Diuretic Effects of α_2-Adrenoceptor Agonists and Their Antagonism by Antagonists in Cats

ネコにおける α_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

Yusuke MURAHATA

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Contents

General Introduction

The α-adrenoceptors are membrane receptors located in both neuronal and non-neuronal tissue. They are responsible for mediating responses to the endogenous catecholamines noradrenaline and adrenaline, and mediate most excitatory functions [7]. The α-adrenoceptors were in turn differentiated in the 1970s into α_1- and α_2-adrenoceptors.The α_1-and α_2-adrenoceptor subtypes were defined pharmacologically by functional and binding studies, and molecular biology. Characterization of both α-adrenoceptors on a pharmacological basis followed the discovery of selective antagonists, prazosin being more potent at α_1-adrenoceptors and yohimbine being more potent at α_2-adrenoceptors [7, 21]. The α_2-adrenoceptors have been classified into 3 subtypes (α_{2A}, α_{2B}, and α_{2C}) based on the differences in drug affinity [4]. The α_{2D}-adrenoceptors in cattle and rodents are considered as a species homologue of the α_{2A}-adrenoceptors [2]. All the subtypes produce cellular action by signaling through G-protein coupled receptors that act pre or post and extrasynaptically in different tissues [18, 21]. The heterogeneity in α_2-adrenoceptor subtype, density and location in animals and humans has led to considerable differences in drug doses and overall effects of α_2-agonists in various species [11]. Furthermore, several α_2- adrenoceptor agonists have strong affinity to nonadrenergic imidazoline preferring receptor sites, because these compounds have an imidazole ring in their structure. It is possible that some of the effects of α_2- adrenoceptor agonists are mediated by imidazoline receptors [18, 21, 31].

The α_2-adrenoceptor agonists medetomidine and xylazine are used in veterinary medicine to induce reliable and dose-dependent sedation, analgesia, and muscle relaxation [24]. Although

both drugs are used similarly in practice, there are differences between the 2 drugs. Medetomidine is a more potent, selective, and specific α_2-adrenoceptor agonist than is xylazine. The ratio of α_2-adrenoceptor selectivity to α_1-adrenoceptor selectivity of medetomidine (1,620:1) is approximately 10-fold as great as that of xylazine (160:1) [55]. In addition, in contrast to xylazine, medetomidine contains an imidazole ring that has an affinity for imidazoline receptors [31].

The α_2-adrenoceptor agonists can induce profound diuresis in several species. It has been suggested that several factors are involved in the mechanism of this diuresis. These factors included inhibition of plasma arginine vasopressin (AVP) secretion from the pituitary gland [5, 36], inhibition of the ability of AVP-induced cyclic adenosine monophosphate (cAMP) formation in the kidneys [9], redistribution of the aquaporin-2 water channel [17], inhibition of renin release [43], increase in plasma atrial natriuretic peptide concentrations [37], inhibition of renal sympathetic activity [26], osmotic diuresis attributable to hyperglycemia and glucosuria as a result of the inhibition of insulin release [48], and inhibition of tubular sodium reabsorption [10]. However, the exact mechanism of the diuretic effect of α_2-adrenoceptor agonists is still unknown, and may differ depending on the particular animal species [9]. Given this effect, medetomidine and xylazine should be used with discretion in hypovolemic or dehydrated animals and avoided in those with urinary tract obstruction. Also, the increase in urine flow must be considered when making decisions regarding anesthetic management [58]. Although a number of previous studies described the diuretic effects of medetomidine or xylazine in several animal species, there are no published reports on the diuretic effects of these drugs in cats.

The α_2-adrenoceptor antagonists, atipamezole and yohimbine, have been clinically used to reverse the sedative and analgesic effects of α_2-adrenoceptor agonists [24, 40]. The α_2/α_1

adrenoceptor selectivity ratios of atipamezole and yohimbine are 8,526:1 and 40:1, respectively [56]. The affinities of atipamezole and yohimbine are similar for the α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors but atipamezole has approximately a 100-fold higher affinity for the α_{2D}-adrenoceptor compared with yohimbine [40]. Prazosin has a selectivity of 1,000:1 for the α_1/α_2 adrenoceptor [14]. In contrast, atipamezole, similar to medetomidine, has an imidazoline ring structure, whereas yohimbine and prazosin are non-imidazoline agents [52]. These differences among atipamezole, yohimbine and prazosin may influence their antagonistic effects in the effects of α_2-adrenoceptor agonists.

Because the regulation of water excretion has implications for a number of clinical situations, medetomidine and xylazine-induced diuresis will influence on hydration conditions in normal cats. In such cases, α_2-adrenoceptor antagonists may be used to reverse the diuretic actions. Therefore, it is important to examine the antagonistic effects on urination associated with medetomidine and xylazine use. However, to the best of our knowledge, there are no published reports regarding the effects of α-adrenoceptor antagonists on medetomidine and xylazine-induced diuresis in cats.

In chapter 1, the study was aimed to investigate dose-related diuretic effects of medetomidine and xylazine on diuretic and hormonal variables; urine volume, pH, and specific gravity; plasma AVP concentrations; and creatinine and electrolyte concentrations as well as osmolality in both urine and plasma in healthy cats.

In chapter 2, the study aimed to investigate and compare the antagonistic effects of a single dose of prazosin and three different doses of either atipamezole or yohimbine on medetomidine-induced diuresis in healthy cats. The variables examined were volume, pH, and specific gravity

of urine; plasma AVP concentration; and creatinine, osmolality, and electrolyte concentrations in both urine and plasma.

In chapter 3, the study was aimed to investigate and compare the antagonistic effects of a single dose of prazosin and three different doses of either atipamezole or yohimbine on xylazine-induced diuresis in healthy cats. The variables examined were volume, pH, and specific gravity of urine; plasma AVP concentration; and creatinine, osmolality, and electrolyte concentrations in both urine and plasma. It is hypothesized that the results of these studies may be best interpreted against this background.

Chapter 1

Comparison of the diuretic effects of medetomidine and xylazine in healthy cats

Introduction

The α_2-adrenoceptor agonists medetomidine and xylazine are used in veterinary medicine to induce reliable and dose-dependent sedation, analgesia, and muscle relaxation [24]. Although both drugs are used similarly in practice, there are differences between the 2 drugs. Medetomidine is a more potent, selective, and specific α_2-adrenoceptor agonist than is xylazine. The ratio of α_2-adrenoceptor selectivity to α_1-adrenoceptor selectivity of medetomidine (1,620:1) is approximately 10-fold as great as that of xylazine (160:1) [55]. In addition, in contrast to xylazine, medetomidine contains an imidazole ring that has an affinity for imidazoline receptors [31].

The α_2-adrenoceptor agonists can induce profound diuresis in several species. It has been suggested that several factors are involved in the mechanism of this diuresis. These factors included inhibition of plasma AVP secretion from the pituitary gland [5, 36], inhibition of the ability of AVP-induced cAMP formation in the kidneys [9], redistribution of the aquaporin-2 water channel independent of changes in vasopressin activity [17], inhibition of renin release mediated directly by specific renal α_2-adrenoceptors in the kidneys [43], increase in plasma atrial natriuretic peptide concentrations [37], inhibition of renal sympathetic activity [26], osmotic diuresis attributable to hyperglycemia and glucosuria as a result of the inhibition of insulin release [48], and inhibition of tubular sodium reabsorption [10]. However, the exact mechanism of the diuretic effect of α_2-adrenoceptor agonists is still unknown. Moreover, this mechanism may differ depending on the particular animal species [9].

In another study conducted by our laboratory group [45], we recently reported that the dose-dependent diuretic response to xylazine was more profound than that to medetomidine in healthy

dogs and that medetomidine decreased plasma AVP concentrations significantly, whereas xylazine did not significantly alter plasma AVP concentrations. Furthermore, the α_2-adrenoceptor antagonists atipamezole and yohimbine antagonize diuresis induced by medetomidine and xylazine without causing meaningful hormonal changes in dogs [46, 47]. Given this effect, medetomidine should be used with discretion in hypovolemic or dehydrated dogs and avoided in those with urinary tract obstruction [3]. Also, the increase in urine flow must be considered when making decisions regarding anesthetic management [58].

To our knowledge, there are no published reports on the diuretic effects of medetomidine and xylazine in cats. Given the differences among species, it is important to examine diuretic effects of both drugs in cats. The purpose of the study reported here was to investigate the effects of both drugs on diuretic and hormonal variables in healthy cats.

Materials and methods

Animals

Five healthy adult mixed-breed cats (4 sexually intact males and 1 sexually intact female) weighing from 2.9 to 5.3 kg were used in the study. They were fed a standard commercial dry food formulated for cats and raised in a laboratory with appropriate animal management facilities. Examinations performed prior to the experiments revealed that all cats were healthy, with physical examination, hematologic, and urinary values within respective reference limits. The study protocol was approved by the Animal Research Committee of Tottori University.

Experimental design and drug administration

The five cats were assigned to receive each of the 11 treatments in a modified randomized design, as described elsewhere [20]. Each cat received intramuscularly (IM) saline solution (0.9% NaCl; 2.0 mL, [control treatment]), medetomidine hydrochloride (20, 40, 80, 160, or 320 μg/kg; Domitor, Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), or xylazine hydrochloride (0.5, 1, 2, 4, or 8 mg/kg; Celactal; Bayer Yakuhin, Ltd., Osaka, Japan). There was at least 1 week between successive treatments for each cat. Food and water was withheld for 12 h before the start of each experiment. After we collected samples at 6 h after injection, food and water were provided. Food was then withheld again from the cats for 12 h prior to collection of samples at 24 h after injection. The experiments were performed in a room with the air temperature maintained at 25°C.

Sample collection

On the day before treatment administration, all cats were anesthetized with propofol, as described elsewhere [19]. A 17-gauge central venous catheter (SMAC plus, Covidien Japan Inc., Tokyo, Japan) was introduced into a jugular vein of each cat. A 4F polyvinyl chloride catheter (Atom Multipurpose Tube, Atom Medical Corp., Tokyo, Japan) and 6F silicon balloon catheter (All Silicone Foley Balloon Catheter, Create Medic Co., Ltd., Yokohama, Japan) were placed in the urinary bladder of male and female cats, respectively. Each cat was then placed in a separate cage, and a maintenance dose of Ringer's solution was administered intravenously (IV) for 10 h to ensure sufficient urine production during the experiment. One hour before the start of each experiment, the bladder of each cat was emptied in preparation for subsequent collection of urine sample. Urine and blood samples were collected 9 times (before injection of the treatment [time

0; baseline] and 0.5, 1, 2, 3, 4, 5, 6, and 24 h after injection) from each cat. After collection of samples at 6 h, each cat again received an infusion of Ringer's solution for 10 h, similar to that administered before the experiment.

Blood samples (2.5 mL) and urine samples were collected from the central venous and urinary catheters, respectively. An aliquot (2.0 mL) of each blood sample was mixed with ethylene diamine tetraacetic acid for measurement of AVP concentrations, and the remaining 0.5 mL was mixed with heparin for other measurements. Blood samples were immediately centrifuged at 2,000 × g at 4°C for 15 minutes, and the plasma was separated and stored at −80°C until analysis. Urine samples were centrifuged at 2,000 × g for 5 minutes, and the supernatant was then collected and stored at −40°C until analysis.

Monitoring of behavior and physical variables

Behavioral responses were observed and physical variables, including heart rate, respiratory rate, and rectal temperature, were measured simultaneously with collection of blood and urine samples. For both medetomidine and xylazine treatments, all cats were sedated and positioned in lateral or sternal recumbency, and behaviors were recorded.

Analytical methods

Urine volume, specific gravity, and pH; urine and plasma creatinine concentrations, osmolality, and electrolyte (sodium, potassium, and chloride) concentrations; and plasma AVP concentration were measured via procedures described elsewhere [45]. The osmolar clearance was calculated as follows: (urine osmolality × urine volume)/plasma osmolality. Free-water clearance was calculated as follows: urine volume−osmolar clearance. The glomerular filtration

rate (GFR) was assessed via creatinine clearance and calculated as follows: (urine creatinine concentration × urine volume)/plasma creatinine concentration. The fractional clearance of electrolytes was calculated as follows: (urine electrolyte concentration/plasma electrolyte concentration) × (plasma creatinine concentration/urine creatinine concentration) × 100.

Data evaluation

Statistical analysis was performed with commercially available statistical programs (StatMate3, ATMS, Tokyo, Japan and GraphPad software version 5; GraphPad Software, Inc., San Diego, CA, USA). A 1-way ANOVA was used to examine the time effect within each treatment and the treatment effect at each time point. When a significant difference was detected, the Tukey test was used to compare the means. The area under the curve (AUC) was measured by calculating the sum of the trapezoids formed by the data points. The total urine volume and the AUC for plasma AVP concentration were plotted against the doses of medetomidine or xylazine, and simple linear regression analysis was applied. When a significant difference was detected, the effect of the drug was considered to be dose related. Results were expressed as mean ± SE. For all tests, values of $P < 0.05$ were considered significant.

Results

Behavior and physical variables

Sternal and lateral recumbency were prolonged in a dose-dependent manner. Abnormal behaviors, such as anxiety, muscle rigidity, or excitation-like movement, were not observed,

even with the highest doses of both drugs. Vomiting or signs of nausea were observed in any cats for both treatments. Heart rate, respiratory rate, and rectal temperature decreased in a dose-dependent manner for both medetomidine and xylazine. The lowest mean ± SE heart rate for the medetomidine and xylazine treatments was 72.4 ± 3.5 beats/min at 2 h for 320 µg/kg of medetomidine and 81.6 ± 4.5 beats/min at 4 h for 4 mg/kg of xylazine. The lowest mean ± SE respiratory rate for the medetomidine and xylazine treatments was 24.6 ± 1.5 breaths/min at 4 h for 160 µg/kg of medetomidine and 28.4 ± 3.6 breaths/min at 4 h for 4 mg/kg of xylazine. Respiratory arrest was not observed in any cats.

Diuretic effects

A diuretic effect was found for all doses of both medetomidine and xylazine, compared with results for the baseline (time 0) values (Fig. 1A, 1B). For both the medetomidine and xylazine treatments, peak diuresis was 3 to 4 h after injection. This diuretic effect persisted up to 5 h after injection. Xylazine had a significant dose effect on the urine volume from 0.5 to 6 h (Fig. 1D), but medetomidine did not (Fig. 1C), which indicated that xylazine, but not medetomidine, induced diuresis in a dose-dependent manner at the administered doses. Similar results were observed for total urine volume from 0.5 to 2, 0.5 to 3, 0.5 to 4, and 0.5 to 5 h after injection for both drugs.

The urine specific gravity decreased significantly for both medetomidine and xylazine, compared with the baseline values, except when cats received 40 µg/kg of medetomidine and 0.5 mg/kg of xylazine (Fig. 2A, 2B). The lowest urine specific gravity was detected 3 to 4 h after injection of medetomidine and 2 to 4 h after injection of xylazine. These decreases in urine

specific gravity corresponded closely with the increase in urine volume for both medetomidine and xylazine. Urine pH did not change significantly for any of the treatments.

Urine osmolality decreased significantly after injection of both medetomidine and xylazine, compared with the baseline values (Fig. 2C, 2D). The lowest urine osmolality was detected 2 to 4 h after injection of medetomidine and 3 to 4 h after injection of xylazine. These decreases in urine osmolality corresponded closely with the increase in urine volume for both medetomidine and xylazine.

