 ± 0.7 |
| | MED | 4.6± 0.2 | 4.8± 0.1 | 5.1± 0.2 | 5.7± 0.3[a] | 5.4± 0.1[a] | 5.2± 0.1 | 4.9± 0.1 | 4.8± 0.1 | 4.8± 0.1 | 4.7± 0.1 | 4.4± 0.1 |
| | ATI50 | 4.4 ± 0.8 | 4.5 ± 0.8 | 5.0 ± 0.9 | 5.0 ± 0.9 | 4.9 ± 0.9 | 4.6 ± 0.8 | 4.5 ± 0.8 | 4.3 ± 0.8 | 4.2 ± 0.8 | 4.2 ± 0.8 | 4.3 ± 0.8 |
| | ATI100 | 4.5 ± 0.8 | 4.4 ± 0.8 | 4.9 ± 0.9 | 4.8 ± 0.9 | 4.7 ± 0.8 | 4.6 ± 0.8 | 4.4 ± 0.8 | 4.4 ± 0.8 | 4.2 ± 0.8 | 4.4 ± 0.8 | 4.3 ± 0.8 |
| | ATI300 | 4.5 ± 0.8 | 4.5 ± 0.8 | 4.6 ± 0.8 | 4.6 ± 0.8 | 4.5 ± 0.8 | 4.6 ± 0.8 | 4.5 ± 0.8 | 4.5 ± 0.8 | 4.6 ± 0.8 | 4.6 ± 0.8 | 4.4 ± 0.8 |
| | YOH50 | 4.5 ± 0.8 | 4.5 ± 0.8 | 4.7 ± 0.8 | 4.7 ± 0.8 | 4.7 ± 0.8 | 4.4 ± 0.8 | 4.1 ± 0.7 | 4.1 ± 0.7 | 4.0 ± 0.7 | 4.0 ± 0.7 | 4.1 ± 0.7 |
| | YOH100 | 4.4 ± 0.8 | 4.5 ± 0.8 | 4.6 ± 0.8 | 4.6 ± 0.8 | 4.6 ± 0.8 | 4.5 ± 0.8 | 4.3 ± 0.8 | 4.3 ± 0.8 | 4.1 ± 0.7 | 4.1± 0.7 | 4.4 ± 0.8 |
| | YOH300 | 4.5 ± 0.8 | 4.5 ± 0.8 | 4.4 ± 0.8 | 4.6 ± 0.8 | 4.5 ± 0.8 | 4.4 ± 0.8 | 4.3 ± 0.8 | 4.3 ± 0.8 | 4.4 ± 0.8 | 4.3 ± 0.8 | 4.3 ± 0.8 |
| Chloride (mmol/L) | Saline | 116 ± 21 | 114 ± 20 | 114 ± 20 | 114 ± 21 | 115 ± 20 | 115 ± 21 | 117 ± 21 | 116 ± 21 | 118 ± 21 | 117 ± 21 | 119 ± 21 |
| | MED | 118 ± 4 | 119 ± 4 | 126 ± 1 | 128 ± 5[a] | 127 ± 2 | 123 ± 2 | 122 ± 22 | 121 ± 3 | 121 ± 2 | 119 ± 3 | 121 ± 2 |
| | ATI50 | 118 ± 21 | 117 ± 21 | 117 ± 21 | 118 ± 21 | 118 ± 21 | 118 ± 21 | 122 ± 21 | 117 ± 22 | 121 ± 22 | 120 ± 22 | 119 ± 21 |
| | ATI100 | 117 ± 21 | 117 ± 21 | 118 ± 21 | 119 ± 22 | 119 ± 21 | 118 ± 21 | 119 ± 21 | 118 ± 21 | 119 ± 21 | 119 ± 22 | 118 ± 21 |
| | ATI300 | 117 ± 21 | 117 ± 21 | 117 ± 21 | 120 ± 21 | 119 ± 21 | 119 ± 21 | 118 ± 21 | 118 ± 21 | 117 ± 21 | 117 ± 21 | 117 ± 21 |
| | YOH50 | 117 ± 1 | 118 ± 1 | 120 ± 2 | 121 ± 2 | 122 ± 2 | 120 ± 1 | 120 ± 1 | 119 ± 2 | 119 ± 2 | 119 ± 1 | 120 ± 2 |
| | YOH100 | 116 ± 2 | 116 ± 2 | 115 ± 2 | 116 ± 2 | 117 ± 2 | 116 ± 2 | 116 ± 2 | 117 ± 2 | 117 ± 1 | 118 ± 2 | 118 ± 1 |
| | YOH300 | 116 ± 21 | 116 ± 21 | 116 ± 21 | 116 ± 21 | 119 ± 23 | 117 ± 21 | 118 ± 21 | 118 ± 21 | 118 ± 21 | 118 ± 21 | 120 ± 22 |

MED – medetomidine 20 μg/kg; ATI50 – medetomidine 20 μg/kg + atipamezole 50 μg/kg; ATI100 – medetomidine 20 μg/kg + atipamezole 100 μg/kg; ATI300 – medetomidine 20 μg/kg + atipamezole 300 μg/kg; YOH50 – medetomidine 20 μg/kg + yohimbine 50 μg/kg; YOH100 – medetomidine 20 μg/kg + yohimbine 100 μg/kg; YOH300 – medetomidine 20 μg/kg + yohimbine 300 μg/kg. Each value represent the mean ± SE (n = 5). Significantly different from the initial value (a: $P < 0.05$; b: $P<0.01$).

## Discussion

The results of this study demonstrated that both atipamezole and yohimbine exert antagonistic effects on medetomidine-induced diuresis in healthy dogs. Our study revealed that the anti-diuretic effect of yohimbine was not dose-dependent at the tested doses in contrast to atipamezole. To our best knowledge, it is the first outlining report that both atipamezole and yohimbine have potent anti-diuretic actions on medetomidine-induced diuresis in dogs, and atipamezole inhibits the diuresis dose-dependently in contrast to yohimbine. However, the low dose of 50 μg/kg yohimbine was more potent in inhibiting the diuresis when compared with the same dose of atipamezole. The inhibitory effects of yohimbine on $\alpha_2$-adrenoceptor agonist-induced diuresis have been reported in rats [11, 13, 25, 28]. However, there is no report regarding the anti-diuretic action of atipamezole. It has been expected that atipamezole will exert anti-diuretic action on medetomidine-induced diuresis more specifically and potentially than yohimbine, since $\alpha_2$-adrenoceptor selectivity and specificity of atipamezole are much higher than yohimbine, and atipamezole has also the imidazoline receptor affinity [1, 2, 19-21]. The precise mechanism of anti-diuretic action of these two agents is unknown. It would be nice if we could make two more control groups for only atipamezole and yohimbine to determine if these drugs are inhibiting the actions of medetomidine or if they have contrary actions of their own. As the main purpose of this study was to examine the antagonistic effects of atipamezole and yohimbine against medetomidine, we did not make the yohimbine or atipamezole alone group. We believe that atipamezole strongly antagonized the medetomidine-induced diuresis compared with yohimbine since medetomidine has also imidazoline receptor affinity [1, 20, 12]. On the other hand, yohimbine affects serotonergic, cholinergic, dopaminergic and GABA receptor-

related mechanism [25, 26]. These effects of yohimbine might be also involved in the mechanisms of the differences of anti-diuretic action between atipamezole and yohimbine. However, it is difficult to explain from this study.

In the present study, the decreases in urine specific gravity, urine osmolality and urine creatinine concentrations were almost simultaneous with the increase of urine volume in both MED-ATI and MED-YOH groups. Higher doses of both atipamezole and yohimbine fasten the recovery from the decreased urine specific gravity, creatinine and osmolality, indicating that higher doses of both agents strongly inhibited the diuresis induced by medetomidine.

Changes in urine and plasma electrolytes were also in correspondence with the anti-diuretic action of both atipamezole and yohimbine in the present study. Higher doses of both agents strongly prevented the increase of plasma electrolytes concentrations induced by medetomidine.

Our study revealed that both atipamezole and yohimbine reversed the medetomidine-inhibited plasma AVP and urine AVP excretion. The present results further revealed that, atipamezole dose-dependently reversed the medetomidine-inhibited plasma AVP as well as diuresis at the tested doses. Both reversals of medetomidine-inhibited AVP release and diuresis by atipamezole were correlated. We had chosen the time point 0.5 to 4 h to calculate total urine AVP excretion and linear regression analyses since diuretic effects persisted up to 4 h. In addition, 0.5 to 4 h time point had chosen for AUC data calculation and linear regression analyses of plasma AVP because peak diuresis occurred at 2 h after medetomidine administration. Elevation of plasma AVP increases water permeability in the collecting duct [50]. It is well known that AVP increases cellular cAMP, the second messenger that leads to an increase of water permeability in the inner medullary collecting duct of rats [50], and that

$\alpha_2$-adrenoceptor agonists inhibit vasopressin-stimulated cAMP formation and subsequently inhibit water permeability in rats [36]. In our study, it might be happened that atipamezole and yohimbine elevated plasma AVP by reversal action and that AVP increases cellular cAMP as well as an increase of water permeability in the collecting duct. Thereby, an increase of water absorption from the kidney might lead to less urine production and inhibited medetomidine-induced diuresis. The effects of AVP on water permeability in the kidney have been found to be mediated though regulation of aquaporin2 (AQP2) water channels [51]. The cAMP has been also found to be a second messenger in AQP2 gene transcription and translocation into the luminal membrane after stimulation of V2 receptor by vasopressin [52]. The $\alpha_2$-adrenoceptor agonist, clonidine stimulates the reduction of AQP2 in whole kidney as early as 1 h after administration [39]. In our study, atipamezole and yohimbine might also exert action on AQP2 water channels to antagonize the medetomidine-induced diuresis. On the other hand, atipamezole and yohimbine might antagonize the action of medetomidine-inhibited plasma AVP via CNS mechanism [2, 19, 23]. Therefore, the reversal effects of both atipamezole and yohimbine on medetomidine-inhibited plasma AVP might be in part influenced on their anti-diuretic actions. Our results further suggest that AVP partly plays a role in the diuretic effect of medetomidine [44]. Furthermore, we do not rule out the possibility of involvement of peripheral factors by which these antagonists exerted anti-diuretic action as suggested by earlier reports [11, 34]. However, it is difficult to explain the mechanism of anti-diuretic action of these agents from the present study.