Plasma osmolality did not change significantly for any of the treatments, compared with the baseline values (Fig. 2E, 2F). However, the value at 6 h after injection of 8 mg/kg of xylazine was significantly increased, compared with the value at 6 h after injection of saline solution (control treatment). Free-water clearance increased significantly for both the medetomidine and xylazine treatments, compared with baseline values, except when cats received 20 µg/kg of medetomidine, 0.5 and 1 mg/kg of xylazine (Fig. 3A, 3B). Peak free-water clearance was detected 3 to 4 h after injection of both medetomidine and xylazine. Osmolar clearance did not change significantly for any of the treatments, compared with the baseline values or the values for the control treatment (Fig. 3C, 3D). Although the GFR did not change significantly for any of the treatments, compared with the baseline values, the values at 5 h after injection of 2 mg/kg of xylazine and 6 h after injection of 4 mg/kg of xylazine were significantly decreased, compared with the values for the control treatment at those time points (Fig. 3E, 3F). Both osmolar clearance and GFR did not have dose-dependent changes for the medetomidine and xylazine treatments.

Plasma AVP concentrations decreased (albeit not significantly) initially (0.5 to 4 h) at the highest dose of medetomidine and increased thereafter (5 to 6 h) for both the medetomidine and

xylazine treatments, compared with the baseline values (Fig. 4A, 4B). A significant difference was detected 6 h after injection only when cats received 160 μg/kg of medetomidine. Result of linear regression of the AUC data for plasma AVP concentrations from 0 to 3 h was significant ($P < 0.01$) for the medetomidine treatments but not for the xylazine treatments (Fig. 4C, 4D). Similar results were obtained from the AUC data for plasma AVP concentration from 0 to 2, 0 to 4, 0 to 5, and 0 to 6 h after injection for both drugs.

Although plasma sodium concentrations did not change significantly for any of the treatments, compared with the baseline values, the value at 6 h after injection of 8 mg/kg of xylazine was significantly increased, compared with the value at 6 h after injection of the control treatment (Fig. 5A, 5B). Plasma potassium and chloride concentrations did not change significantly for any of the medetomidine or xylazine treatments, compared with the baseline values or values for the control treatment. Fractional clearances of all electrolytes typically tended to increase or increased significantly 5 to 6 h after injection of higher doses of both medetomidine and xylazine (Fig. 6). At 6 h after injection, there was a significant increase in potassium concentrations when cats received 4 mg/kg of xylazine and a significant increase in all electrolyte concentrations when cats received 8 mg/kg of xylazine.

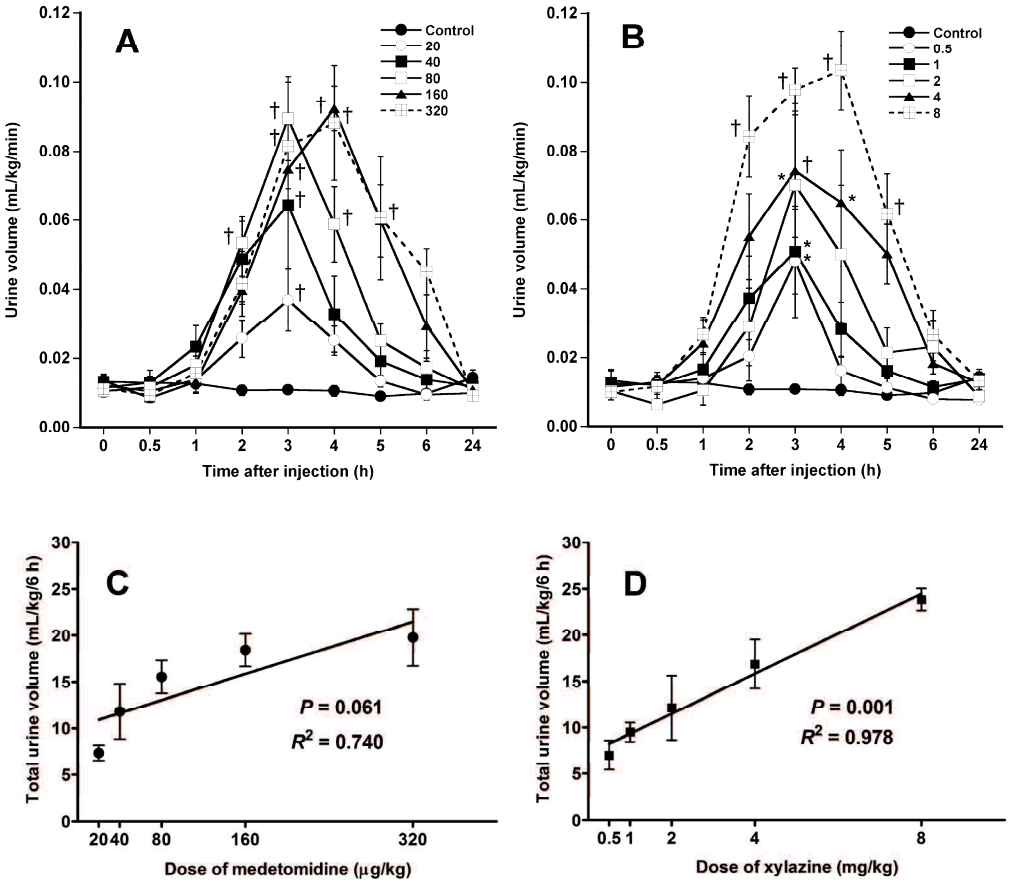

Fig. 1. Urine volume (mean ± standard error) of five cats after injection of saline (0.5% NaCl) solution (control treatment) or various doses of A) medetomidine (µg/kg) or B) xylazine (mg/kg) and linear regression of the total urine volume after injection of the various doses of C) medetomidine or D) xylazine. Time of injection was designated as time 0 (baseline). *†Value differs significantly (*$P < 0.05$; †$P < 0.01$) from the baseline value.

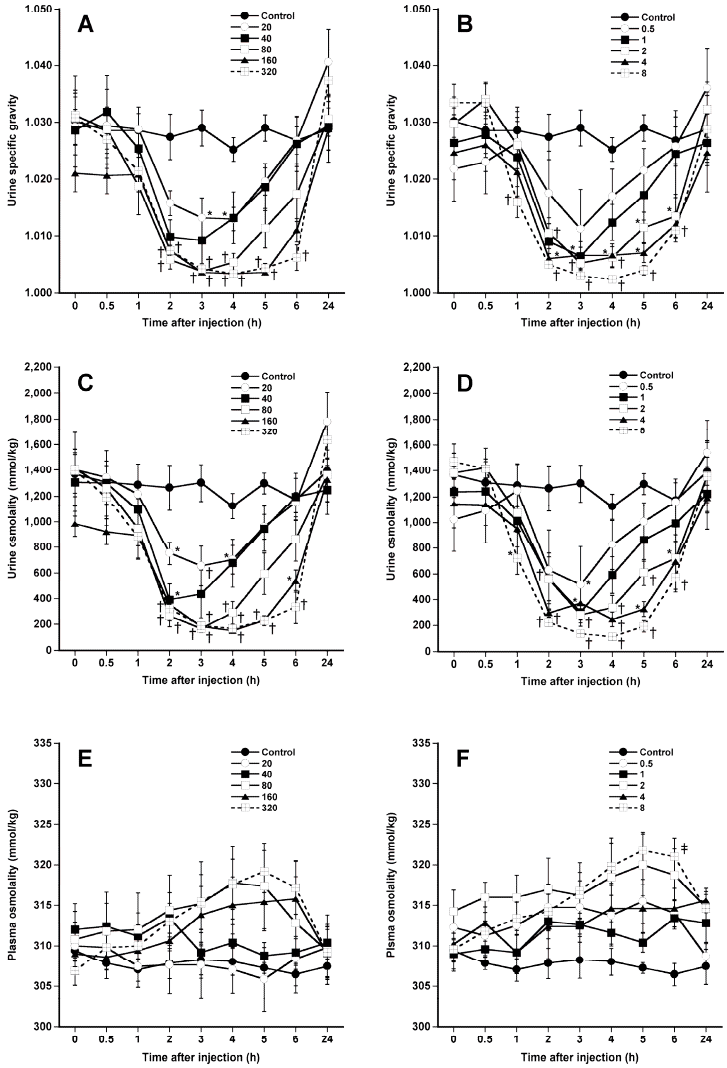

Fig. 2. Urine specific gravity (A and B), urine osmolality (C and D), and plasma osmolality (E and F) (mean ± standard error) of five cats after injection of saline solution (control treatment) or various doses of medetomidine (µg/kg; A, C, and E) or xylazine (mg/kg; B, D, and F). ‡Within a time point, values differs significantly ($P < 0.05$) from the value for the control treatment. See Fig. 1 for remainder of key.

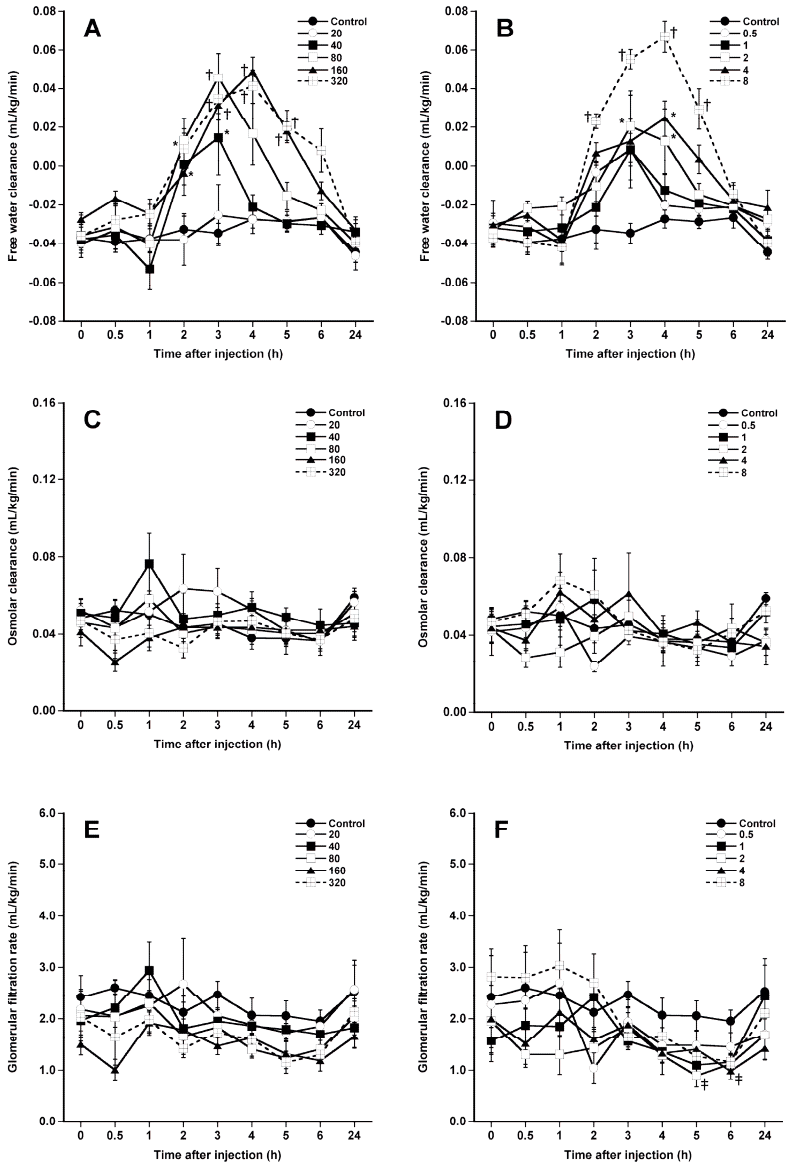

Fig. 3. Free-water clearance (A and B), osmolar clearance (C and D), and GFR (E and F) (mean ± standard error) of five cats after injection of saline solution (control treatment) or various doses of medetomidine (µg/kg; A, C, and E) or xylazine (mg/kg; B, D, and F). See Fig. 1 and 2 for remainder of key.

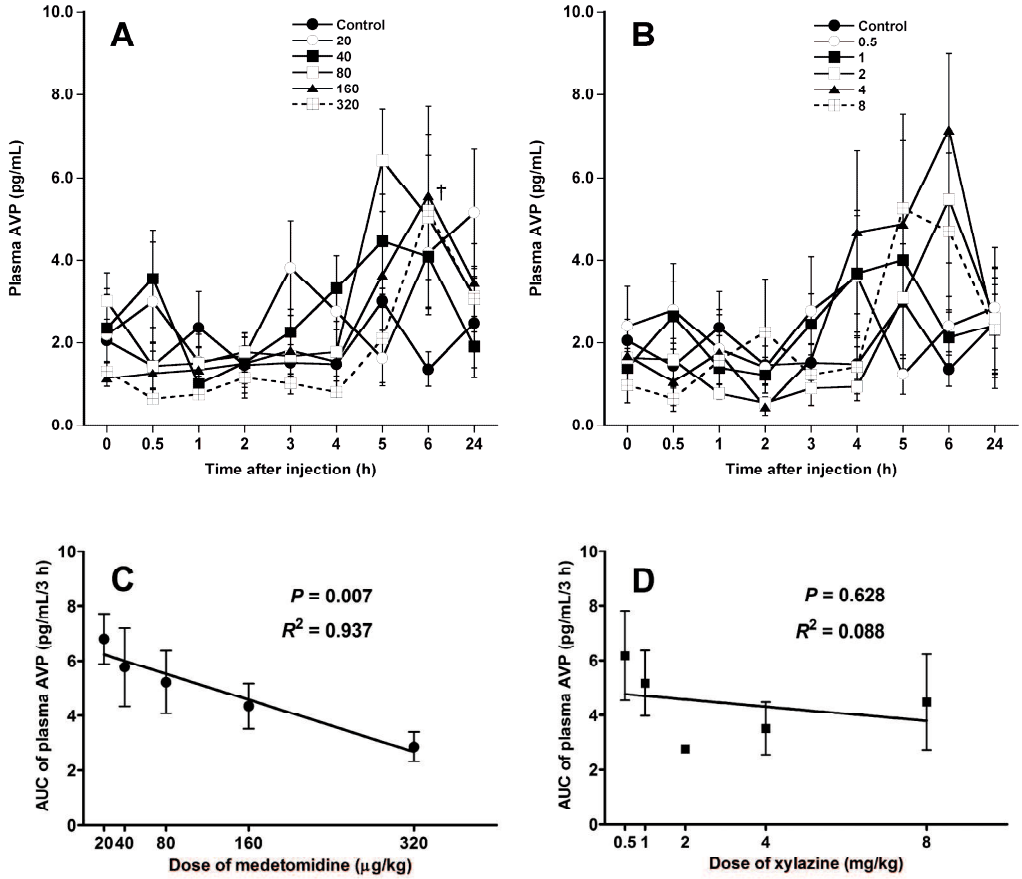

Fig. 4. Plasma AVP concentration (mean ± standard error) of five cats after injection of saline solution (control treatment) or various doses of medetomidine (µg/kg; A) or xylazine (mg/kg; B) and linear regression of the AUC data for plasma AVP concentration after injection of the various doses of medetomidine (C) or xylazine (D). See Fig. 1 for remainder of key.

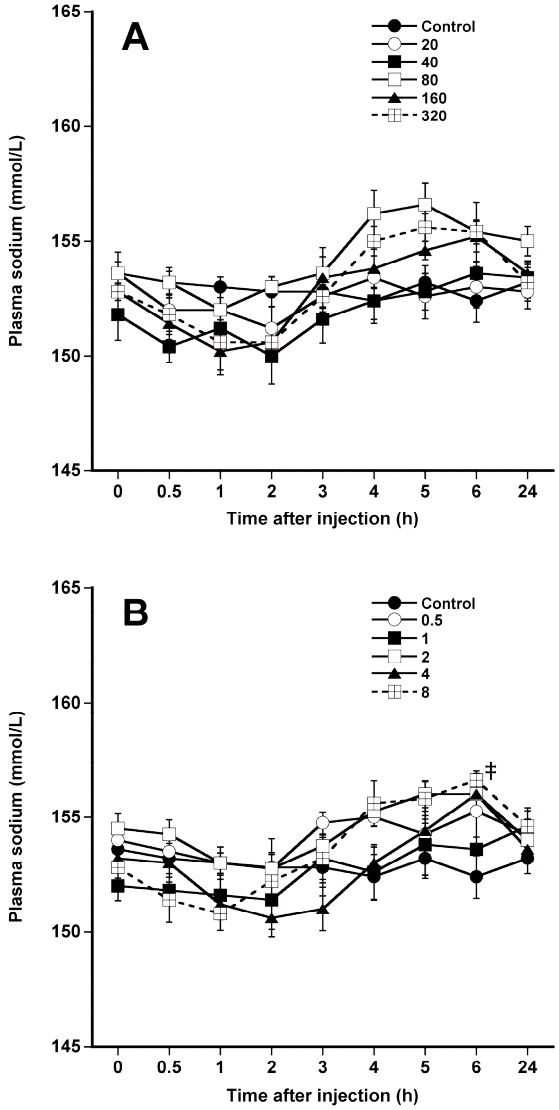

Fig. 5. Plasma sodium concentration (mean ± standard error) of five cats after administration of saline solution (control treatment) or various doses of medetomidine (µg/kg; A) or xylazine (mg/kg; B). See Fig. 2 for remainder of key.

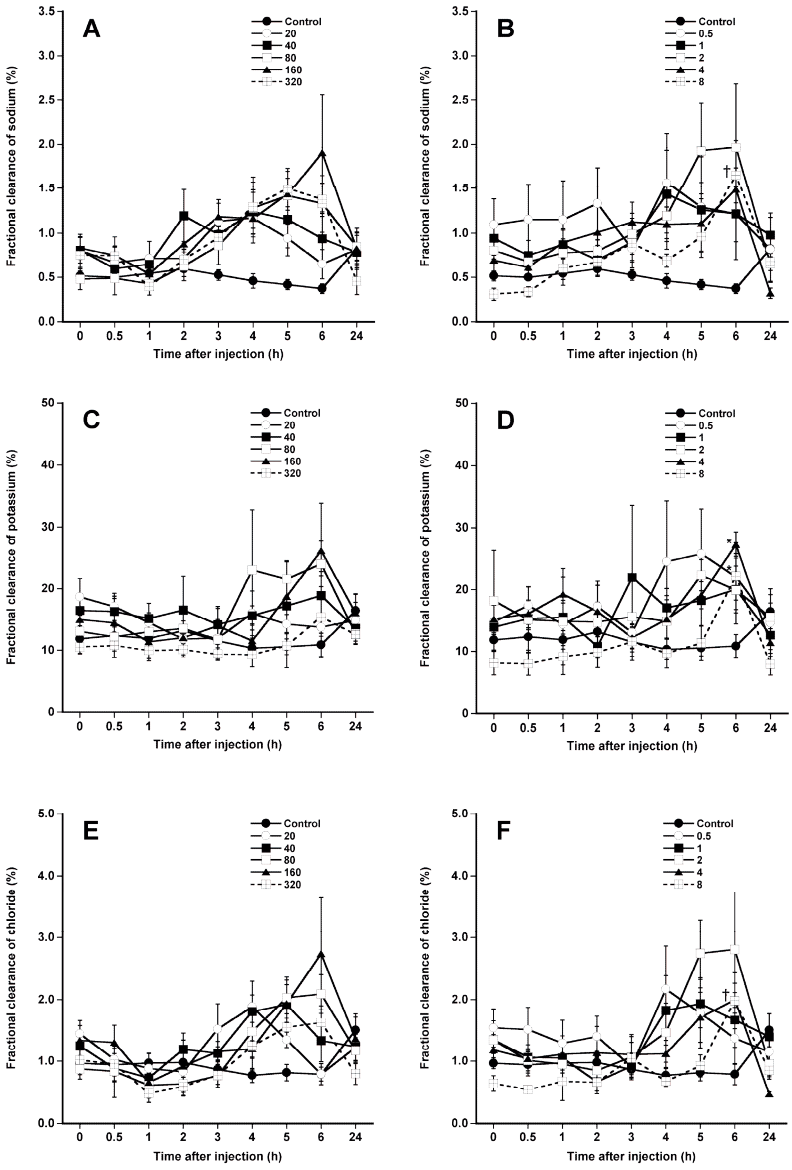

Fig. 6.Fractional clearance of sodium (A and B), potassium (C and D), and chloride (E and F) (mean ± standard error) for 5 cats after administration of saline solution (control treatment) or various doses of medetomidine (µg/kg; A, C, and E) and xylazine (mg/kg; B, D, and F). *See* Fig. 1 for remainder of key.

Discussion

Analysis of results for the study reported here revealed that IM administration of both medetomidine and xylazine significantly increased urine volume in healthy cats. To our knowledge, this is the first study in which a diuretic effect of both medetomidine and xylazine in cats has been reported. These effects are consistent with results of studies on medetomidine-induced diuresis in dogs [3, 38, 45, 47] and rats [37] and xylazine-induced diuresis in dogs [45, 46], cattle [48], horses [32, 49, 50, 58], and rats [15, 27, 28, 29]. The present study also found that the diuretic effects of medetomidine were not a dose-dependent phenomenon at the evaluated doses; however, xylazine induced dose-dependent diuresis in cats. The differences in the dose-dependent effects between medetomidine and xylazine were similar to the findings of another study [45] in dogs conducted by our laboratory group. Because medetomidine is a more selective and specific α_2-adrenoceptor agonist than is xylazine, the different diuretic responses to both drugs could not be explained by the difference in the affinity of α_2-adrenoceptors. Therefore, this difference may have been attributable to the distinct α_2-adrenoceptor and α_1-adrenoceptor selectivity or the imidazoline-receptor selectivity for the 2 drugs, as has been suggested in dogs [45].

In the present study, urine specific gravity and urine osmolarity decreased in proportion with the increase in free-water clearance after medetomidine and xylazine administration. These changes were detected at the same time as the increase in urine volume induced by both drugs. In addition, the present study revealed that the osmolar clearance did not change significantly for both drugs. In rats, osmolar clearance increases after administrations of clonidine [17] and xylazine [28]. Furthermore, it has been suggested that the osmotic diuresis caused by

hyperglycemia and glucosuria attributable to xylazine-induced inhibition of insulin release is involved in the diuretic action of xylazine in cattle and ponies [48, 50]. However, our results in cats indicated that the diuretic effects of medetomidine and xylazine can largely be explained by a decrease in the absorption of water in the renal tubules of the kidneys.

A significant decrease in GFR, compared with the value for the control treatment, was detected 5 h after injection of 2 mg/kg of xylazine and 6 h after injection of 4 mg/kg of xylazine. However, there were no significant differences, compared with the baseline values, for any of the treatments. In dogs, the GFR decreases after IM administration of medetomidine, and it has been suggested that this effect may be the result of a decrease in renal blood flow [38]. Results of the present study indicated that there was a significant decrease in GFR after the peak diuretic effect. Therefore, this effect may have been attributable to a secondary change on the basis of dehydration by diuresis.

In contrast, urine pH did not change significantly change for any of the treatments in the present study. Studies on cattle [48] and dogs [3, 45] have found that urine pH decreased after medetomidine or xylazine administration, which may have been attributable to arterial hypercapnia.

An increase in plasma osmolarity, compared with the control value, was detected 6 h after injection of the highest dose of xylazine, but the increase was not significant for any of the treatments. Serum or plasma osmolarity increases after medetomidine or xylazine administration, probably as a result of renal loss of water in dogs [3, 45], but there is no change in osmolarity after xylazine injection in horses [49, 50]. Differences between animal species may be responsible for differences in renal function. In addition, cats in the present study a received an infusion of Ringer's solution the night preceding treatment. This infusion may have influenced

the changes in urine pH and plasma osmolality in the present study. The precise reasons for the differences among the animal species for the variables are unknown.

In another study [45] conducted by our laboratory group on dogs, we found that medetomidine significantly decreased plasma AVP concentrations, whereas xylazine did not significantly alter the AVP concentrations. The present study revealed that plasma AVP concentrations did not change significantly during the diuretic period after administration of medetomidine and xylazine in cats, although it typically decreased initially after injection of the highest dose of medetomidine. Investigators in 1 study [34] found that cats have a higher osmotic threshold than do other animals. In the present study, the cats received Ringer's solution to maintain urine volume during sample collection by increasing extracellular fluid compartments; therefore, this infusion may have influenced changes in plasma AVP concentrations because the cats received a high sodium load. It has also been reported that α_2-adrenoceptor agonists (eg, clonidine, medetomidine, and dexmedetomidine) inhibit the secretion of AVP from the pituitary gland and thereby decrease plasma AVP concentrations in rats and dogs [5, 35, 36, 38, 54]. One in vitro study [9] revealed that α_2-adrenoceptor agonists (eg, epinephrine, clonidine, guanabenz, and oxymetazoline) inhibit AVP-stimulated accumulation of cAMP in the collecting tubes of rats but not of dogs, rabbits, or pigs. Furthermore, the increase in free-water clearance after clonidine administration is associated with a reduction in whole kidney aquaporin-2 mRNA and independent of the changes in vasopressin activity in rats [17]. Therefore, the increase in urine volume detected in the study reported here may have been independent of the changes in plasma AVP concentrations in cats.

In the present study, linear regression analysis of the AUC data revealed that medetomidine decreased plasma AVP concentrations in a dose-dependent manner, whereas xylazine did not.

These results were consistent with those of a study [45] in dogs. These findings indicated that medetomidine, in contrast to xylazine, more clearly inhibited AVP release. Although the precise mechanism for the difference between medetomidine and xylazine is unknown, it may be partly attributable to differences in receptor selectivities in that medetomidine has affinity for the imidazoline receptor [31] and also has α_2-adrenoceptor selectivity that is approximately 10-fold as great as that of xylazine [55]. Investigators in 1 study [21] reported that imidazoline α_2-adrenoceptor agonists (eg, clonidine, moxonidine, and dexmedetomidine) mediate their actions via both α_2-adrenoceptors and imidazoline receptors.

An increase in plasma sodium concentrations, compared with the value for the control treatment, was detected 6 h after injection of the highest dose of xylazine; however, there were no significant increases, compared with the baseline values, for any of the treatments. This change is associated with the increase in plasma osmolality. In contrast, plasma potassium and chloride concentrations did not change significantly after medetomidine and xylazine injections in the present study. These effects differ from the results of a study [45] on dogs. In the present study, the fractional clearances of sodium, potassium, and chloride typically tended to increase or increased significantly 4 to 6 h after medetomidine and xylazine injections in cats. The increase in fractional clearance or excretion of electrolytes in cats is consistent with results of studies on dogs [3], horses [49, 50], and rats [27, 28, 29. 37]. Although there is a simultaneous increase in the excretion of sodium and potassium with the increase in urine volume in rats [27, 28, 29. 37], results of the present study indicated that there was a significant increase in plasma sodium concentrations and fractional clearance of electrolytes after the peak diuretic effect for high doses of xylazine. This effect may have been attributable to a compensatory response to the increase in the plasma electrolyte concentrations in cats.

In the present study, diuretic effects for a wide range of doses of medetomidine and xylazine were evaluated for the purpose of determining a dose-related effect in clinically normal cats. The lower doses of medetomidine (20 to 40 µg/kg) and xylazine (0.5 to 1 mg/kg), which would be recommended clinically, had mild and similar diuretic effects in this study. It appeared that the diuretic effects induced by both drugs at the lower doses would not pose problems in healthy cats. Given that both drugs cause diuresis, even when the drugs are administered at lower doses, careful administration may be needed for the use of either drug in cats with urinary tract obstruction, hypovolemia, or dehydration. In contrast, this study revealed that administration of both medetomidine and xylazine at higher doses induced profound and prolonged diuresis and that the diuretic effect of medetomidine was also accompanied with a change in AVP concentrations. Therefore, the use of higher doses of both drugs is not recommended for clinical practice and requires concurrent administration of fluids.

In the present study, both medetomidine and xylazine induced profound diuretic effects in healthy cats. At the evaluated doses, xylazine induced a dose-dependent diuretic response, whereas medetomidine induced a diuretic response that was not dose dependent. The AUC of plasma AVP concentrations after administration of medetomidine, in contrast to that after administration of xylazine, decreased in a dose-dependent manner, but this was not related to diuresis. The present study further revealed that changes in plasma AVP concentrations, GFR, and osmolar clearance are not part of the diuretic effects of either drug in cats. Other factors, such as the difference in α_2- and α_1-adrenoceptor selectivity or imidazoline receptor selectivity on the renal system, may be involved in the diuretic mechanism.

Chapter 2

Antagonistic effects of atipamezole, yohimbine, and prazosin on medetomidine-induced diuresis in healthy cats

Introduction

The effects of α_2-adrenoceptor agonists in cats include reliable, dose-dependent sedation, analgesia and muscle relaxation that can be readily reversed by administration of selective antagonists [24]. Pharmacologically, the α_2-adrenoceptors are classified into 3 subtypes (α_{2A}, α_{2B} and α_{2C}) on the basis of radioligand affinity and molecular cloning [4]. The α_{2D}-adrenoceptor subtype in cattle and rodents is considered a species-specific homologue of the α_{2A}-adrenoceptor [2]. Medetomidine is a potent and highly specific α_2-adrenoceptor agonist [42], and the α_2/α_1 receptor-binding selectivity ratio of medetomidine is 1,620:1 [57]. In addition, medetomidine contains an imidazole ring that has an affinity for imidazoline receptors [31].

Medetomidine has been shown to induce diuresis in rats [37] and dogs [3, 38, 45, 47]. Recently, our study showed that both medetomidine and xylazine induced profound diuresis in cats by decreasing water reabsorption in the kidneys [30]. In addition, the same study demonstrated a dose-dependent diuretic effect of xylazine, but not medetomidine, suggesting that some factors, such as differences in α_2- and α_1-adrenoceptor selectivity or imidazoline receptor selectivity of the renal system, may be involved in the diuretic mechanism [30].

The α_2-adrenoceptor antagonists, atipamezole and yohimbine, have been clinically used to reverse the sedative and analgesic effects of α_2-adrenoceptor agonists [24, 40]. The α_2/α_1 adrenoceptor selectivity ratios of atipamezole and yohimbine are 8,526:1 and 40:1, respectively [56]. The affinities of atipamezole and yohimbine are similar for the α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors of HT29 (a human colon adenocarcinoma grade II cell line), rat neonatal lung and opossum kidney epithelial cells, but atipamezole has approximately a 100-fold higher affinity for the α_{2D}- adrenoceptor of PC12 cells (derived from pheochromocytoma of the rat adrenal

medulla) transfected using rat RG-20 (derived from the abdominal ganglion) and sheep brainstem cells compared with yohimbine [40]. Prazosin has a selectivity of 1,000:1 for the α_1/α_2 adrenoceptor [14]. In contrast, atipamezole, similar to medetomidine, has an imidazoline ring structure, whereas yohimbine and prazosin are non-imidazoline agents [52]. These differences among atipamezole, yohimbine and prazosin may influence their antagonistic effects in medetomidine-induced diuresis in cats.