This study revealed for the first time that atipamezole treatment increased plasma ANP release significantly and dose-dependently in contrast to yohimbine. Atipamezole potently and dose-dependently antagonized the medetomidine-induced diuresis in presence of

increased plasma ANP. On the other hand, all doses of yohimbine strongly antagonized the medetomidine-induced diuresis without the elevated plasma ANP at the tested doses. Therefore, ANP might have a weak diuretic action on medetomidine-induced diuresis and that atipamezole has an agonistic effect on ANP release. In addition, it is suggested that the level of ANP stimulated by atipamezole at the tested doses was not enough to induce the diuresis. The stimulation of plasma ANP release has been reported to be related with the atrial stretch due to hypertension [13-16, 28]. Atipamezole produces stronger and relatively longer hypertension [23-26], which may be a cause of the stimulation of ANP release by atipamezole treatment in this study.

In conclusion, both atipamezole and yohimbine had profound antagonistic effects on medetomidine-induced diuresis in healthy dogs. Although yohimbine did not dose-dependently inhibit diuretic action in contrast to atipamezole, it had potent inhibitory action at our tested doses. The results of the present study suggested that AVP in part plays a role in the anti-diuretic effects of both atipamezole and yohimbine. This study also demonstrated for the first time that atipamezole stimulates ANP release significantly. The differences in the mechanism of the anti-diuretic action of atipamezole and yohimbine might be due to their selectivity and specificity on the $\alpha_2$-adrenoceptor subtypes and/or imidazoline receptors. In addition, other cellular messengers, receptor subtypes, or AQP2 water channels might have biological roles in anti-diuretic action of atipamezole and yohimbine, since urine is the net products of multiple hemodynamic, neural, hormonal and local factors in the kidney. However, the precise mechanism can not be explained from this study. Both drugs can be used as antagonistic agents against medetomidine-induced diuresis in healthy dogs.

# Chapter 3

## Antagonistic effects of atipamezole and yohimbine on xylazine-induced diuresis in healthy dogs

## Introduction

Xylazine (2(2,6 dimethylphenylamino)-5,6-dihydro-4H-1,3 thiazine hydrochloride) is a potent $\alpha_2$-adrenoceptor agonist, clonidine analogue, and non-narcotic drug [54]. The $\alpha_2/\alpha_1$ receptor binding selectivity of xylazine is 160, whereas that of medetomidine, detomidine or clonidine is 1620, 260 or 220, respectively [55]. Xylazine is less lipophilic than medetomidine, detomidine or clonidine [55]. It acts on presynaptic and postsynaptic receptors of the central and peripheral nervous systems as an $\alpha_2$-adrenoceptor agonist, and inhibits the effects of postganglionic nerve stimulation. It is used primarily for sedation, anesthesia, analgesia and muscle relaxation in many species of small and large animals, often in combination with ketamine [54]. At the recommended dose rates, xylazine has considerable and variable pharmacodynamic effects [54]. Xylazine is known to induce diuresis in cattle, horses and rats [8, 9, 11, 56]. Recently, we have reported for the first time that xylazine induce a profound dose-related diuresis associated with changes in urine specific gravity, pH, creatinine values, and osmolality, sodium, potassium and chloride ions in both urine and plasma in healthy dogs [44]. In that study, we have also demonstrated that inspite of non-significant changes in urine and plasma AVP, xylazine had a profound diuretic effect in dogs. From our recent study, the mechanism of xylazine induced diuresis is unknown and the role of AVP is not clear. In addition, we revealed that xylazine slightly stimulate plasma ANP release at the early phase after administration. Previous studies have suggested that the decrease in plasma AVP played a partial role in the profound diuretic effect of xylazine though its action on $\alpha_2$-adrenoceptors [8-12, 56]. In our recent study [44], we suggested that ANP might partially influence on xylazine-induced diuresis in dogs, because it exerts a diuretic and natriuretic action on renal proximal tubules and inner medullary duct

cells of the kidney in rats [13, 28]. The regulation of water excretion has implications for a number of clinical situations. The $\alpha_2$-adrenoceptor antagonists, atipamezole and yohimbine, have been shown to reverse a variety of clinicophysiological effects produced by $\alpha_2$-adrenoceptor agonists [1-4, 19-27].

The $\alpha_2/\alpha_1$ selectivity of atipamezole and yohimbine are 8526/1 and 40/1, respectively [19, 23-26]. Atipamezole is a potent and highly specific antagonist of centrally and peripherally located $\alpha_2$-adrenoceptors compared with yohimbine [25]. The affinities of atipamezole and yohimbine are similar at the $\alpha_{2A}$-, $\alpha_{2B}$- and $\alpha_{2C}$-adrenoceptors, but differ by approximately 100-folds at the $\alpha_{2D}$-adrenoceptors [19]. In addition, yohimbine affects serotonergic, cholinergic, dopaminergic and GABA receptor-related mechanisms [26], whereas atipamezole lacks these receptor activities [25]. Furthermore, atipamezole has a similar structure to imidazoline, whereas yohimbine has no imidazoline receptor affinity [23-25]. These differences between atipamezole and yohimbine may influence on the antagonistic effects of xylazine-induced actions. The xylazine-induced diuresis during sedation or analgesia is beneficial for the animals. But in case of animals with urinary tract obstruction, dehydration or hypovolemia, may limit the use of xylazine. In such cases atipamezole or yohimbine may be used to reverse the diuretic action. Since both drugs are clinically used to reverse xylazine induced sedative and analgesic effects, if necessary [19, 23, 25]. It is reported that yohimbine reverses the diuretic effects of xylazine in rats [11, 34, 56]. However, to our best knowledge, there are no published reports on the antagonistic effects of atipamezole and yohimbine against xylazine-induced diuresis in dogs. In addition, there are no available data about the effects of atipamezole or yohimbine on the AVP and ANP changes after xylazine administrations in dogs. We hypothesize that the results of the present study

may be best interpreted against this background. The findings may have implications for the potential treatment of disorders associated with impaired regulation of water balance. This study aimed to investigate and compare the antagonistic effects of three different doses of either atipamezole or yohimbine on the diuresis induced by xylazine in healthy dogs. The variables examined were urine volume, specific gravity, pH, creatinine values, and osmolality, electrolytes and AVP values in both urine and plasma, and plasma ANP.

## Materials and methods

### Animals

Five adult male healthy beagle dogs, with a mean age of 5.8 (standard deviation 2.7) years old and mean body weight of 11.8 (1.1) kg were used. All the dogs were raised at the laboratory providing animal management facilities and fed a standard commercial dry canine food. Routine hematologic examination was done before the experiment; all values were within normal physiologic ranges. The study protocols were approved by the Animal Research Committee of Tottori University, Tottori, Japan.

### Experimental protocol

The experiment consists of 8 treatment groups. Five dogs were assigned to each of the 8 treatment groups in a randomized design at 1 week interval in the same dog. Each dog in a group was given an IM injection of 2.0 mL /head physiological saline solution as non-medicated control. The dogs in other groups received an IM 1st treatment of 2 mg/kg of xylazine hydrochloride (2% solution, Celactal®, Bayer, Tokyo, Japan) at the beginning of the experiment. This was followed 0.5 h later by a 2nd IM treatment of 0.5 mL /head physiological saline solution, 50, 100, and 300 μg/kg atipamezole hydrochloride (0.5% solution; Antisedan®, Meiji Seika, Tokyo, Japan); and 50, 100 and 300 μg/kg yohimbine

hydrochloride (Sigma Chemical, St. Louis, MO, USA). Yohimbine was dissolved in distilled water at the concentration of 0.5 mg/m*l*. The groups will be referred to as Saline, XYL, XYL-ATI 50, XYL-ATI 100, XYL-ATI 300, XYL-YOH 50, XYL-YOH 100 and XYL-YOH 300. As the $\alpha_2$-adrenoceptor agonists have been often used for an IM injection, this route was preferred in our study. The quadriceps muscle was used for injection site. Food was withheld for 12 h prior to drug injection. The dogs were not accessed to food and water during the experiment. After sample collection of 8 h, food and water were provided once, and again fasted for 12 h to collect the sample at 24 h in the next day. We did not measure the volume of urine voided by the dogs after urinary catheter removal and before placement in the following day, because we have observed that urine volume returns to baseline within 6 to 8 h after injection of xylazine in dogs during the trial experiment. The experiments were performed in a room with air temperature at 25°C.