Because the regulation of water excretion has implications for a number of clinical situations, medetomidine-induced diuresis will influence on hydration conditions in normal cats. Especially, urinary tract obstruction, dehydration or hypovolemia may limit the use of medetomidine in cats. In such cases, α_2-adrenoceptor antagonists may be used to reverse the diuretic actions. Therefore, it is important to examine the antagonistic effects on urination associated with medetomidine use. However, to the best of our knowledge, there are no published reports regarding the effects of α-adrenoceptor antagonists on medetomidine-induced diuresis in cats. Therefore, the aims of this study were to investigate and compare the antagonistic effects of a single dose of prazosin and three different doses of either atipamezole or yohimbine on medetomidine-induced diuresis in healthy cats.

Materials and methods

Animals

Five healthy adult mixed-breed cats (3 sexually intact males, 1 neutered male and 1 sexually intact female) aged from 1 to 2 years and weighing from 2.5 to 4.0 kg were used in this study. They were fed a standard commercial dry food formulated for cats and raised in an appropriate

animal management facility. Examinations performed prior to the experiments revealed that all cats were healthy with physical examination, hematological and urinary values within respective reference limits. The study protocol was approved by the Animal Research Committee of Tottori University, Tottori, Japan.

Experimental design and drug administration

The five cats were randomly assigned to receive each of the 9 treatment regimens. IM administrations of saline solution (0.9% NaCl; 0.1 mL/kg) as the 1st treatment at the beginning of the experiment and 2nd IV dose of saline solution (0.1 mL/kg) were given 0.5 h later to each cat in 1 group (control). Those in the other eight groups received medetomidine hydrochloride (40 μg/kg; Domitor, Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan) as the 1st treatment and 2nd treatment of saline solution (0.1 mL/kg), prazosin hydrochloride (160 μg/kg; Sigma-Aldrich Japan, Tokyo, Japan), atipamezole hydrochloride (40, 160 or 480 μg/kg; Antisedan, Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan) or yohimbine hydrochloride (40, 160 or 480 μg/kg; Sigma-Aldrich Japan, Tokyo, Japan) were administered. Prazosin hydrochloride was dissolved in distilled water at a concentration of 1.6 mg/mL and yohimbine hydrochloride was dissolved in distilled water at a concentration of 5.0 mg/mL. The groups are referred to as SAL, MED, PRA, ATI 40, ATI 160, ATI 480, YOH 40, YOH 160, and YOH 480, hereafter. There was at least 1 week between successive treatments for each cat. Food and water were withheld for 12 h before the start of each experiment. After collecting the samples at 8 h after injection, food and water were provided. The experiments were performed in a room with the temperature maintained at 25°C.

Sample collection

One day before treatment, all cats were anesthetized with propofol, as described elsewhere [19]. A 17-gauge central venous catheter (SMAC plus, Covidien Japan Inc., Tokyo, Japan) was inserted into a jugular vein of each cat. Lidocaine (Xylocaine injection 2%, AstraZeneca, Osaka, Japan) was used to assist with local analgesia at the catheterization site. A 4F polyvinyl chloride catheter (Atom Multipurpose Tube, Atom Medical Corp., Tokyo, Japan) and a 6F silicon balloon catheter (All Silicone Foley Balloon Catheter, Create Medic Co., Ltd., Yokohama, Japan) were placed in the urinary bladder of male and female cats, respectively, and each cat was subsequently placed in a separate cage. One hour before the start of each experiment, the bladder of each cat was emptied in preparation for subsequent collection of urine samples. Urine and blood samples were collected 10 times (before agent injection [time 0; baseline] and 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h after injection) from each cat. Blood samples (2.5 mL) and urine samples were collected from the central venous and urinary catheters, respectively. An aliquot of 2.0 mL from each blood sample was mixed with ethylenediaminetetraacetic acid for measurement of plasma AVP concentrations, and the remaining 0.5 mL was mixed with heparin for other measurements. The blood samples were immediately centrifuged at 2,000 × g at 4°C for 15 min, and the plasma was separated and stored at −80°C until analysis. Urine samples were centrifuged at 2,000 × g for 5 min, and the supernatant was stored at −40°C until assayed.

Analytical methods

Urine volume, specific gravity, and pH, urine and plasma creatinine concentrations, osmolality and electrolyte (sodium, potassium and chloride) concentrations and plasma AVP concentrations were measured using previously described procedures [45]. The osmolar

clearance was determined as follows: urine osmolality × urine volume/plasma osmolality. The free-water clearance was determined as follows: urine volume − osmolar clearance. The GFR was assessed using creatinine clearance, which was determined as urine creatinine × urine volume/plasma creatinine. The fractional clearance of electrolytes was determined as follows: (urine electrolyte/plasma electrolyte) × (plasma creatinine/urine creatinine) × 100.

Statistical analysis

All data obtained were analyzed together using the Prism statistical software (GraphPad software version 5; GraphPad Software, Inc., San Diego, CA, USA). One-way analysis of variance was used to examine the time effect within each treatment and the treatment effect at each time point. When a significant difference was detected, the Tukey test was used to compare the means. Total urine volume was plotted against atipamezole or yohimbine doses and evaluated using simple linear regression analysis. When a significant difference was detected, the effect of the drug was considered to be dose related. The AUC was assessed using the data points by prism. Pearson's correlation coefficient was used to examine the correlation between the total urine volume and the AUC of plasma AVP. Results were expressed as mean ± standard error. $P<0.05$ was considered statistically significant.

Results

For all variables, there were no significant differences between groups at baseline, and no significant changes in urine volume or other biochemical and hormonal variables before and

after treatment were observed in the SAL group. Urine volume increased significantly and similarly in the MED and PRA groups compared with baseline values (Fig. 7A). The peak diuresis occurred 2 h after injection. Both ATI and YOH treatments reversed the increase in the urine volume induced by medetomidine (Fig. 7B, 7C). The total urine volume from 0.5 to 5 h increased significantly in the MED and PRA groups compared with the SAL group (Fig. 8A). Both ATI and YOH treatments reversed the medetomidine-induced increase in total urine volume, except for the YOH 40 and 160 groups. The ATI 160, 480 and YOH 480 groups showed significantly inhibited medetomidine-induced increases in the total urine volume. The linear regression of the total urine volume from 0.5 to 5 h after injection was significant in the YOH groups, but not in the ATI groups (Fig. 8B, 8C), which indicated that yohimbine, but not atipamezole, inhibited medetomidine-induced diuresis in a dose-dependent manner at the tested doses. Similar linear regression results were observed with the total urine volume from 0.5 to 4, 0.5 to 6, 0.5 to 7 and 0.5 to 8 h after injection.

Urine specific gravity decreased significantly and similarly in the MED and PRA groups compared with baseline values (Table 1). Both ATI and YOH treatments reversed the medetomidine-induced decrease in urine specific gravity. The ATI 160, ATI 480 and YOH 480 groups significantly inhibited the medetomidine-induced decrease in urine specific gravity. The decrease in urine specific gravity corresponded mostly to the increase in urine volume. Urine pH did not change significantly in response to any of the treatments. Urine osmolality decreased significantly and similarly in the MED and PRA groups compared with the baseline values. Both ATI and YOH treatments reversed the medetomidine-induced decrease in urine osmolality, as evidenced in the ATI 480 and YOH 480 groups. The decrease in urine osmolality also corresponded to an increase in urine volume. Plasma osmolality increased significantly in the

MED and PRA groups for 2 to 3 h after treatment compared with the baseline values. The mean values at 2 to 3 h after treatment in the ATI 160, ATI 480 and YOH 480 groups were significantly lower than that in the MED group.

Free-water clearance increased significantly and similarly in the MED and PRA groups compared with the baseline values (Fig. 9A). All doses of ATI and YOH 480 treatments significantly inhibited the medetomidine-induced increase in free-water clearance (Fig. 9B, 9C). Osmolar clearance and GFR did not significantly change in any group.

Plasma AVP concentrations increased significantly in the MED and PRA groups compared with the baseline values after peak diuresis (Fig. 10A). The mean value at 3 h after treatment in the PRA group was significantly higher than that in the MED group. Both ATI and YOH treatments tended to prevent the increase in plasma AVP concentrations, and a significant difference was detected at 4 h after treatment in the ATI 160 and ATI 480 groups (Fig. 10B, 10C). Plasma AVP concentrations in the ATI 480 and YOH 480 groups increased significantly at 1 h post-treatment compared with the SAL group. The AUC value for plasma AVP from 0.5 to 2 h post-treatment increased significantly in the ATI 480 and YOH 480 groups compared with the MED group (Fig. 11). There were no correlations between the total urine volume and the AUC of plasma AVP from 0.5 to 2 h after treatment in any group.

Plasma sodium concentrations increased significantly and similarly in the MED and PRA groups compared with the baseline values after peak diuresis (Table 2). The ATI and YOH treatments tended to prevent the increase in plasma sodium observed at 4 to 7 h after treatment in the MED group. Plasma potassium and chloride concentrations did not significantly change in any group. The fractional clearance of sodium increased significantly at 3 h after treatment in the MED group and at 3 to 4 h after treatment in the PRA group compared with the baseline values.

Significant increases were also observed in the ATI 40, ATI 160, YOH 40 and YOH 160 groups compared with the baseline values. The fractional clearance of potassium increased significantly at 5 to 6 h after treatment in the PRA group compared with the baseline values. Significant increases were also observed at 2 h after treatment in the ATI 160 group and at 4 h in the YOH 40 group compared with the baseline values. The fractional clearance of potassium significantly decreased at 4 h after treatment in the ATI 160, ATI 480, YOH 160 and YOH 480 groups compared with the MED group. The fractional clearance of chloride increased significantly and at 3 to 4 h after treatment in the MED group compared with the baseline values. Significant increases were also observed at 3 to 4 h after treatment in the ATI 40, YOH 40 and YOH 160 groups compared with the baseline values.

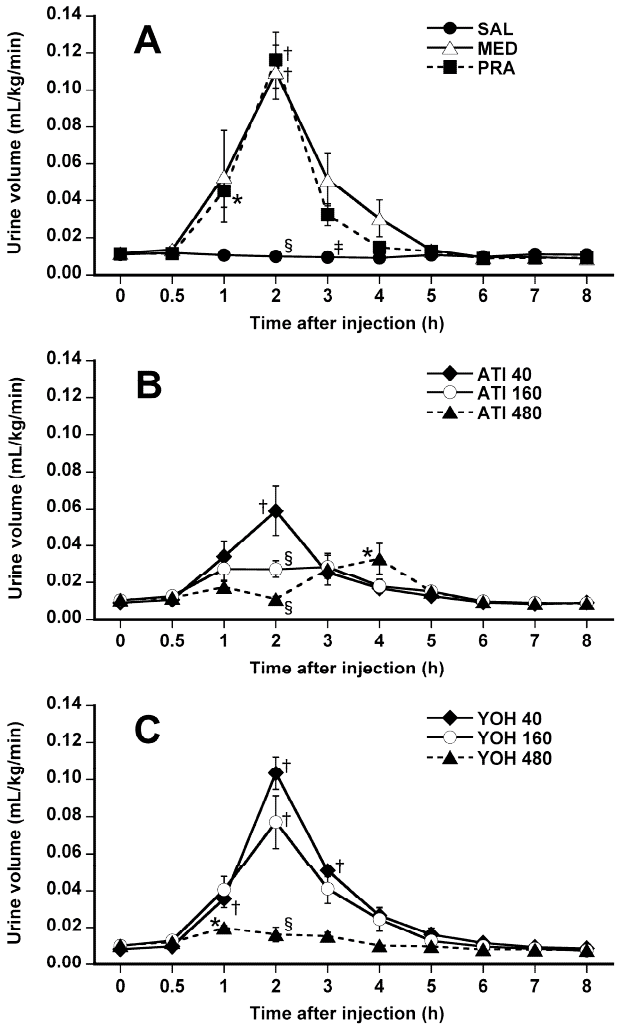

Fig. 7. Urine volume (mean ± standard error) of five cats before and after injection of A) physiological saline (SAL), medetomidine followed by saline (MED), prazosin (PRA); B) atipamezole (ATI); C) yohimbine (YOH), the last two drugs in doses of 40, 160, and 480 µg/kg. Time of 1st injection was designated as time 0 (baseline). *†Value differs significantly (* $p < 0.05$; † $p < 0.01$) from the baseline value. ‡§Within a time point, value differed significantly (‡ $p < 0.05$; § $p < 0.01$) from the MED value.

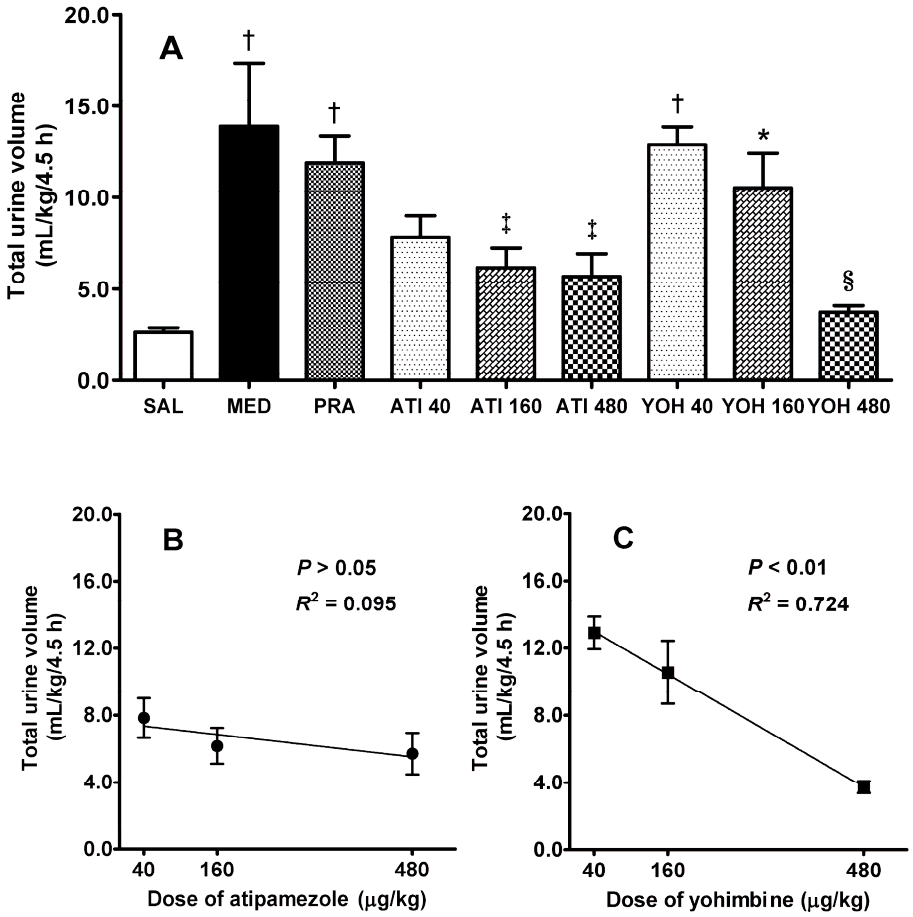

Fig. 8. Total urine volume (mean ± standard error) of five cats at 0.5 to 5 h post-injection of prazosin or various doses of atipamezole or yohimbine (A). Simple linear regression analysis of the total urine volume of five cats at 0.5 to 5 hr after injection of various atipamezole doses (B) or yohimbine (C). See Fig. 7 for the remainder of the key.