**Sample collection**

A 6- or 8-Fr Silicon balloon catheter (All Silicon Foley Catheter, Cliny Medical Corp, Tokyo, Japan) was inserted prior to 1 h of the experiment to empty the bladder and for subsequent urine sampling. The catheter was withdrawn after sampling at 8 h. On next day at 22 h, the catheter was again inserted and the bladder was made empty. Subsequently, urine sample was collected at 24 h. Urine and blood samples were taken for the following 11 times: prior to injection of the agent (0), 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24 h after injection of xylazine. Blood samples (5.5 mL) were collected from the jugular vein by a 6 mL disposable syringe with a 21-gauze needle, at the same time points of urine samples were collected. An aliquot of 4.0 mL from each sample was mixed with ethylene diamine tetraacetic acid and aprotinin (Trasylol®, Bayer, Leverksen, Germany) for AVP and ANP measurements, and the remaining

1.5 mL was mixed with heparin for osmolality measurement. The blood samples were immediately centrifuged at 2,000 × g at 4 °C for 15 minutes, and the plasma was separated and kept at -40 °C for analysis. Urine samples were centrifuged at 2,000 × g for 5 minutes, and then the supernatant was collected and stored at -40 °C until analyzed.

### Analytical methods

Urine volume was measured at each time point by a measuring cylinder after collection from the urine bag. Urine specific gravity and pH were measured by a refractor photometer (Erma®, Tokyo, Japan) and pH meter (pH meter F-52®, Horiba Corp, California, USA), respectively. Urine creatinine concentrations were measured by creatinine assay kit (Wako Pure Chemical Industries Corp, Osaka, Japan) with Jaffe method using spectrophotometer. In both urine and plasma, osmolality and electrolytes were measured by using vapor pressure osmometer (VAPRO®, Wescor, Utah, USA) and Na-K-Cl ion-concentrations auto analyzer (DRI-CHEM800V®, Tokyo, Japan), respectively. Plasma AVP was extracted following the procedure for solid phase column extraction (Sep-Pak® Cartridges, Waters, Ireland). For the extraction, Sep-Pak C18 cartridges were attached with plastic 10 mL syringe and kept in a test tube rack. Each was washed with 10 mL methanol (100 %) and then washed two times with 10 mL ultra pure water. Then, plasma sample (0.5 mL) and 1 mL of 0.1 M hydrochloric acid were mixed and poured into each syringe. After dropping out the solution, the syringes were washed with 10 mL of 4 % acetic acid, and all water was taken out by using the plunger. Then, AVP was collected in tubes after putting 1 mL of 100% methanol into the syringe. Using nitrogen gas with solvent evaporation apparatus, all the AVP solution was desiccated and stored at -40 °C until analyzed. Buffered solution (0.5 mL) was added into the desiccated AVP tubes, and the tubes were shaked for 15 minutes using shaking apparatus before

measurement. Urine and plasma AVP concentrations were measured by a double antibody radioimmunoassay (RIA) technique with the use of commercially available AVP kit (Mitsubishi Chemical, Tokyo, Japan). The intra-assay coefficients of variation (CVs) were 10 % and the limits of detection and quantification were 0.063 to 8.0 pg/tube. ANP was also assayed by a double antibody RIA kit (HANP kit®, Eiken Chemical Company, Tokyo, Japan). The intra-assay CV was 15 %. The detection and quantification limits were 10 and 1280 pg/mL, respectively.

### Statistical analysis

All data obtained were analyzed together with Prism statistical software (Graph Pad Software; version 4, San Diego, California, USA). One-way analysis of variance for repeated measures was used to examine the time effect within each group and the group effect at each time point. When a significant difference was found, the Tukey test was used to compare the means. The area under the curve (AUC) was calculated for each biochemical variable. The AUC was measured by calculating the sum of the trapezoids formed by the data points. The AUC data were plotted against the doses of atipamezole or yohimbine, and simple linear regression analysis was applied. When a significant difference was found, the effect of the drug on the plasma level of the examined biochemical was claimed to be dose-related. Mean values are presented with standard error. The level of significance in all tests was set at $P < 0.05$.

## Results

For all the variables, there were no significant differences between groups at baseline (0 h). No significant changes of urine volume and other biochemical and hormonal variables were observed in the Saline-treated group. Xylazine increased urine production significantly

($P<0.01$; Figure 15A, 15B) from 1 to 3 h after injection. The diuretic effects of xylazine persisted approximately up to 4 h with a peak at 2 h after xylazine administration. While, comparing with the peak means value of urine volume at 2 h with the XYL group, it was recognized that both ATI and YOH groups significantly inhibited xylazine-induced diuresis (Figure 15A, 15B). The doses of ATI 300 and YOH 300 antagonized the XYL-induced diuresis effectively with almost similar potencies. The results of total urine volume data from 0.5 to 4 h revealed that all the tested doses of ATI and YOH had anti-diuretic action. Moreover, the linear regression of the total urine volume from 0.5 to 3 h was significant ($P<0.05$) in the XYL-ATI groups but not in the XYL-YOH groups ($P>0.05$; Figure 15C, 15D), indicating that atipamezole inhibited xylazine-induced diuresis dose-dependently in contrast to yohimbine at the tested doses. Similar results were observed with the linear regression of the total urine volume data from 0.5 to 2, 0.5 to 4, and 0.5 to 6 h.

Mean urine specific gravity increased slightly and gradually during 8 h in the Saline group. Xylazine decreased significantly urine specific gravity during 0.5 to 3 h. The XYL-ATI 50, 100 and XYL-YOH 50, 100 groups decreased significantly mean urine specific gravity when compared with their respective baseline values (Figure 16A, 16B). ATI 300 and YOH 300 did not significantly decrease mean specific gravity, although the values were lower compared with the Saline. These decreases in urine specific gravity were in correspondence with the decrease in urine volume in both XYL-ATI and XYL-YOH groups.

Mean urine pH decreased significantly only in the XYL, XYL-ATI and XYL-YOH 100 groups during 1 to 4 h after injection of the agents and then gradually returned to baseline values. Thereafter, the urine pH in all groups increased over the value in the Saline group during 6 to 8 h.

Urine creatinine concentration was increased gradually during 8 h in the Saline group, whereas it was decreased significantly in the XYL, XYL-ATI 50, 100 and XYL-YOH 50, 100 groups during 2 to 3 h (Figure 16C, 16D). The lowest mean concentration of urine creatinine was found at 2 h in the XYL-ATI 50, 100 and XYL-YOH 50, 100 groups. Both ATI and YOH fastened dose-dependently the recovery from the decrease of urine creatinine concentrations induced by XYL.

Mean urine osmolality in both XYL-ATI and XYL-YOH groups decreased significantly and similarly during 1 to 4 h compared to their respective baseline values (Figure 17A, 17B). Higher doses of both atipamezole and yohimbine reduced the decrease of mean urine osmolality induced by XYL. On the other hand, mean plasma osmolality in the XYL group significantly increased during 2 to 5 h compared to the baseline value (Figure 17C, 17D). Treatments with all doses of both ATI and YOH prevented dose-dependently the increase of plasma osmolality induced by XYL.

Urine AVP concentrations decreased significantly from 1 to 4 h in the XYL, XYL-ATI 50, 100 and XYL-YOH 50, 100 groups from 1 to 3 h compared to baseline values. Higher doses of both ATI and YOH prevented the decrease in urine AVP concentrations induced by XYL (Figure 18A, 18B). The decrease in urine AVP concentrations returned to baseline in a dose-dependent manner in both ATI and YOH groups. Actual amounts of urine AVP excretion from 0.5 to 4 h did not significantly differ between XYL and Saline groups (Figure 18C, 18D). There were no significant differences on actual amounts of urine AVP excretion in both ATI and YOH groups compared with XYL group (Figure 18C, 18D). Similar results were obtained with the total urine AVP excretion from 0.5 to 2 h and 0.5 to 3 h.

Plasma AVP concentrations in the XYL group were gradually increased from 0.5 to 8 h

compared to baseline, and significantly increased at 5 and 6 h (Figure 19A, 19B). In the XYL-ATI groups, the plasma AVP concentrations tended to be lower during 0.5 to 5 h compared with the XYL group. The plasma AVP was increased at 2 and 3 h in the XYL-YOH 300 and at 4 h in the XYL-YOH 50 groups (Figure 19B). The AUC data of plasma AVP from 0.5 to 3 h in the XYL group were not significantly differ from those in the Saline group. The mean of AUC data of plasma AVP from 0.5 to 3 h was lower in all the XYL-ATI groups compared with the XYL group but not significantly (Figure 19C). In the YOH treated groups, the mean of AUC of plasma AVP value was higher only in the YOH 300 group compared with the XYL group but not significantly (Figure 19D). Similar results in the AUC data of plasma AVP were obtained from 0.5 to 2 h and 0.5 to 4 h.

Plasma ANP concentrations tended to be increased at 1 and 2 h only in the XYL-ATI 300 group, but were not significantly altered in all of the groups (Figure 20A, 20B). The AUC data of plasma ANP from 1 to 2 h also revealed that either ATI or YOH did not significantly increase ANP release (Figure 20C, 20D). Similar results were obtained with the linear regression of AUC data of plasma ANP from 1 to 3 h and 1 to 4 h.