Table 1. Urine specific gravity, plasma and urine osmolality (mmol/kg) in 5 healthy cats after an intramuscular injection of saline (SAL), 0.1 mL/kg or medetomidine 40 µg/kg followed 0.5 h later by an intravenous injection of saline (MED) 0.1 mL/kg, prazosin (PRA), 160 µg/kg, atipamezole (ATI), 40, 160, or 480 µg/kg, or yohimbine (YOH), 40, 160, or 480 µg/kg

Variable Group	Time after initial injection (h)									
	0	0.5	1	2	3	4	5	6	7	8
Urine specific gravity										
SAL	1.040 ± 0.003	1.037 ± 0.004	1.034 ± 0.003‡	1.039 ± 0.003§	1.041 ± 0.003§	1.041 ± 0.003§	1.040 ± 0.005	1.040 ± 0.005	1.039 ± 0.005	1.040 ± 0.004
MED	1.034 ± 0.003	1.029 ± 0.003	1.015 ± 0.004†	1.003 ± 0.001†	1.010 ± 0.002†	1.018 ± 0.002*	1.028 ± 0.003	1.036 ± 0.004	1.040 ± 0.003	1.043 ± 0.004
PRA	1.035 ± 0.003	1.031 ± 0.003	1.010 ± 0.002†	1.003 ± 0.001†	1.013 ± 0.003†	1.026 ± 0.004	1.032 ± 0.005	1.041 ± 0.003	1.044 ± 0.002	1.044 ± 0.003
ATI 40	1.041 ± 0.001	1.034 ± 0.001	1.015 ± 0.002†	1.007 ± 0.002†	1.013 ± 0.001†	1.023 ± 0.001	1.028 ± 0.002	1.036 ± 0.003	1.037 ± 0.003	1.039 ± 0.003
ATI 160	1.040 ± 0.002	1.035 ± 0.003	1.023 ± 0.005*	1.019 ± 0.003†‡	1.018 ± 0.004†	1.025 ± 0.004	1.028 ± 0.002	1.036 ± 0.002	1.041 ± 0.002	1.046 ± 0.002
ATI 480	1.038 ± 0.005	1.033 ± 0.003	1.023 ± 0.002	1.034 ± 0.004§	1.023 ± 0.005	1.018 ± 0.005	1.027 ± 0.004	1.034 ± 0.003	1.038 ± 0.003	1.041 ± 0.004
YOH 40	1.042 ± 0.003	1.037 ± 0.002	1.013 ± 0.001†	1.005 ± 0.001†	1.008 ± 0.001†	1.017 ± 0.001†	1.024 ± 0.003*	1.032 ± 0.004	1.039 ± 0.005	1.044 ± 0.006
YOH 160	1.034 ± 0.003	1.029 ± 0.002	1.012 ± 0.001†	1.005 ± 0.001†	1.015 ± 0.002†	1.023 ± 0.003	1.029 ± 0.003	1.036 ± 0.003	1.040 ± 0.002	1.041 ± 0.002
YOH 480	1.041 ± 0.002	1.035 ± 0.001	1.024 ± 0.002	1.033 ± 0.003§	1.034 ± 0.003§	1.040 ± 0.002§	1.045 ± 0.003	1.046 ± 0.002	1.046 ± 0.002	1.051 ± 0.002
Urine osmolality										
SAL	1632 ± 100	1396 ± 142	1346 ± 129‡	1453 ± 102§	1542 ± 136§	1551 ± 139	1461 ± 219	1479 ± 213	1458 ± 209	1452 ± 192
MED	1384 ± 92	1056 ± 102	604 ± 137†	178 ± 16†	460 ± 79†	931 ± 146	1282 ± 113	1468 ± 128	1520 ± 141	1580 ± 161
PRA	1371 ± 69	1129 ± 104	419 ± 60†	156 ± 13†	572 ± 127†	1137 ± 146	1349 ± 164	1590 ± 104	1666 ± 69	1622 ± 149
ATI 40	1698 ± 93	1386 ± 124	865 ± 223*	630 ± 242†	881 ± 171*	1285 ± 80	1375 ± 107	1564 ± 109	1463 ± 92	1512 ± 85
ATI 160	1712 ± 97	1390 ± 116	970 ± 208	805 ± 186†	869 ± 195*	1135 ± 203	1350 ± 83	1519 ± 74	1679 ± 73	1809 ± 77
ATI 480	1423 ± 148	1097 ± 166	782 ± 92	1362 ± 167§	947 ± 215	792 ± 208	1111 ± 163	1290 ± 163	1411 ± 141	1512 ± 164
YOH 40	1696 ± 65	1390 ± 61	544 ± 48†	209 ± 13†	440 ± 53†	876 ± 81†	1170 ± 76†	1399 ± 83	1612 ± 104	1705 ± 100
YOH 160	1463 ± 64	1113 ± 96	544 ± 73†	298 ± 64†	637 ± 79†	1012 ± 125*	1314 ± 114	1386 ± 73	1460 ± 51	1448 ± 62
YOH 480	1681 ± 102	1388 ± 63	991 ± 40†	1342 ± 160§	1503 ± 142§	1706 ± 83	1772 ± 100	1763 ± 84	1766 ± 81	1846 ± 52
Plasma osmolality										
SAL	318.0 ± 0.5	317.6 ± 0.8	318.6 ± 0.5	317.8 ± 0.7‡	317.6 ± 0.5	318.4 ± 0.5	317.8 ± 0.6	317.0 ± 0.9	316.6 ± 0.9	317.2 ± 1.2
MED	316.8 ± 0.9	317.2 ± 0.9	321.0 ± 1.2	323.2 ± 0.5*	322.2 ± 0.9*	320.8 ± 1.2	320.6 ± 1.3	320.8 ± 1.1	320.0 ± 1.3	320.2 ± 1.6
PRA	316.2 ± 0.8	316.8 ± 0.4	319.8 ± 0.9	321.6 ± 1.0	322.8 ± 0.9	319.8 ± 1.4	319.0 ± 1.5	319.2 ± 1.4	319.6 ± 1.3	320.0 ± 1.7
ATI 40	317.8 ± 0.8	318.2 ± 0.8	318.8 ± 1.0	320.0 ± 0.5	319.0 ± 1.0	317.4 ± 0.9	317.8 ± 1.1	318.0 ± 1.4	318.6 ± 1.1	318.0 ± 1.1
ATI 160	317.4 ± 1.3	318.6 ± 1.5	317.2 ± 0.7	316.8 ± 0.7§	317.8 ± 1.4	316.0 ± 0.7	315.8 ± 1.0	317.0 ± 0.9	315.8 ± 1.3	316.6 ± 1.3
ATI 480	318.6 ± 1.3	318.0 ± 1.2	320.2 ± 1.0	316.8 ± 1.2§	316.2 ± 0.8‡	317.2 ± 1.0	317.0 ± 1.6	317.8 ± 1.0	317.6 ± 1.3	317.6 ± 1.0
YOH 40	318.2 ± 1.3	318.0 ± 1.3	319.2 ± 1.0	321.6 ± 1.6	319.0 ± 1.4	320.0 ± 1.7	319.2 ± 1.3	318.6 ± 1.8	317.2 ± 1.1	318.4 ± 1.1
YOH 160	316.8 ± 0.7	318.8 ± 1.2	318.4 ± 0.9	320.0 ± 1.4	318.4 ± 1.0	317.4 ± 1.2	316.8 ± 1.1	317.2 ± 0.8	317.2 ± 0.5	316.8 ± 1.0
YOH 480	317.6 ± 0.7	317.8 ± 0.8	320.6 ± 0.5	318.4 ± 0.8	316.6 ± 0.2‡	317.2 ± 0.8	318.6 ± 0.5	318.2 ± 0.8	317.6 ± 0.5	318.4 ± 0.4

Data are shown as the mean ± standard error.

† Value differs significantly ($p < 0.05$; † $p < 0.01$) from the baseline value (0 h). ‡§ Within a time point, value differed significantly (‡ $p < 0.05$; § $p < 0.01$) from the MED group.

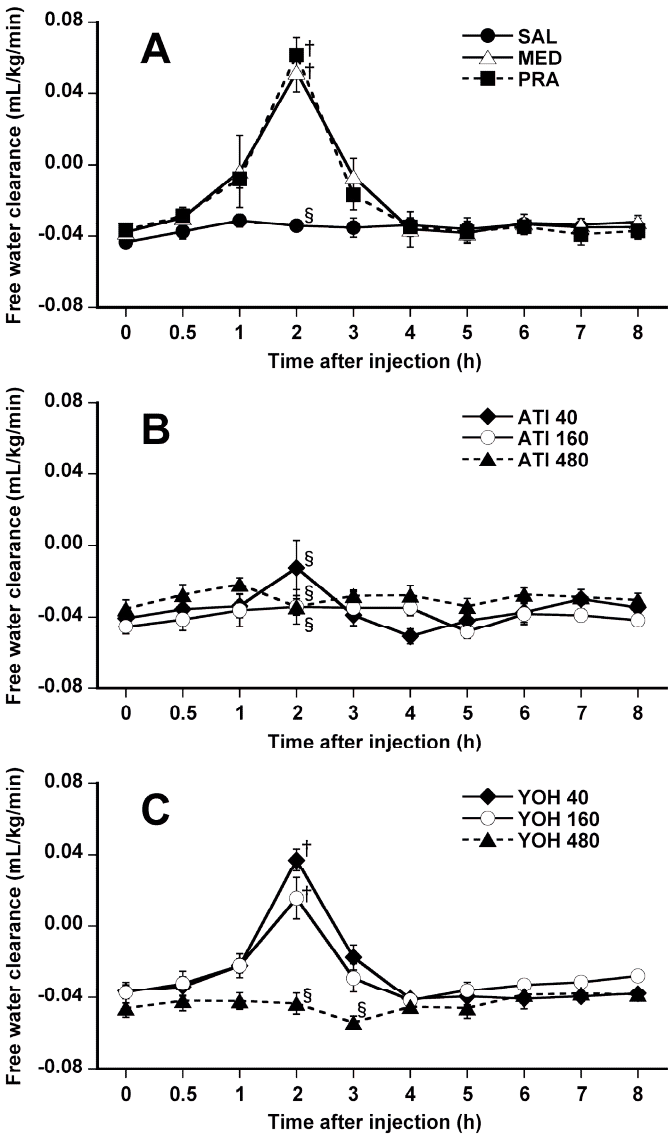

Fig. 9. Free-water clearance (mean ± standard error) of five cats (A, B, and C) before and after injection of physiological saline or medetomidine. See Fig. 7 for the remainder of the key.

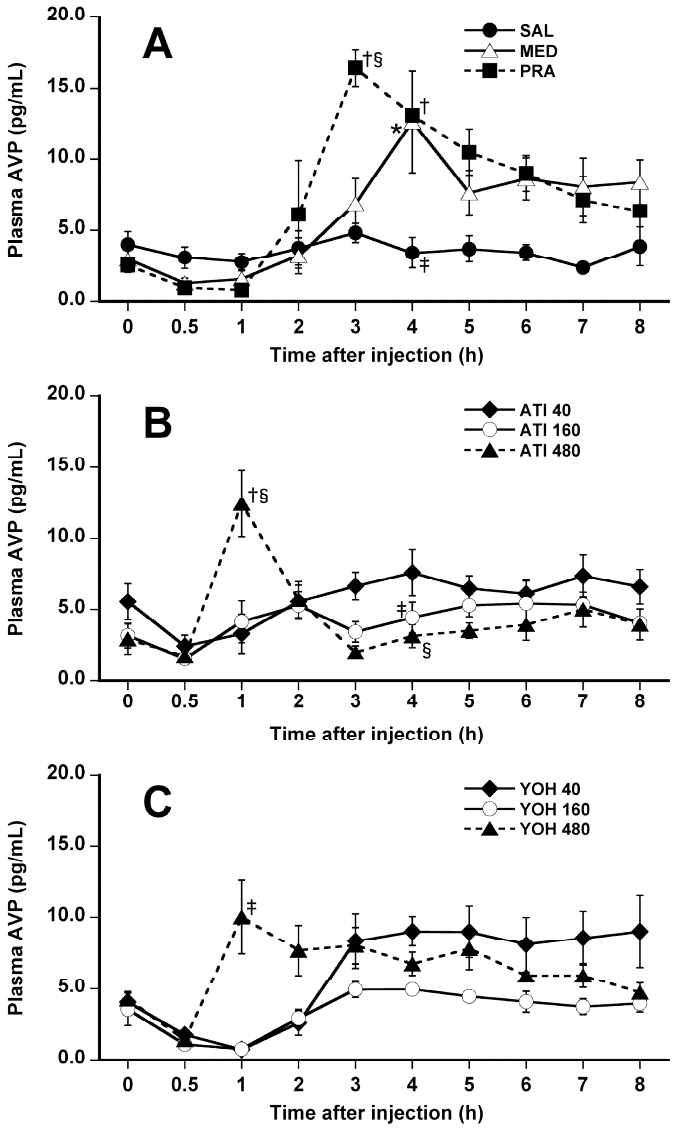

Fig. 10. Plasma AVP concentration (mean ± standard error) of five cats (A, B, and C) before and after injection of physiological saline or medetomidine. See Fig. 7 for the remainder of the key.

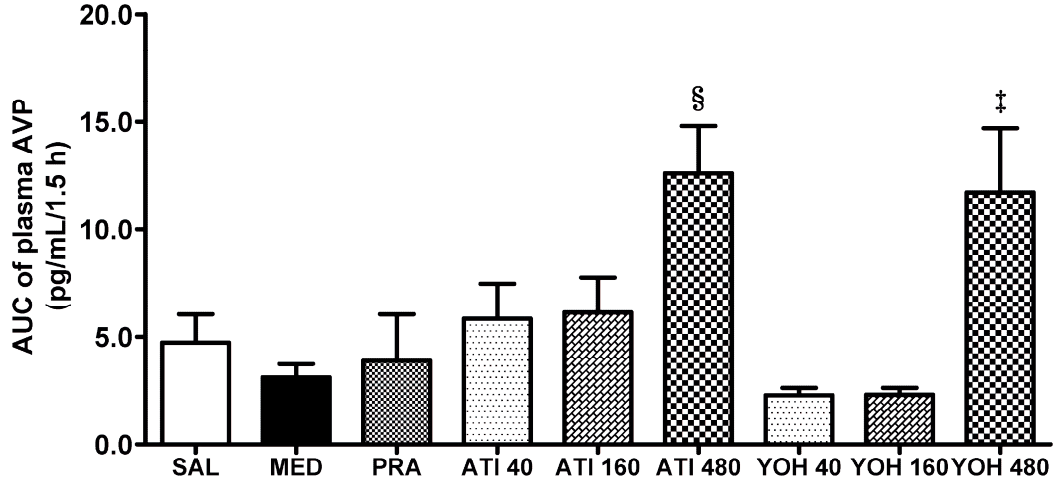

Fig. 11. Area under the curve (AUC) data for plasma AVP concentration (mean ± standard error) of five cats after injection of the prazosin or various doses of atipamezole or yohimbine at 0.5 to 2 h. The symbols indicate a significant difference from the value of the MED group (‡ $p < 0.05$; § $p < 0.01$).

Table 2. Plasma sodium, potassium, and chloride concentrations (mmol/L) and fractional clearance of sodium, potassium, and chloride (%) in the same experiment

Variable	Group	Time after initial injection (h)									
		0	0.5	1	2	3	4	5	6	7	8
Plasma sodium	SAL	153.6 ± 0.5	153.8 ± 0.3	153.6 ± 0.5	153.4 ± 0.4	153.8 ± 0.3	153.8 ± 0.3	153.8 ± 0.5	154.2 ± 0.3	153.6 ± 0.4	154.2 ± 0.3
	MED	153.2 ± 0.6	152.0 ± 0.3	151.4 ± 0.2	154.4 ± 0.5	154.4 ± 0.5	155.6 ± 0.4*	155.0 ± 0.5	155.4 ± 0.5	155.0 ± 0.3	155.0 ± 0.6
	PRA	152.8 ± 0.3	151.0 ± 0.5	150.8 ± 0.5	152.8 ± 0.5	155.2 ± 0.4*	155.0 ± 0.0*	154.6 ± 0.5	154.4 ± 0.4	154.6 ± 0.4	154.8 ± 0.2
	ATI 40	154.2 ± 0.3	152.6 ± 0.7	153.2 ± 0.7	154.0 ± 0.4	154.8 ± 0.3	153.8 ± 0.7	154.6 ± 0.6	154.4 ± 0.5	155.0 ± 0.4	154.2 ± 0.4
	ATI 160	152.8 ± 0.7	151.4 ± 0.8	153.2 ± 0.6	152.6 ± 0.6	152.2 ± 0.5	152.6 ± 0.4§	153.2 ± 0.3	152.8 ± 0.6‡	153.0 ± 0.6‡	153.2 ± 0.5
	ATI 480	153.6 ± 0.4	151.6 ± 0.6	153.8 ± 0.5	153.8 ± 0.5	153.2 ± 0.7	153.4 ± 0.5	153.6 ± 0.5	153.6 ± 0.6	153.6 ± 0.5	154.2 ± 0.3
	YOH 40	154.0 ± 0.5	152.6 ± 0.2	152.0 ± 0.6	153.6 ± 0.2	154.6 ± 0.4	155.6 ± 0.7	154.6 ± 0.5	154.6 ± 0.4	154.8 ± 0.2	155.0 ± 0.3
	YOH 160	153.8 ± 0.4	152.2 ± 0.7	152.4 ± 0.8	154.4 ± 0.5	154.0 ± 0.4	154.4 ± 0.7	153.6 ± 0.4	153.6 ± 0.4	153.4 ± 0.4	153.2 ± 0.4
	YOH 480	153.6 ± 0.2	152.4 ± 0.6	155.2 ± 0.5§	155.0 ± 0.3	154.2 ± 0.2	154.0 ± 0.3	154.2 ± 0.3	154.2 ± 0.3	154.4 ± 0.2	154.6 ± 0.2
Plasma potassium	SAL	3.60 ± 0.09	3.70 ± 0.14	3.72 ± 0.10	3.64 ± 0.12	3.76 ± 0.10	3.74 ± 0.06	3.64 ± 0.14	3.70 ± 0.11	3.70 ± 0.13	3.70 ± 0.12
	MED	3.70 ± 0.05	3.68 ± 0.06	3.78 ± 0.13	3.68 ± 0.08	3.80 ± 0.08	3.82 ± 0.12	3.66 ± 0.09	3.60 ± 0.12	3.56 ± 0.07	3.54 ± 0.08
	PRA	3.80 ± 0.09	3.72 ± 0.10	3.76 ± 0.08	3.86 ± 0.10	3.84 ± 0.18	3.78 ± 0.13	3.66 ± 0.12	3.62 ± 0.11	3.56 ± 0.14	3.60 ± 0.11
	ATI 40	3.64 ± 0.06	3.64 ± 0.13	3.68 ± 0.14	3.80 ± 0.07	3.92 ± 0.12	3.98 ± 0.13	3.80 ± 0.13	3.54 ± 0.12	3.64 ± 0.11	3.56 ± 0.06
	ATI 160	3.90 ± 0.14	3.78 ± 0.11	3.92 ± 0.12	3.78 ± 0.17	3.86 ± 0.08	3.78 ± 0.12	3.72 ± 0.07	3.62 ± 0.10	3.60 ± 0.07	3.58 ± 0.13
	ATI 480	3.94 ± 0.15	3.78 ± 0.11	3.68 ± 0.20	3.96 ± 0.13	3.96 ± 0.18	4.02 ± 0.11	4.02 ± 0.13	4.06 ± 0.07	3.86 ± 0.07	3.86 ± 0.12
	YOH 40	3.84 ± 0.15	3.66 ± 0.12	3.96 ± 0.13	3.80 ± 0.13	3.92 ± 0.06	3.80 ± 0.11	3.72 ± 0.11	3.74 ± 0.12	3.70 ± 0.11	3.70 ± 0.10
	YOH 160	3.72 ± 0.06	3.74 ± 0.05	3.84 ± 0.06	3.76 ± 0.08	3.76 ± 0.10	3.74 ± 0.04	3.68 ± 0.09	3.60 ± 0.07	3.62 ± 0.05	3.64 ± 0.07
	YOH 480	3.98 ± 0.03	3.98 ± 0.08	3.46 ± 0.09	3.66 ± 0.17	3.64 ± 0.12	3.70 ± 0.04	3.92 ± 0.19	3.96 ± 0.14	3.82 ± 0.11	3.80 ± 0.09
Plasma chloride	SAL	117.0 ± 1.4	117.0 ± 1.6	117.4 ± 1.5	117.4 ± 1.3	117.6 ± 1.5	118.0 ± 1.4	118.2 ± 1.7	118.0 ± 1.4	118.6 ± 1.3	118.8 ± 1.4
	MED	117.8 ± 0.7	116.8 ± 0.7	117.0 ± 0.8	118.8 ± 1.0	118.6 ± 0.9	119.2 ± 0.9	119.8 ± 0.9	119.8 ± 0.9	119.8 ± 0.7	120.2 ± 0.7
	PRA	116.0 ± 1.1	114.6 ± 0.9	114.8 ± 1.0	116.0 ± 0.7	117.4 ± 2.2	117.4 ± 1.2	118.2 ± 1.1	118.6 ± 0.9	119.0 ± 1.1	119.6 ± 0.5
	ATI 40	118.0 ± 1.1	116.2 ± 1.2	116.8 ± 1.0	117.8 ± 0.8	117.8 ± 1.0	118.6 ± 1.0	119.2 ± 1.3	119.0 ± 1.4	119.8 ± 1.1	118.8 ± 1.3
	ATI 160	118.4 ± 1.0	116.4 ± 0.8	117.8 ± 1.0	116.8 ± 0.9	117.8 ± 0.8	117.8 ± 0.8	118.2 ± 1.1	118.0 ± 0.8	118.2 ± 0.8	118.4 ± 0.8
	ATI 480	118.2 ± 0.2	116.0 ± 0.4	117.2 ± 0.8	117.6 ± 1.0	118.2 ± 0.7	118.0 ± 0.6	119.2 ± 0.5	119.0 ± 0.6	118.8 ± 0.6	120.6 ± 0.5
	YOH 40	118.6 ± 0.7	116.6 ± 0.8	116.4 ± 0.9	118.2 ± 1.2	118.6 ± 1.0	119.6 ± 1.3	118.6 ± 1.3	119.2 ± 1.2	120.0 ± 0.9	119.2 ± 1.2
	YOH 160	117.2 ± 1.0	115.6 ± 1.1	115.4 ± 1.2	117.6 ± 1.2	118.2 ± 1.2	118.0 ± 1.2	118.0 ± 1.2	118.8 ± 1.1	118.2 ± 1.0	118.8 ± 1.1
	YOH 480	118.0 ± 1.2	115.4 ± 1.2	117.8 ± 0.8	118.2 ± 1.1	117.8 ± 1.0	117.8 ± 0.9	118.8 ± 1.1	120.0 ± 1.0	120.0 ± 0.9	120.6 ± 0.7
Fractional clearance of sodium	SAL	0.35 ± 0.08	0.42 ± 0.13	0.48 ± 0.14	0.30 ± 0.06	0.27 ± 0.04‡	0.25 ± 0.04§	0.28 ± 0.05	0.25 ± 0.05	0.29 ± 0.07	0.32 ± 0.08
	MED	0.34 ± 0.09	0.32 ± 0.06	0.40 ± 0.09	0.65 ± 0.06	1.56 ± 0.32†	1.57 ± 0.23†	1.01 ± 0.31	0.56 ± 0.17	0.39 ± 0.10	0.32 ± 0.05
	PRA	0.27 ± 0.10	0.27 ± 0.07	0.54 ± 0.21	0.76 ± 0.19	1.13 ± 0.20*	1.03 ± 0.21	0.67 ± 0.14	0.41 ± 0.07	0.35 ± 0.36	0.31 ± 0.07
	ATI 40	0.37 ± 0.09	0.34 ± 0.06	0.81 ± 0.15	1.16 ± 0.15*	1.38 ± 0.19†	1.27 ± 0.22†	0.79 ± 0.21	0.41 ± 0.09	0.33 ± 0.40	0.28 ± 0.04
	ATI 160	0.29 ± 0.07	0.26 ± 0.05	0.60 ± 0.08	1.15 ± 0.25†	1.22 ± 0.23†	0.96 ± 0.07	0.80 ± 0.10	0.48 ± 0.06	0.36 ± 0.63	0.25 ± 0.04
	ATI 480	0.14 ± 0.03	0.27 ± 0.06	0.78 ± 0.23	0.58 ± 0.19	0.64 ± 0.15	0.68 ± 0.11	0.59 ± 0.08	0.46 ± 0.08	0.36 ± 0.46	0.30 ± 0.10
	YOH 40	0.42 ± 0.19	0.38 ± 0.18	0.60 ± 0.12	1.17 ± 0.34	1.65 ± 0.30*	1.83 ± 0.19†	1.12 ± 0.19	0.63 ± 0.11	0.49 ± 0.18	0.46 ± 0.13
	YOH 160	0.47 ± 0.09	0.47 ± 0.08	0.96 ± 0.26	1.54 ± 0.39	1.96 ± 0.40†	1.66 ± 0.30	0.96 ± 0.15	0.54 ± 0.07	0.33 ± 0.36	0.28 ± 0.04
	YOH 480	0.54 ± 0.20	0.48 ± 0.18	0.69 ± 0.20	0.84 ± 0.21	0.78 ± 0.10	0.80 ± 0.13	0.56 ± 0.09	0.46 ± 0.08	0.35 ± 0.22	0.29 ± 0.06
Fractional clearance of potassium	SAL	14.2 ± 2.5	10.3 ± 1.5	11.0 ± 1.9	8.5 ± 1.5	10.2 ± 1.9	9.7 ± 2.2§	10.7 ± 2.3	8.7 ± 1.9	8.1 ± 1.8	8.2 ± 1.8
	MED	18.1 ± 4.6	12.4 ± 2.6	13.9 ± 1.1	15.9 ± 1.4	18.5 ± 2.1	32.0 ± 5.0	22.9 ± 4.6	19.6 ± 4.4	15.2 ± 2.8	13.7 ± 3.1
	PRA	9.9 ± 1.2	10.9 ± 0.9	15.4 ± 1.6	15.6 ± 3.1	22.2 ± 7.2	28.2 ± 2.8*	28.1 ± 2.0*	25.5 ± 2.0	23.4 ± 2.3	20.4 ± 3.1
	ATI 40	17.9 ± 4.0	15.2 ± 3.6	20.1 ± 1.2	19.9 ± 3.0	21.2 ± 5.0	19.6 ± 2.8	15.5 ± 2.8	13.6 ± 2.5	11.6 ± 2.0	10.6 ± 1.6
	ATI 160	12.8 ± 2.4	12.0 ± 2.2	20.8 ± 2.9	26.8 ± 2.7*	19.4 ± 3.7	15.7 ± 2.2‡	15.4 ± 2.1	14.3 ± 2.3	12.4 ± 1.9	12.1 ± 2.9
	ATI 480	7.7 ± 1.8	9.1 ± 2.1	18.5 ± 3.0	10.7 ± 1.8	9.1 ± 1.7	9.7 ± 2.3§	8.3 ± 1.6‡	9.2 ± 1.5	9.4 ± 2.2	7.9 ± 2.2
	YOH 40	11.6 ± 2.3	11.1 ± 2.4	14.5 ± 2.6	16.3 ± 1.2	13.7 ± 2.5	25.5 ± 2.7*	21.8 ± 3.3	17.0 ± 2.5	16.6 ± 2.4	14.4 ± 1.6
	YOH 160	15.3 ± 2.9	11.8 ± 1.5	16.8 ± 3.1	17.1 ± 3.0	15.2 ± 2.9	13.2 ± 1.0§	11.3 ± 1.1	10.8 ± 1.2	9.3 ± 1.4	8.3 ± 1.4
	YOH 480	15.3 ± 2.9	15.8 ± 2.0	23.6 ± 2.3	19.7 ± 5.3	15.7 ± 3.5	13.0 ± 2.3§	11.2 ± 1.3	13.2 ± 1.8	14.1 ± 2.4	11.7 ± 1.8
Fractional clearance of chloride	SAL	0.72 ± 0.17	0.65 ± 0.15	0.62 ± 0.14	0.44 ± 0.07	0.43 ± 0.06	0.45 ± 0.10‡	0.46 ± 0.09	0.39 ± 0.08	0.43 ± 0.10	0.40 ± 0.06
	MED	0.55 ± 0.12	0.42 ± 0.04	0.37 ± 0.06	0.63 ± 0.07†	1.84 ± 0.40†	2.12 ± 0.37	1.30 ± 0.28	0.72 ± 0.12	0.52 ± 0.10	0.41 ± 0.08
	PRA	0.60 ± 0.14	0.48 ± 0.09	0.41 ± 0.14	0.65 ± 0.16	1.37 ± 0.30	1.43 ± 0.31	0.82 ± 0.20	0.44 ± 0.07	0.32 ± 0.06	0.29 ± 0.05
	ATI 40	0.77 ± 0.21	0.51 ± 0.12	0.72 ± 0.13	1.31 ± 0.29*	2.09 ± 0.38	1.74 ± 0.32	1.04 ± 0.21	0.51 ± 0.09	0.39 ± 0.05	0.32 ± 0.05
	ATI 160	0.78 ± 0.16	0.49 ± 0.08	0.80 ± 0.19	1.53 ± 0.30	1.76 ± 0.37	1.45 ± 0.16	1.20 ± 0.19	0.73 ± 0.06	0.56 ± 0.08	0.45 ± 0.04
	ATI 480	0.36 ± 0.06	0.37 ± 0.09	0.82 ± 0.26	0.60 ± 0.21	0.81 ± 0.17	0.81 ± 0.17	0.76 ± 0.12	0.58 ± 0.11	0.45 ± 0.10	0.37 ± 0.09
	YOH 40	0.70 ± 0.27	0.54 ± 0.25	0.46 ± 0.13	0.86 ± 0.19	2.01 ± 0.49†	2.65 ± 0.29	1.56 ± 0.39	0.86 ± 0.24	0.60 ± 0.17	0.49 ± 0.14
	YOH 160	1.02 ± 0.26	0.65 ± 0.10	0.84 ± 0.24	1.63 ± 0.41*	2.85 ± 0.54	2.11 ± 0.43	1.22 ± 0.29	0.72 ± 0.19	0.45 ± 0.11	0.37 ± 0.07
	YOH 480	1.15 ± 0.34	0.76 ± 0.25	0.82 ± 0.21	1.16 ± 0.29	1.10 ± 0.18	0.82 ± 0.17	0.49 ± 0.08	0.45 ± 0.04	0.41 ± 0.03	0.38 ± 0.05

Data are shown as the mean ± standard error.