In the XYL group, the mean concentrations of urine sodium, potassium and chloride were lower during 1 to 4 h compared with the baseline values, and then increased over baseline values at 5 to 8 h. In the XYL-ATI and XYL-YOH groups, higher doses of ATI or YOH prevented the decreases in the urine concentrations of sodium, potassium and chloride induced by XYL (Figure 21A, 21B, 21C, 21D, 21E and 21F). Treatments with higher doses of both ATI and YOH did not significantly change urine concentrations of sodium, potassium and chloride during 24 h after administration of XYL. Total amounts of excreted urine sodium, potassium and chloride did not significantly change during 1 to 3 h in both XYL-ATI

and XYL-YOH groups compared to the XYL group and Saline group, respectively.

Plasma sodium and chloride concentrations were not significantly changed in all the groups. Both ATI and YOH treatments prevented the increase of plasma potassium concentration induced by XYL (Figure 22A, 22B).

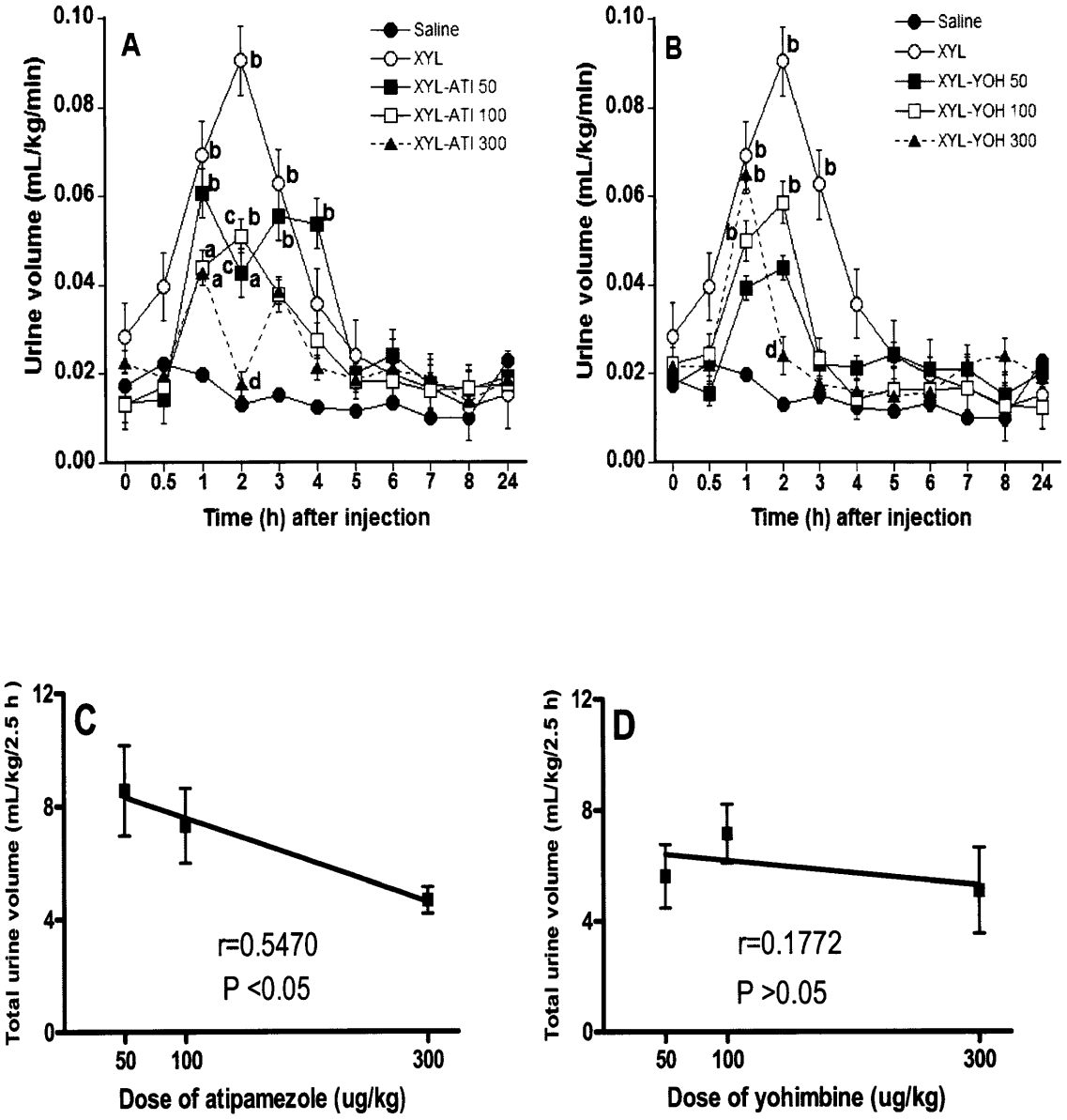


Figure 15. Urine volume (XYL-ATI, μg/kg, A; XYL-YOH, μg/kg, B) and simple linear regression of total urine volume (XYL-ATI, μg/kg, C; XYL-YOH, μg/kg, D) during 0.5 to 3 hrs after the administration of xylazine followed by atipamezole and yohimbine in dogs. Each point and vertical bar represent the mean and standard error (n=5). Significantly different from the 0 h value (a: $P<0.05$, b: $P<0.01$); significantly different from XYL group (c: $P<0.05$, d: $P<0.01$).

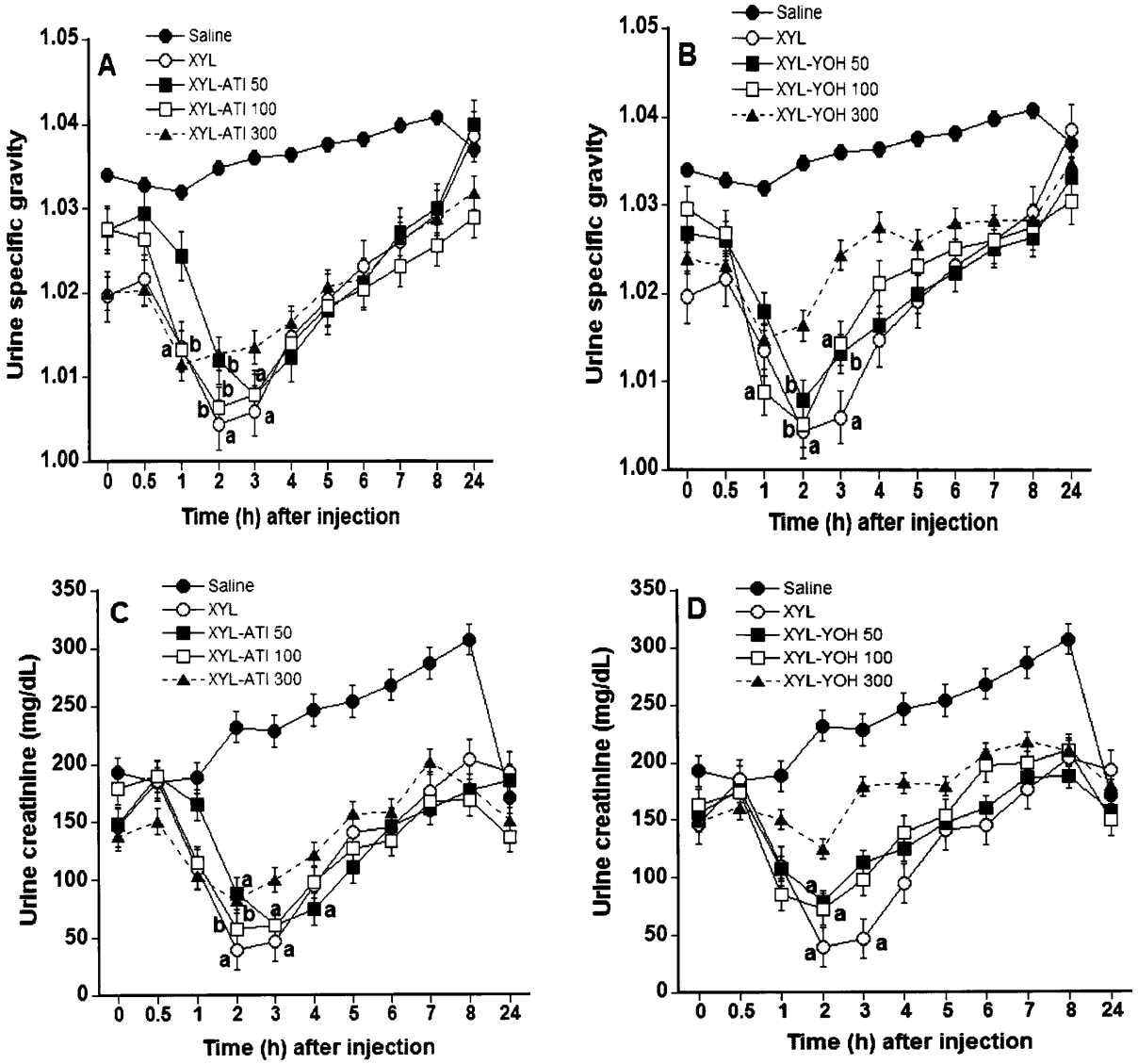


Figure 16. Urine specific gravity (XYL-ATI, A; XYL-YOH, B) and creatinine concentrations (XYL-ATI, C; XYL-YOH, D) after the administrations of xylazine followed by atipamezole and yohimbine in dogs. Meanings of points, bars, "a" or "b" as for Figure 15.

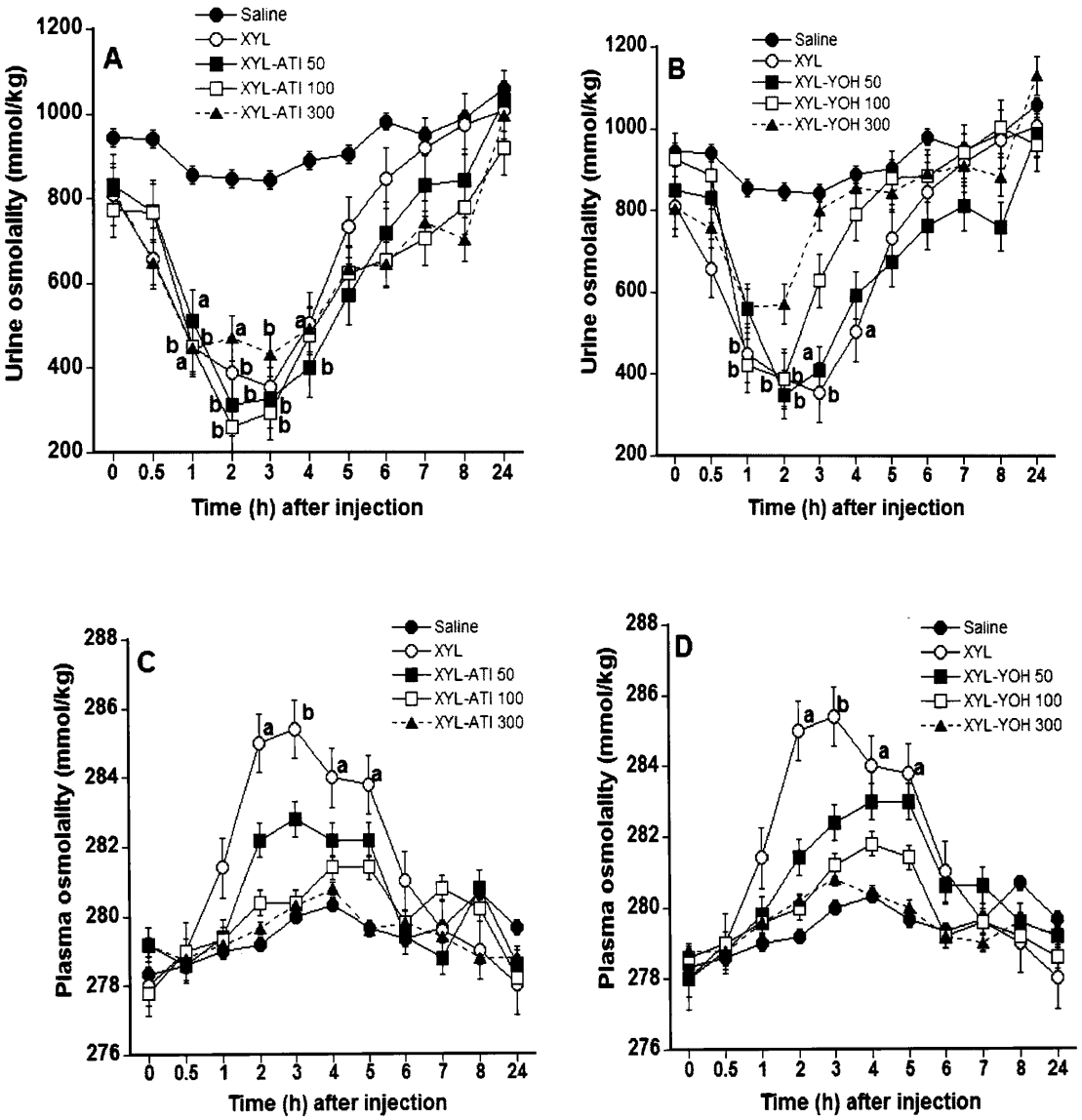


Figure 17. Urine osmolality (XYL-ATI, μg/kg, A; XYL-YOH μg/kg, B) and plasma osmolality (XYL-ATI, μg/kg, C; XYL-YOH, μg/kg, D) after the administrations of xylazine followed by atipamezole and yohimbine in dogs. Meanings of points, bars, "a" or "b" as for Figure 15.

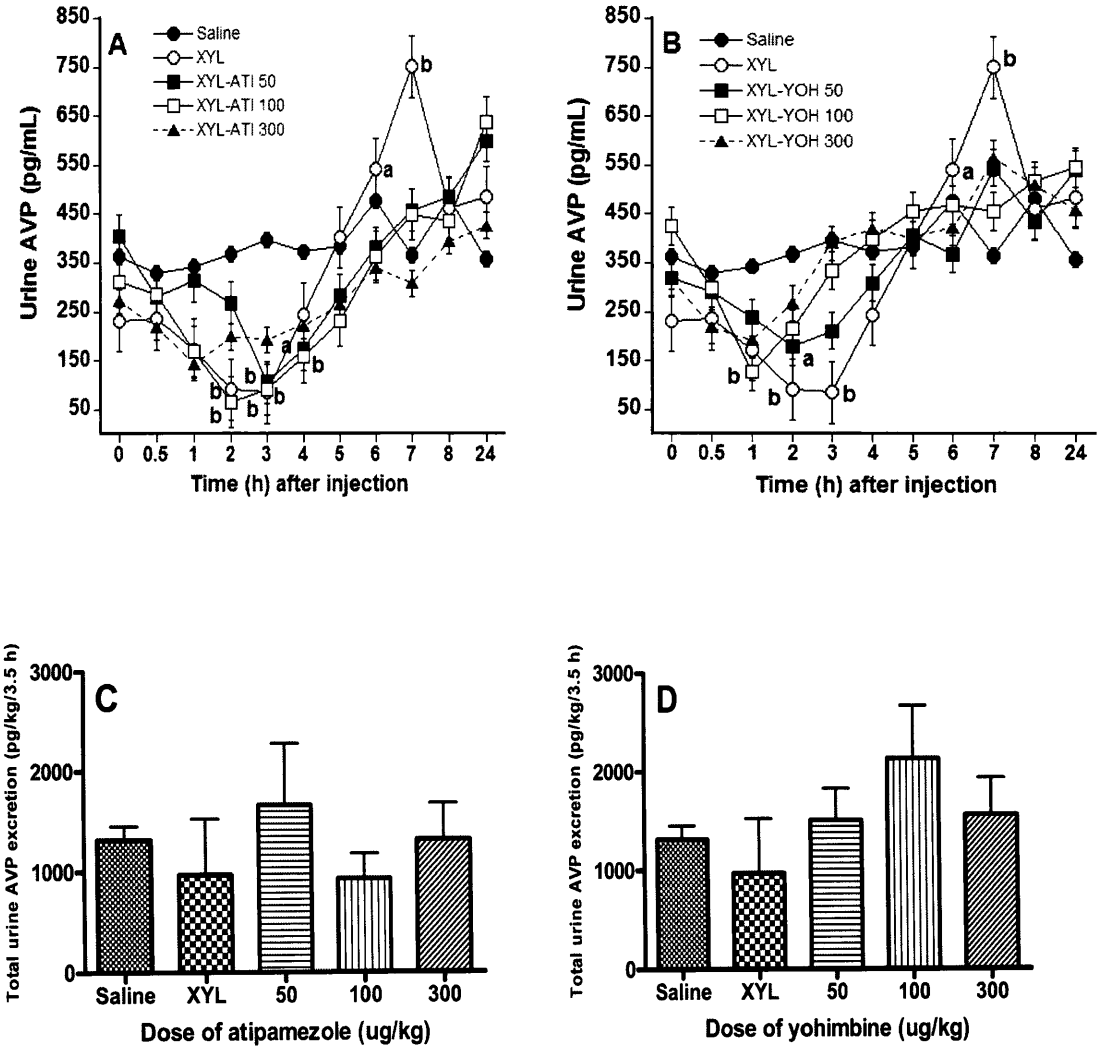


Figure 18. Urine AVP concentrations (XYL-ATI μg/kg, A; XYL-YOH, μg/kg, B) and total urine AVP excretion (XYL-ATI μg/kg, C; XYL-YOH, μg/kg, D) during 0.5 to 4 hrs after the administrations of xylazine followed by atipamezole and yohimbine in dogs. Meanings of points, bars, "a" or "b" as for Figure 15.