† Value differs significantly ($p < 0.05$; † $p < 0.01$) from the baseline value (0 h). ‡§ Within a time point, value differed significantly (‡ $p < 0.05$; § $p < 0.01$) from the MED group.

Discussion

Clinically, the recommended label dose of medetomidine as a sedative in cats is 40 to 80 μg/kg IM in cats [42]. Thus, 40 μg/kg medetomidine and low dose of atipamezole and yohimbine were selected to compare the potency of these drugs on anti-diuretic effects by use of equal doses. Middle dose (160 μg/kg) of atipamezole was clinically recommended dose to antagonize sedative effect of medetomidine [51], and high dose was decided based on a previous study [53]. To the best of our knowledge, there is no report on the effect of prazosin that may influence on the diuresis in cats. We expected that prazosin probably does not have the antidiuretic effect in the preliminary study. Therefore, we used a dose of prazosin to compare the potencies of other 2 drugs.

The results of this study indicated that both atipamezole and yohimbine, but not prazosin, displayed antagonistic effects against medetomidine-induced diuresis in healthy cats. To our knowledge, this is the first study to report the effect of α-adrenoceptor antagonists on medetomidine-induced diuresis in cats. These effects were consistent with previous results regarding the antagonistic action of atipamezole or yohimbine on medetomidine- or xylazine-induced diuresis in dogs [46, 47]. Prazosin has been known to cause an inconsistent decrease in the elevated urinary output caused by clonidine, guanabenz, guanfacine [1, 41] and xylazine [28] administration in rats, and it is known to be a highly selective α_1-adrenoceptor antagonist [14]. Our results indicated that the medetomidine-induced diuretic effect in cats was not mediated by the α_1-adrenoceptor because it was not antagonized by prazosin. In the present study, yohimbine dose-dependently inhibited medetomidine-induced diuresis, in contrast to atipamezole, at the tested doses. Atipamezole is known to be a highly selective and specific antagonist compared

with yohimbine for centrally and peripherally located α_2-adrenoceptors [56]. In addition, atipamezole has an imidazoline ring structure in contrast to yohimbine [31]. Therefore, the difference in atipamezole and yohimbine responses may have been attributable to the differences in the affinity and selectivity of the α_2-adrenoceptor and/or imidazoline receptors.

In the present study, urine pH did not significantly change in response to any of the treatments. In contrast, urine specific gravity and osmolality decreased significantly in proportion to the increase in urine volume after medetomidine administration. These effects were consistent with the results of previous studies in cats [30]. The changes in urine specific gravity and osmolality also corresponded to the antidiuretic action of atipamezole and yohimbine. In addition, both atipamezole and yohimbine caused dose-dependent reversals in the medetomidine-induced changes in urine specific gravity and osmolality. Higher doses of atipamezole and yohimbine tended to accelerate recovery from the decreases in urine specific gravity and osmolality.

Osmolar clearance in the present study did not significantly change in any of the groups. In contrast, there was an almost perfect correlation between the increase in free-water clearance and the increase in urine volume after medetomidine administration. Both atipamezole and yohimbine, but not prazosin, caused dose-dependent inhibitions of the medetomidine-induced increase in free-water clearance. A previous study on rats proposed that xylazine caused an increase in osmolar clearance, but significant change in free-water clearance was also observed [28]. It has also been reported that low doses of clonidine induced increased water excretion in rats and higher clonidine doses increased sodium and potassium excretion, in addition to water excretion [1]. Moreover, it has been reported that clonidine induced diuresis and increased both free-water and osmolar clearance, but the clonidine-induced increase in free-water clearance was blocked by prazosin [16]. However, our results in cats revealed that the diuretic effect of

medetomidine was caused only by an increase in free-water clearance, which was blocked by atipamezole and yohimbine, which are α_2-adrenoceptor antagonists, but not by prazosin, an α_1-adrenoceptor antagonist. Therefore, our results suggested that the mechanism of medetomidine-induced diuresis in cats differed from that in rats.

The present study revealed that plasma AVP concentrations significantly increased in the MED group after the medetomidine-induced diuretic effect had returned to the baseline value. However, the AUC value for plasma AVP from 0.5 to 2 h after treatment did not significantly change in the MED group compared with the SAL group. These findings were in agreement with previous reports that demonstrated that plasma AVP concentrations did not significantly decrease after medetomidine administration in cats [30] and dogs [45]. Furthermore, a previous study revealed that the increase in free-water clearance after clonidine administration was associated with a decrease in whole kidney aquaporin-2 mRNA concentrations and was independent of the changes in vasopressin activity in rats [17]. Therefore, the medetomidine-induced diuretic effect in the present study may have been independent of the changes in plasma AVP concentrations. Plasma AVP accelerates free-water reabsorption through its action on vasopressin V2 receptors in the distal nephron and subsequently promotes cAMP-dependent trafficking of aquaporin-2 water channels to the luminal membrane of principal cells [44]. It is well known that α_2-adrenoceptor agonists inhibit AVP-stimulated cAMP accumulation in the collecting tubes and the antidiuretic effect of AVP in rats [8, 9]. However, it has also been reported that, in contrast to rat kidneys, α_2-adrenoceptor agonists, including epinephrine and clonidine, did not inhibit AVP-induced cAMP formation in the inner medullary of cortical collecting tubule cells dissected from canine, laprine or human kidneys [9]. Therefore, there is a

difference between animal species in the ability of α_2-adrenoceptor agonists to inhibit AVP-induced cAMP formation at the tubular level.

In the present study, the plasma AVP concentration at 3 h post-treatment in the PRA group was significantly higher than that in the MED group. Plasma AVP is released in response to hypotension, hemorrhage and water deprivation [25]. In this study, the mean values of plasma osmolality increased significantly at 3 h post-treatment in the PRA group and similarly increased in the MED group. Prazosin is known to have a hypotensive effect attributed to the blockade of central and peripheral α_1-adrenoceptors [46]. Therefore, the difference in plasma AVP concentrations between the MED and PRA groups at 3 h post-treatment may have been attributable to differences in blood pressure. On the other hand, both atipamezole and yohimbine tended to prevent the increase in plasma AVP concentrations following the medetomidine-induced diuretic effect. Furthermore, the highest dose of both treatments caused a transient, but significant, increase in plasma AVP concentrations. An overdose of α_2-adrenoceptor antagonists is known to cause neurological (i.e., excitement and muscle tremors) and cardiovascular (i.e., hypotension and tachycardia) side effects [24]. Although the precise mechanisms of the increase in plasma AVP following the administration of higher doses of atipamezole and yohimbine are unknown, this increase may be involved in the antidiuretic action of both agents in medetomidine-induced diuresis in cats. However, our results showed that the medetomidine-induced diuretic effect was inhibited by both atipamezole and yohimbine, except at the highest doses of both agents. In addition, there was no association between the total urine volume and the AUC of plasma AVP from 0.5 to 2 h after treatment in any group. An *in vitro* study revealed that α_2-adrenoceptor agonists (i.e., dexmedetomidine and clonidine) inhibited AVP-stimulated osmotic water permeability, which was reversed by the α_2-adrenoceptor antagonists yohimbine

and atipamezole, but not prazosin, in the rat collecting duct [22]. Therefore, the antagonistic effects of atipamezole and yohimbine on medetomidine-induced diuresis observed in this study may have been attributed to changes in water permeability of the α_2-adrenoceptors in the collecting duct.

Plasma sodium concentration increased significantly and similarly in the MED and PRA groups after peak diuresis compared with the baseline values. A previous study showed that plasma sodium concentrations did not significantly increase after IM administration of 40 μg/kg medetomidine in cats [30]. Thus, this difference may be attributable to the fluid infusion to the cats in the previous study. Both atipamezole and yohimbine prevented the medetomidine-induced increase in plasma sodium concentrations. These results were consistent with those of a previous study in dogs [47]. In the present study, plasma potassium and chloride concentrations did not significantly change in any group. A previous study reported that plasma potassium and chloride concentrations significantly increased after IM administration of 20 μg/kg medetomidine and higher doses of atipamezole and yohimbine prevented the medetomidine-induced increase in plasma potassium and chloride concentrations in dogs [47]. In the present study, our result showed that, in contrast to dogs, plasma potassium concentrations did not increase in cats. Therefore, we propose that plasma ionic regulation to maintain appropriate plasma potassium and chloride concentrations is well controlled in cats compared with dogs.

Fractional clearance of sodium, potassium and chloride increased after diuresis and peaked following medetomidine administration, suggesting that this event was a rebound phenomenon. High doses of both atipamezole and yohimbine tended to prevent the medetomidine-induced increases in fractional clearance of sodium, potassium and chloride in the present study. These results indicated that the antidiuretic actions of atipamezole and yohimbine contributed to

decrease the medetomidine-induced changes in fractional clearances of sodium, potassium and chloride. Fractional electrolyte excretion tests have been used to evaluate renal dysfunction, particularly tubule impairments, in veterinary nephrology [23]. Therefore, when medetomidine is administered in cats, its influence on the interpretation of urinalysis results should be considered, even if α_2-adrenoceptor antagonists are used.

In the present study, 3 different doses of both atipamezole and yohimbine were evaluated for determination of the effective dose in antagonizing medetomidine-induced diuresis in normal cats. Administration of 40 μg/kg medetomidine yielded a potent diuresis, and subsequently caused dehydration in cats. It is recommended that medetomidine-induced diuresis should be antagonized, especially in cats with urinary tract obstruction, dehydration or hypovolemia.

In conclusion, both atipamezole and yohimbine, but not prazosin, showed profound antidiuretic effects against medetomidine-induced diuresis. Although atipamezole did not cause dose-dependent inhibitions on diuresis, it had a greater inhibitory effect than yohimbine at our tested doses. The medetomidine-induced diuretic effect in cats may be mediated by α_2-adrenoceptors, but not by α_1-adrenoceptors. Furthermore, increases in plasma AVP concentrations after administration of high-dose atipamezole and yohimbine may be involved in the antidiuretic actions of both agents for medetomidine-induced diuresis in cats. Therefore, both drugs can be used as antagonists against medetomidine-induced diuresis in healthy cats.

Chapter 3

Antagonistic effects of atipamezole, yohimbine, and prazosin on xylazine-induced diuresis in healthy cats

Introduction

The effects of α_2-adrenoceptor agonists in cats include reliable, dose-dependent sedation, analgesia, and muscle relaxation [24]. Pharmacologically, the α_2-adrenoceptors have been classified into 3 subtypes (α_{2A}, α_{2B}, and α_{2C}) based on the differences in drug affinity [4]. The α_{2D}-adrenoceptors in cattle and rodents are considered as a species homologue of the α_{2A}-adrenoceptors [2]. Xylazine has been widely used as a sedative in veterinary practice [12]. The α_2/α_1 adrenoceptor selectivity ratio of xylazine is 160:1 [57], whereas it does not have selectivity for the 3 α_2-adrenoceptor subtypes [39].

Xylazine is known to induce diuresis in dogs [45, 46], cattle [48], horses [32, 49, 50, 58], and rats [5, 6, 26-29]. Recently, we found that both medetomidine and xylazine induce profound diuresis in cats by decreasing the reabsorption of water in the kidneys [30]. In a previous study, we demonstrated that xylazine had a dose-dependent diuretic effect, but medetomidine did not, suggesting that some factors such as the difference in α_2- and α_1- adrenoceptor selectivity or imidazoline receptor selectivity in the renal system may be involved in the diuretic mechanism. The α_2-adrenoceptor antagonists atipamezole and yohimbine have been clinically used to reverse the sedative and analgesic effects of α_2-adrenoceptor agonists [24, 40]. The α_2/α_1 adrenoceptor selectivity ratios of atipamezole and yohimbine are 8,526:1 and 40:1, respectively [56]. The affinities of atipamezole and yohimbine are similar for the α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors, but atipamezole has an approximately 100-fold higher affinity for the α_{2D}- adrenoceptor compared to yohimbine [40]. Prazosin has a selectivity of 1,000:1 for the α_1/α_2 adrenoceptor [14]. In contrast, atipamezole has an imidazoline structure, whereas yohimbine and prazosin are non-imidazoline

agents [52]. These differences among atipamezole, yohimbine, and prazosin may influence their antagonistic effects for xylazine-induced diuresis in cats.

Because the regulation of water excretion has implications for a number of clinical situations, xylazine-induced diuresis will influence on hydration conditions in normal cats. Especially, urinary tract obstruction, dehydration, or hypovolemia may limit the use of xylazine in cats. In such cases, α_2-adrenoceptor antagonists may be used to reverse the diuretic actions. Therefore, it is important to examine the antagonistic effects for urination associated with xylazine use. However, to the best of our knowledge, there are no published reports on the effects of α-adrenoceptor antagonists on xylazine-induced diuresis in cats. This study aimed to investigate and compare the antagonistic effects of a single dose of prazosin and three different doses of either atipamezole or yohimbine on xylazine-induced diuresis in healthy cats.

Materials and Methods

Animals

Five healthy adult mixed-breed cats (1 sexually intact male, 2 neutered males and 1 sexually intact female and 1 neutered males) with a mean age of 2.8 y [standard deviation (*s*) = 0.84] and a mean weight of 3.4 (*s* = 0.76) kg were used in this study. They were fed a standard commercial dry food formulated for cats and raised in a laboratory with appropriate animal management facilities. Examinations performed prior to the experiments revealed that all cats were healthy, with physical examination, hematologic, and urinary values within respective reference limits. The study protocol was approved by the Animal Research Committee of Tottori University.

Experimental design and drug administration

The five cats were assigned to receive each of the 9 treatment groups in a modified randomized design. In group 1 each cat was given an IM administration of physiological saline solution (0.1 mL/kg) as non-medicated control treatment. In groups 2-9 each cat received an IM of 2 mg/kg xylazine hydrochloride as the 1st treatment (Celactal; Bayer Yakuhin, Ltd., Osaka, Japan) at the beginning of the experiment. In group 2 each cat received 2nd IV treatment of 0.1 mL/kg physiological saline, similarly group 3; 160 μg/kg prazosin hydrochloride (Sigma-Aldrich Japan K.K., Tokyo, Japan), group 4; 40 μg/kg atipamezole hydrochloride (Antisedan Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), group 5; 160 μg/kg atipamezole hydrochloride, group 6; 480 μg/kg atipamezole hydrochloride, group 7; 40 μg/kg yohimbine hydrochloride (Sigma-Aldrich Japan K.K., Tokyo, Japan), group 8; 160 μg/kg yohimbine hydrochloride, and group 9; 480 μg/kg yohimbine hydrochloride were administered 0.5 h later. Prazosin was dissolved in sterile water to obtain a concentration of 1.6 mg/mL. Yohimbine was dissolved in sterile water to obtain a concentration of 5.0 mg/mL. The groups are denoted as SAL, XYL, PRA, ATI 40, ATI 160, ATI 480, YOH 40, YOH 160, and YOH 480. A gap of at least 1 wk was maintained between successive treatments for each cat.

Food and water were withheld for 12 h before the start of each experiment. Food and water were provided after sample collection at 8 h after injection. The experiments were performed in a room in which the room temperature was maintained at 25°C.