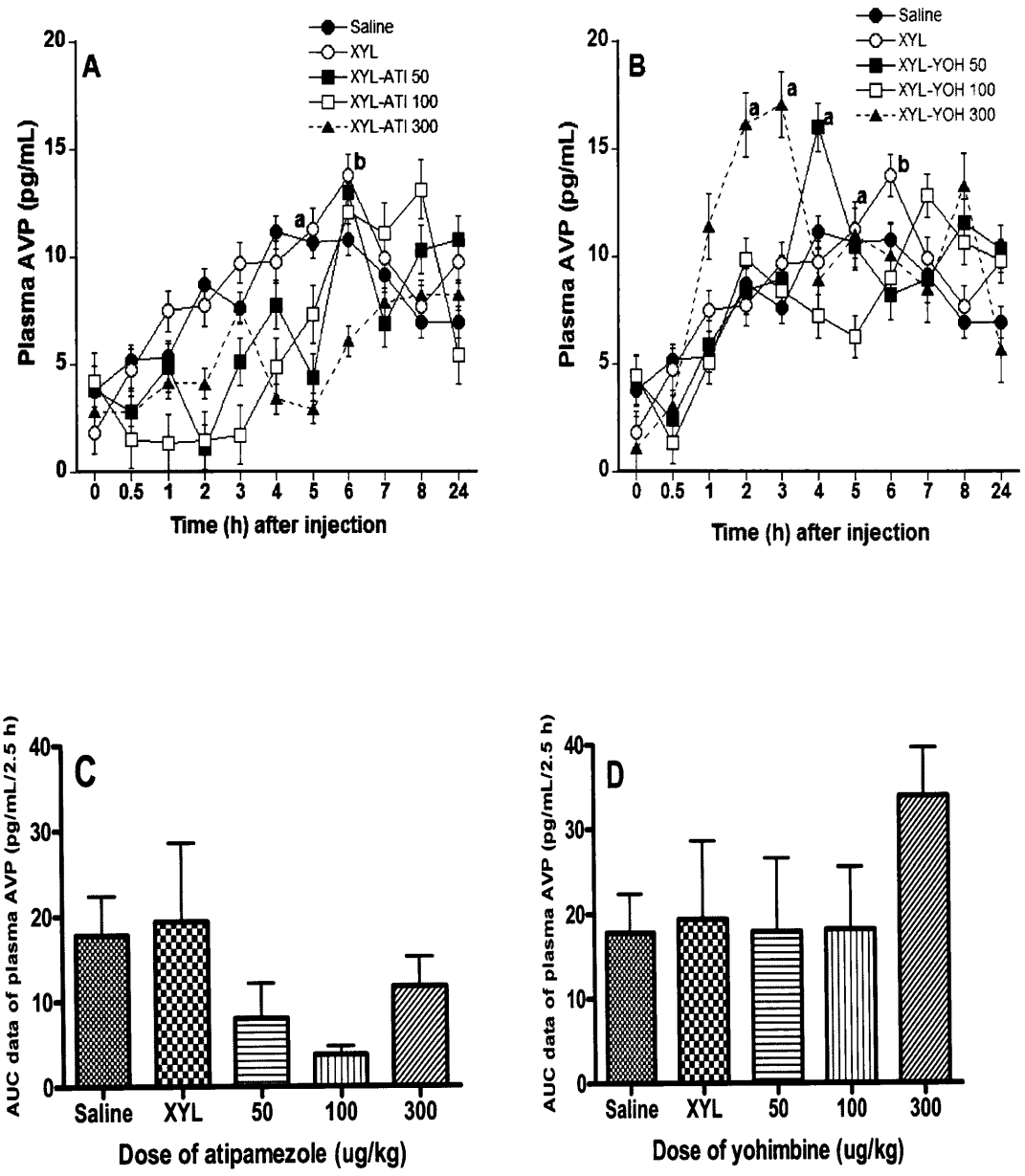


Figure 19. Plasma AVP concentrations (XYL-ATI μg/kg, A; XYL-YOH, μg/kg, B) and AUC data of plasma AVP during 0.5 to 3 hrs (XYL-ATI μg/kg, C; XYL-YOH, μg/kg, D) after the administrations of xylazine followed by atipamezole and yohimbine in dogs. Meanings of points, bars, and "a" or "b" as for Figure 15.

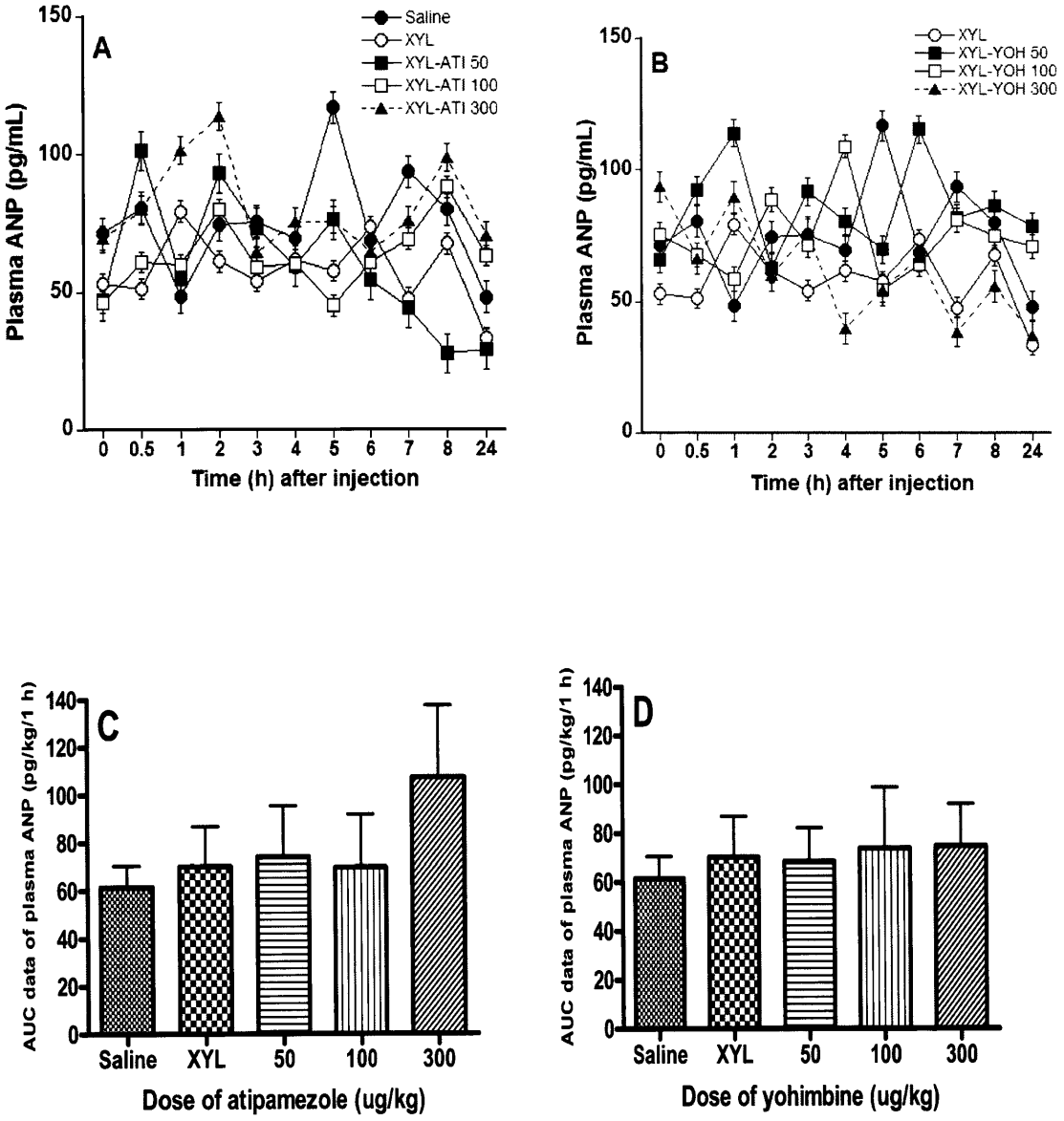


Figure 20. Plasma ANP concentrations (XYL-ATI μg/kg, A; XYL-YOH, μg/kg, B) and AUC data of plasma ANP (XYL-ATI μg/kg, C; XYL-YOH, μg/kg, D) during 1 to 2 hrs after the administrations of xylazine followed by atipamezole and yohimbine in dogs. Meanings of points, bars, and "a" or "b" as for Figure 15.

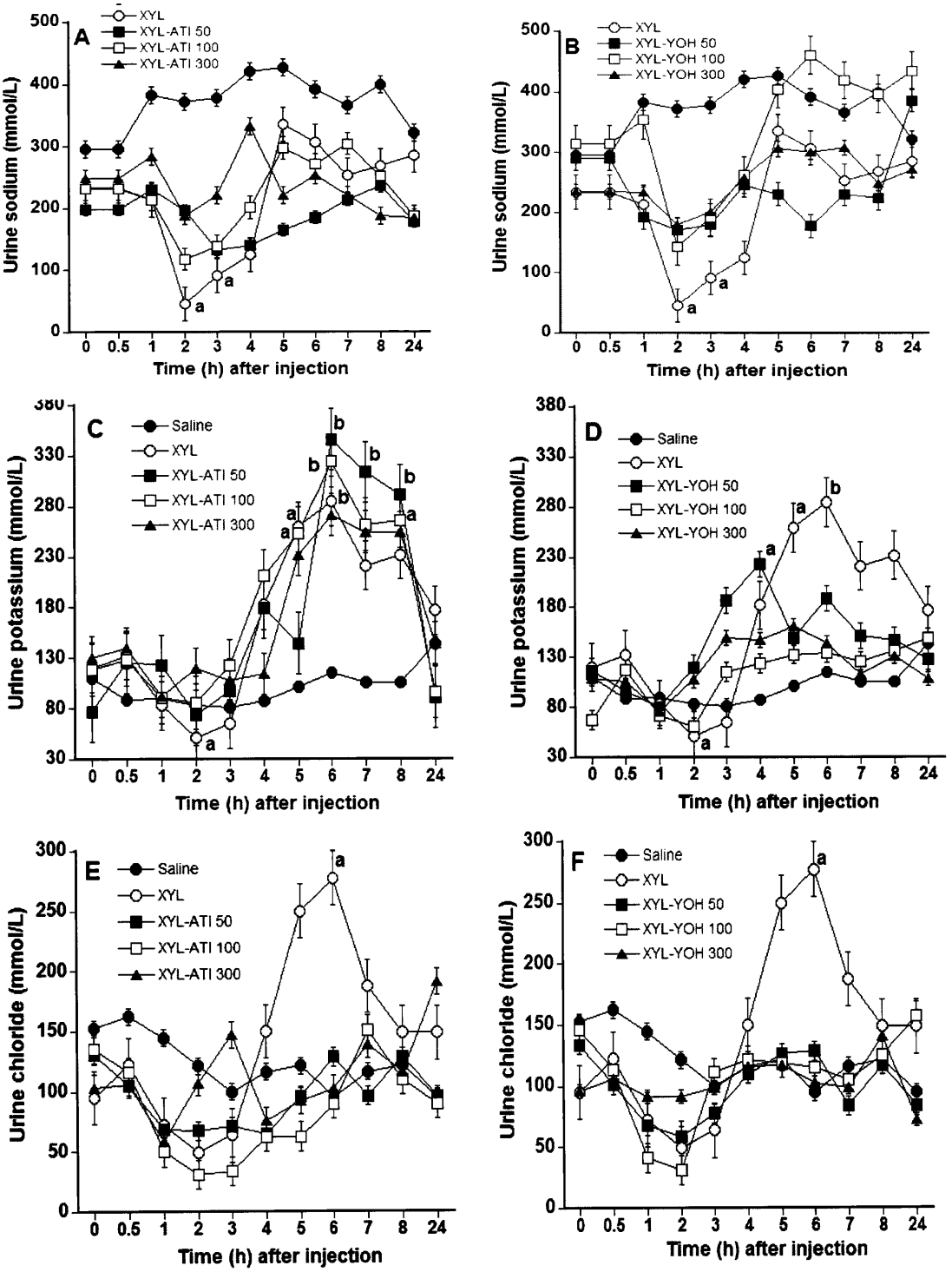


Figure 21. Urine electrolytes concentrations after the administrations of xylazine followed by atipamezole (XYL-ATI, μg/kg, A: sodium, C: potassium, E: chloride) and yohimbine (XYL-YOH, μg/kg, B: sodium, D: potassium, F: chloride) in dogs. Meanings of points, bars, and "a" or "b" as for Figure 15.

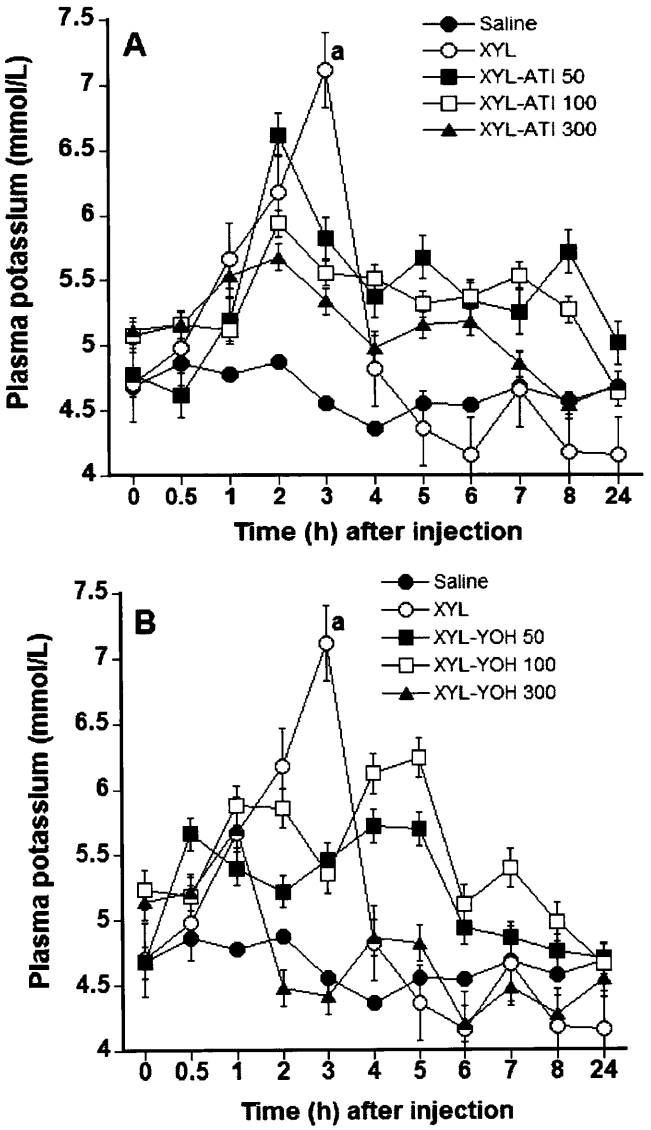


Figure 22. Plasma potassium concentrations after the administrations of xylazine followed by atipamezole (XYL-ATI, μg/kg, A) and yohimbine (XYL-YOH, μg/kg, B) in dogs. Meanings of points, bars, and "a" or "b" as for Figure 15.

## Discussion

The results of this study demonstrated that both atipamezole and yohimbine have antagonistic effects on xylazine-induced diuresis in healthy dogs. Most importantly, both antagonistic agents inhibited xylazine-induced diuresis without significantly altering the plasma AVP and ANP profiles. Furthermore, our study revealed that the anti-diuretic effect of yohimbine was not dose-dependent at the tested doses in contrast to atipamezole. To our best knowledge, it is the first outlining report that both atipamezole and yohimbine have potent anti-diuretic actions on xylazine-induced diuresis in dogs, and atipamezole inhibits the diuresis dose-dependently in contrast to yohimbine. It was also revealed from this study that, although anti-diuretic effect of yohimbine was not dose-dependent at the tested doses, the low dose of 50 μg/kg yohimbine was more potent in inhibiting the diuresis compared with the same dose of atipamezole. The inhibitory effects of yohimbine on $\alpha_2$-adrenoceptor agonist-induced diuresis have been reported in rats [11, 28, 34, 56]. The anti-diuretic effects of yohimbine in dogs in this study are in agreement with the previous results of xylazine-induced diuresis in rats. However, there is no report regarding the anti-diuretic action of atipamezole. It has been expected that atipamezole exerted anti-diuretic action on xylazine-induced diuresis more specifically and potentially than yohimbine, since $\alpha_2$-adrenoceptor selectivity and specificity of atipamezole are much higher than yohimbine [15, 19, 23-26]. Although the precise mechanism of the differences in anti-diuretic actions of these two agents is unknown, these effects would be mediated by the blockade of $\alpha_2$-adrenoceptors. On the other hand, yohimbine affects serotonergic, cholinergic, dopaminergic and GABA receptor-related mechanism [19, 26]. These effects of yohimbine might also be the reasons in the mechanisms of the differences of anti-diuretic action between atipamezole and yohimbine. However, it is difficult to explain

from this study.

In the present study, the decreases in urine specific gravity, urine osmolality and urine creatinine concentrations were almost simultaneous with the increase of urine volume in both XYL-ATI and XYL-YOH groups. Higher doses of both atipamezole and yohimbine tended to fasten the recovery from the decreased urine specific gravity, creatinine and osmolality, indicating that higher doses of both agents strongly inhibit the diuresis induced by xylazine. Changes in urine and plasma electrolytes were also in correspondence with the anti-diuretic action of both atipamezole and yohimbine in the present study. Higher doses of both agents prevented the increase of plasma potassium concentration induced by xylazine, which are in agreement with the earlier reports in rats and sheep [11, 19, 28, 56].

Our study revealed that both atipamezole and yohimbine reversed the xylazine- inhibited urine AVP concentrations. Importantly, XYL itself did not significantly change plasma AVP concentrations except for 6 h compared with the Saline group. In addition, the AUC data of XYL-ATI groups did not significantly differ from that of the XYL group. The present results further revealed that, atipamezole effectively reversed the xylazine-induced diuresis dose-dependently at the tested doses without significantly affecting the plasma AVP. On the other hand, except for YOH 300, yohimbine antagonized the diuretic action of xylazine without significantly altering the change of plasma AVP induced by xylazine. The anti-diuretic actions of either atipamezole or yohimbine were not correlated with the effect on AVP release. Elevation of plasma AVP increases water permeability in the collecting duct [36]. It is well known that AVP increases cellular cAMP, the second messenger that leads to an increase of water permeability in the inner medullary collecting duct of rats [11, 34, 36,] and that $\alpha_2$-adrenoceptors inhibit vasopressin-stimulated cAMP formation and subsequently inhibit water permeability in rats [34, 36, 51, 57]. From our study, it is not clear that how atipamezole and yohimbine exert anti-diuretic actions

without much affecting plasma AVP. However, it is difficult to explain the mechanism of anti-diuretic action of these agents from the present study.

Importantly, atipamezole effectively and dose-dependently and yohimbine strongly antagonized the xylazine-induced diuresis without significantly affecting plasma AVP and ANP at the tested doses. Our previous study has also demonstrated that xylazine induce a profound diuresis without altering the plasma AVP as well as plasma ANP levels [44]. Our previous results [44] and this study suggest that the higher dose of atipamezole has an agonistic-like effect on ANP release. However, it is suggested that the level of ANP stimulated by atipamezole at the tested doses is not enough to induce the diuresis. The stimulation of plasma ANP release has been reported to be related with the atrial stretch due to hypertension [13, 23, 28]. Atipamezole produces stronger and relatively longer hypertension [23, 25, 26,]. Moreover, the $\alpha_2$-adrenoceptor selectivity of yohimbine is 100-folds lower at $\alpha_{2D}$ subtypes compared with atipamezole, and yohimbine has no imidazoline receptor affinity [23, 26, 55, 57]. Therefore, we also suggest that yohimbine does not stimulate ANP release.

In conclusion, both atipamezole and yohimbine had profound anti-diuretic effects on xylazine-induced diuresis without altering the hormonal profile in healthy dogs. Although yohimbine did not dose-dependently inhibit the diuretic action in contrast to atipamezole, it had a potent inhibitory action at our tested doses. The anti-diuretic effects of both atipamezole and yohimbine on xylazine-induced diuresis mainly may be due to the blockade of $\alpha_2$-adrenoceptors, but other factors may be involved. The differences in the mechanism of the anti-diuretic action of atipamezole and yohimbine might be due to their selectivity and specificity on the $\alpha_2$-adrenoceptor subtypes and/or imidazoline receptors. Both drugs can be used as antagonistic agents against xylazine-induced diuresis in healthy dogs.

# General Conclusion

In chapter 1, both medetomidine and xylazine dose-dependently increased urine production up to 4 h. The dose-dependency of diuretic effect of medetomidine compared to xylazine was greatly lower at tested doses. This is our first outlining report of dose-dependent diuretic action of these agents in dogs. In addition, the profound diuretic action of xylazine in healthy dogs was first demonstrated from this study. Urine specific gravity, pH, osmolality, creatinine, sodium, potassium, chloride and AVP concentrations were decreased dose-dependently in both groups. Plasma osmolality, sodium, potassium and chloride concentrations were increased significantly in both groups. Total amounts of urine AVP excretion were significantly decreased by higher doses of medetomidine but not by xylazine. In addition, higher doses of medetomidine decreased significantly plasma AVP values, whereas xylazine did not significantly decrease. The results of both urine and plasma AVP, suggest that AVP alone may not be responsible for the dose-dependent diuretic effects of both medetomidine and xylazine. The present study has also demonstrated for the first time that medetomidine stimulate ANP release with greater potency in comparison with xylazine, which partially may influence diuresis. Medetomidine and xylazine may have differences in the mechanism of the diuresis, because the diuresis is the net products of multiple hemodynamic, neural, hormonal and local factors in kidney, and there are definitely differences between medetomidine and xylazine in the selectivity and specificity on $\alpha_2$-adrenoceptors and/or imidazoline receptors. Both drugs can be used as effective diuretic agents accompanied by sedation.

In chapter 2, the results of the study revealed that both atipamezole and yohimbine

inhibited medetomidine-induced diuretic and hormonal effects in dogs, and that anti-diuretic action of yohimbine was more potent, but not dose-dependent in contrast with atipamezole. Although yohimbine did not dose-dependently inhibit diuretic action in contrast to atipamezole, it had potent inhibitory action at our tested doses. This is the first outlining report on the anti-diuretic action of atipamezole and yohimbine on medetomidine-induced diuresis in healthy dogs. Medetomidine-induced decreases in urine specific gravity, pH, osmolality, creatinine, sodium, potassium, chloride and AVP concentrations were inhibited by both atipamezole and yohimbine. Atipamezole and yohimbine reversed medetomidine-induced increase in plasma sodium, potassium and chloride. Medetomidine-induced decreases in plasma AVP were reversed by both atipamezole and yohimbine. From this study it is suggested that AVP in part plays a role in the anti-diuretic effects of both atipamezole and yohimbine. This study also demonstrated for the first time that atipamezole stimulates ANP release significantly. The differences in the mechanism of the anti-diuretic action of atipamezole and yohimbine might be due to their selectivity and specificity on the $\alpha_2$-adrenoceptor subtypes and/or imidazoline receptors. In addition, other cellular messengers, receptor subtypes, or AQP2 water channels might have biological roles in anti-diuretic action of atipamezole and yohimbine. Both drugs can be used as antagonistic agents against medetomidine-induced diuresis in healthy dogs.

In chapter 3, both atipamezole and yohimbine had shown profound anti-diuretic effects on xylazine-induced diuresis without altering the hormonal profile in healthy dogs. Although yohimbine did not dose-dependently inhibit the diuretic action in contrast to atipamezole, it had a potent inhibitory action at our tested doses. Both atipamezole and yohimbine reversed the decreases in urine specific gravity, pH, osmolality, creatinine, sodium and chloride concentrations, and the increase in plasma potassium concentration

induced by xylazine. Higher dose of atipamezole tended to increase plasma ANP concentration.

The anti-diuretic effects of both atipamezole and yohimbine on xylazine-induced diuresis mainly may be to the blockade of $\alpha_2$-adrenoceptors, but other factors may be involved. The differences in the mechanism of the anti-diuretic action of atipamezole and yohimbine might be due to their selectivity and specificity on the $\alpha_2$-adrenoceptor subtypes and/or imidazoline receptors. In addition, other cellular messengers, receptor subtypes, or AQP2 water channels might have biological roles in anti-diuretic action of atipamezole and yohimbine. Both drugs can be used as antagonistic agents against xylazine-induced diuresis in healthy dogs.

# Abstract

In chapter 1, the study was aimed to investigate and compare the effects of medetomidine and xylazine on diuretic and hormonal variables in blood plasma and urine of healthy dogs. Five dogs were used randomly in each of 11 groups, which were injected intramuscularly with physiological saline (control), 5, 10, 20, 40 and 80 μg/kg medetomidine and 0.25, 0.5, 1, 2 and 4 mg/kg xylazine. Urine and blood samples were taken for 11 times during 24 h. Both medetomidine and xylazine dose-dependently increased urine production up to 4 h. The dose-dependency of diuretic effect of medetomidine compared to xylazine was greatly lower at tested doses. Urine specific gravity, pH, osmolality, creatinine, sodium, potassium, chloride and AVP concentrations were decreased dose-dependently in both groups. Plasma osmolality, sodium, potassium and chloride concentrations were increased significantly in both groups. Total amounts of urine AVP excretion were significantly decreased by higher doses of medetomidine but not by xylazine. In addition, higher doses of medetomidine decreased significantly plasma AVP values, whereas xylazine did not significantly decrease. Medetomidine increased plasma ANP levels significantly with greater potency than xylazine. The results revealed that both medetomidine and xylazine induce a profound diuresis, but medetomidine has a lower dose-dependency in comparison with xylazine. Although changes of plasma AVP and ANP partially might influence on diuresis induced by medetomidine, other factors may be involved in the mechanism of diuretic responses of both drugs. Both agents can be used clinically as transient but effective diuretic agents accompanied by sedation.

In chapter 2, the study was aimed to investigate and compare the antagonistic effects of atipamezole and yohimbine on medetomidine-induced diuresis in healthy dogs. Five dogs

were used repeatedly in each of 8 groups. One group was not medicated. Dogs in other groups received 20 μg/kg medetomidine intramuscularly and followed 0.5 h later by a treatment of saline (control), 50, 100, or 300 μg/kg atipamezole, or 50, 100, or 300 μg/kg yohimbine intramuscularly. Urine and blood samples were taken for 11 times during 24 h. Urine volume, pH, specific gravity and creatinine values, and osmolality, electrolytes and AVP values in both urine and plasma, and plasma ANP were measured. Both atipamezole and yohimbine antagonized diuretic effect of medetomidine. Medetomidine-induced decreases in urine specific gravity, pH, osmolality, creatinine, sodium, potassium, chloride and AVP concentrations were inhibited by both atipamezole and yohimbine. Atipamezole and yohimbine reversed medetomidine-induced increase in plasma sodium, potassium and chloride. Medetomidine-induced decreases in plasma AVP were reversed by both atipamezole and yohimbine. Atipamezole significantly stimulated ANP release. The results revealed that both atipamezole and yohimbine inhibited medetomidine-induced diuretic and hormonal effects in dogs, and that anti-diuretic action of yohimbine was more potent, but not dose-dependent in contrast with atipamezole. This may not be due only to actions mediated by $\alpha_2$-adrenoceptors.

In chapter 3, the study was aimed to investigate and compare the antagonistic effects of atipamezole and yohimbine on xylazine-induced diuresis in healthy dogs. Five healthy male beagle dogs were assigned to each of the 8 treatment groups in a randomized design at 1 week interval in the same dog. One group was not medicated. Dogs in other groups received 2 mg/kg xylazine intramuscularly (IM) and followed 0.5 h later by a treatment of saline (control), 50, 100, or 300 μg/kg of each atipamezole or yohimbine IM. Urine and blood samples were taken for 11 times during 24 h. Urine volume, specific gravity, pH and creatinine values, and osmolality, electrolytes and AVP values in both urine and plasma,

and plasma ANP were measured. Both atipamezole and yohimbine antagonized xylazine-induced diuresis. This reversal effect of yohimbine was more potent, but not dose-dependent at the tested doses, in contrast with atipamezole. Both atipamezole and yohimbine with similar potencies reversed the decreases in urine specific gravity, pH, osmolality, creatinine, sodium and chloride concentrations, and the increase in plasma potassium concentration induced by xylazine. Both atipamezole and yohimbine inhibited xylazine-induced diuresis without significantly altering the hormonal profile in dogs. A higher dose of atipamezole tended to increase plasma ANP concentration. This may not be due only to actions mediated by $\alpha_2$-adrenoceptors. Both drugs can be used as antagonistic agents against xylazine-induced diuresis in healthy dogs.

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