Sample collection

A day before treatment, all cats were anesthetized using propofol (Propofol 1%; Intervet K.K, Tokyo, Japan), as described elsewhere [19]. A 17-gauge central venous catheter (SMAC plus;

Covidien Japan Inc., Tokyo, Japan) was introduced into a jugular vein of each cat. Lidocaine (Xylocaine injection 2%; AstraZeneca K.K., Osaka, Japan) was used to assist with local analgesia at the catheterization site. A 4-Fr polyvinyl chloride catheter (Atom Multipurpose Tube; Atom Medical Corp., Tokyo, Japan) and 6-Fr silicon balloon catheter (All Silicone Foley Balloon Catheter; Create Medic Co., Ltd. Kanagawa, Japan) were inserted in the urinary bladder of male and female cats, respectively. Each cat was subsequently placed in a separate cage. In order to keep bladder catheter, the cats were continually put on a diaper and elizabethan collar except for when cats were provided food and water. In addition, a soft catheter was used, and a tip of catheter was appropriately put in a bladder. One hour before the start of the experiments, the bladder of each cat was emptied by suction through the indwelling catheter in preparation for subsequent collection of urine sample. Urine and blood samples were collected 10 times (before injection of the treatment [time 0; baseline] and 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h after injection) from each cat. Blood samples (2.5 mL) and urine samples were collected from the central venous and urinary catheters, respectively. Behavioral responses were observed simultaneously with the collection of blood and urine samples. An aliquot (2.0 mL) of each blood sample was mixed with ethylene diamine tetraacetic acid for measurement of plasma arginine vasopressin (AVP) concentrations, and the remaining 0.5 mL was mixed with heparin for other measurements. Blood samples were immediately centrifuged at 2,000 × g at 4°C for 15 min, and the plasma was separated and stored at −80°C until analysis. Urine samples were centrifuged at 2,000 × g for 5 min, and the supernatant was subsequently collected and stored at −40°C until analysis.

Analytical methods

Urine volume, specific gravity, and pH; urine and plasma creatinine and electrolyte (sodium, potassium, and chloride) concentrations as well as osmolality; and plasma AVP concentrations were measured via procedures described elsewhere [45]. The osmolar clearance was calculated as follows: (urine osmolality × urine volume)/plasma osmolality. Free water clearance was calculated as follows: urine volume − osmolar clearance. The GFR was assessed via creatinine clearance and calculated as follows: (urine creatinine concentration × urine volume)/plasma creatinine concentration. In these formulas, urine volume was calculated from total urine volume for 0.5 or 1 h each time point. The fractional clearance of electrolytes was calculated as follows: (urine electrolyte concentration/plasma electrolyte concentration) × (plasma creatinine concentration/urine creatinine concentration) × 100.

Data evaluation

Statistical analysis was performed using commercially available statistical programs (GraphPad software version 5; GraphPad Software, Inc., San Diego, CA, USA). A 1-way ANOVA was used to examine the time effect within each treatment and the treatment effect at each time point. When a significant difference was detected, Tukey test was used to compare the means. Total urine volume was plotted against atipamezole or yohimbine doses and simple linear regression analysis was applied. When a significant difference was detected, the effect of the drug was considered to be dose-related. The AUC was measured by calculating the sum of trapezoids formed by the data points. Pearson's correlation coefficient was used to examine the correlation between the total urine volume and AUC of plasma AVP. Results were expressed as mean ± standard error. For all tests, values of $P < 0.05$ were considered significant.

Results

All cats in the XYL and PRA groups showed transiently profound sedation including sternal and lateral recumbency for approximately 1 h, and subsequently light sedation with slight drowsiness for 2 to 3 h after xylazine administration. Both atipamezole and yohimbine dose-dependently antagonized the sedative effect of xylazine, but the highest dose of both treatments transiently induced abnormal behaviors, such as excitement, vocalization, salivation, or defecation. Vomiting was observed before sedation in all xylazine-administered cats.

For urine and plasma variables, there were no significant differences between the groups at baseline. No significant changes in urine volume or other biochemical and hormonal variables were observed in the SAL group. Urine volume significantly and similarly increased in the XYL and PRA groups compared with the baseline values (Fig. 12A). The peak diuresis was observed at 2 h after injection. Comparison with the peak mean value of urine volume at 2 h with the XYL group showed that the all ATI groups and YOH 480 group significantly inhibited xylazine-induced diuresis (Fig. 12B, 12C). Total urine volume for 0.5 to 5 h significantly increased in the XYL and PRA groups compared with SAL group (Fig. 13A). The ATI 160, 480, and YOH 480 groups showed significantly inhibited xylazine-induced increases in the total urine volume. The linear regression of the total urine volume for 0.5 to 5 h after injection was significant in the YOH groups (Fig. 13C), but not in the ATI groups (Fig. 13B), indicating that yohimbine dose-dependently inhibited xylazine-induced diuresis, in contrast to atipamezole, at the tested doses. Similar results were observed in case of linear regression analysis of the total urine volume from 0.5 to 2, 0.5 to 3, 0.5 to 4, 0.5 to 6, 0.5 to 7, and 0.5 to 8 h.

Urine pH significantly increased at 6 h in the XYL group and 5 h in the PRA group (Table 3). In both the ATI and YOH groups, urine pH did not significantly change. Urine specific gravity significantly decreased from 1 to 4 h in both the XYL and PRA groups compared with baseline values. The ATI 480 and YOH 480 groups showed significantly inhibited xylazine-induced decreases in urine specific gravity.

Urine osmolality significantly and similarly decreased in the XYL and PRA groups compared with baseline values (Table 4). The ATI 160, 480, and YOH 480 groups significantly inhibited xylazine-induced decrease in urine osmolality. Plasma osmolality did not significantly change in any of the groups compared with the baseline values, but the mean value at 3 h in the XYL group significantly increased compared with that in the SAL group. The mean value at 3 h in the YOH 480 group was significantly lower than that in the XYL group. Osmolar clearance did not significantly change in any of the groups. Free water clearance significantly and similarly increased in the XYL and PRA groups compared with the baseline values. All doses of ATI and YOH 480 groups significantly inhibited the xylazine-induced increase in free water clearance. GFR did not significantly change in any of the groups.

Plasma AVP concentrations significantly and similarly increased at 4 to 7 h in the XYL and PRA groups compared with the baseline values after the xylazine-induced increase in urine volume had stopped (returned to the baseline) (Fig. 14A). Plasma AVP concentrations in the ATI 480 and YOH 480 groups significantly increased at 1 h compared with the value in the XYL group (Fig. 14B, 14C). The AUC value for plasma AVP from 0.5 to 2 h significantly increased in the ATI 480 and YOH 480 groups compared with the SAL or XYL group (Fig. 15). There were no correlations between the total urine volume and the AUC of plasma AVP from 0.5 to 2 h in any of the groups.

Plasma sodium, potassium, and chloride concentrations did not significantly change in any of the groups (Table 5). The fractional clearance of sodium tended to increase at 3 to 5 h similarly in the XYL and PRA groups. The mean value of the fractional clearance of sodium at 4 h in the XYL group significantly increased compared with the value in the SAL group. The fractional clearance of sodium did not significantly change in any of the ATI groups. Significant increases were also observed at 3 h in the YOH 160 group and at 4 h in the YOH 40 group compared with the baseline values. The fractional clearance of potassium significantly increased at 5 to 8 h in the PRA group compared with the baseline values. The fractional clearance of potassium significantly decreased at 4 h in the ATI 160 and ATI 480 groups and at 4 and 7 h in the YOH 480 group compared with the XYL group. The fractional clearance of chloride did not significantly change in any of the groups.

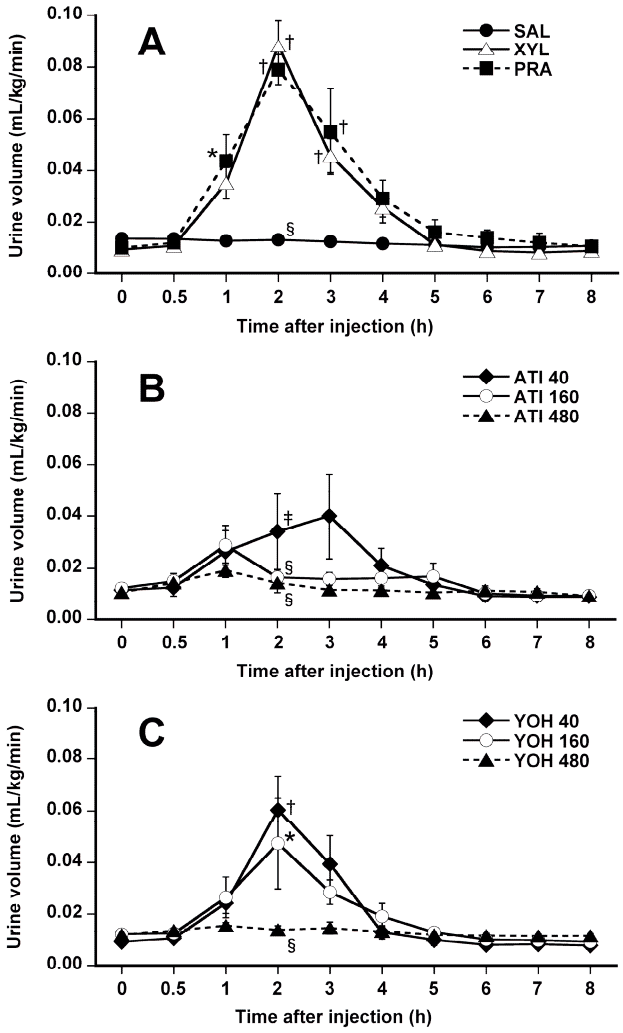

Fig. 12. Urine volume (mean ± standard error) of five cats before and after injection of A) physiological saline (SAL), xylazine followed by saline (XYL), prazosin (PRA); B) atipamezole (ATI); C) yohimbine (YOH), the last two drugs in doses of 40, 160, and 480 µg/kg. Time of 1st injection was designated as time 0 (baseline). *†Value differs significantly (* $p < 0.05$; † $p < 0.01$) from the baseline value. ‡§Within a time point, value differed significantly (‡ $p < 0.05$; § $p < 0.01$) from the XYL value.

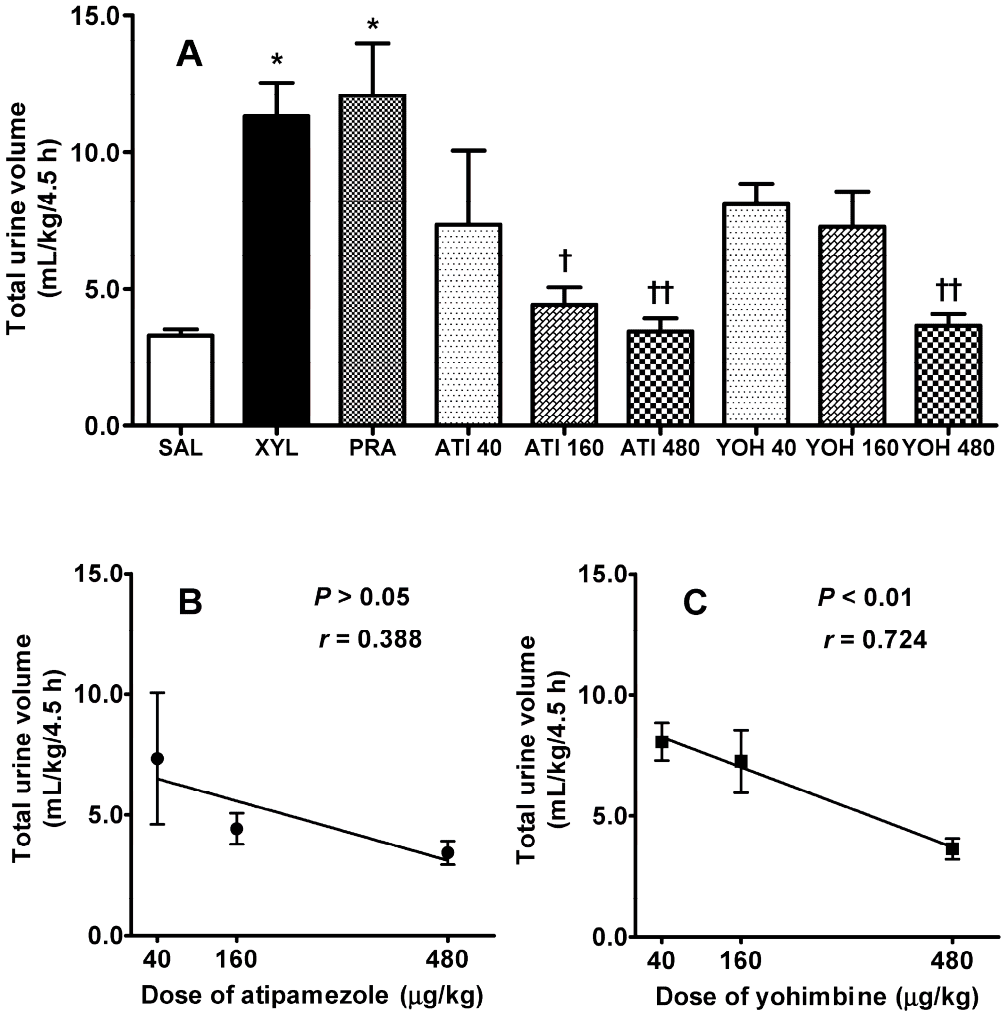

Fig. 13. Total urine volume (mean ± standard error) of five cats at 0.5 to 5 h post-injection of prazosin or various doses of atipamezole or yohimbine (A). Simple linear regression analysis of the total urine volume of five cats at 0.5 to 5 h after injection of various atipamezole doses (B) or yohimbine (C). See Fig. 12 for the remainder of the key.

Table 3. Urine pH and urine specific gravity in cats after an intramuscular injection of saline (SAL) 0.1 mL/kg or xylazine 2 mg/kg followed 0.5 h later by an intravenous injection of saline (XYL) 0.1 mL/kg, prazosin (PRA) 160 µg/kg, atipamezole (ATI) 40, 160, or 480 µg/kg, or yohimbine (YOH) 40, 160, or 480 µg/kg

Variable	Time after xylazine injection (h)									
Group	0	0.5	1	2	3	4	5	6	7	8
Urine pH										
SAL	6.3 ± 0.3	6.3 ± 0.3	6.4 ± 0.3	6.6 ± 0.4	6.4 ± 0.3	6.4 ± 0.3	6.1 ± 0.2	6.3 ± 0.2	6.3 ± 0.2	6.4 ±0.1
XYL	6.2 ± 0.1	6.3 ± 0.1	6.9 ± 0.2	7.0 ± 0.1	7.0 ± 0.2	7.0 ± 0.1	6.8 ± 0.2	7.2 ± 0.3*	7.1 ± 0.3	6.8 ±0.2
PRA	6.5 ± 0.2	6.3 ± 0.1	6.9 ± 0.2	6.6 ± 0.2	6.9 ± 0.1	7.2 ± 0.1	7.5 ± 0.1*	7.2 ± 0.2	7.0 ± 0.2	6.8 ±0.3
ATI 40	6.0 ± 0.3	6.2 ± 0.2	6.5 ± 0.2	6.7 ± 0.3	6.6 ± 0.3	6.7 ± 0.3	6.7 ± 0.4	6.8 ± 0.5	6.8 ± 0.5	6.7 ±0.4
ATI 160	6.1 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.6 ± 0.3	6.4 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.5 ± 0.2	6.3 ± 0.2	6.4 ±0.1
ATI 480	6.1 ± 0.1	6.3 ± 0.2	6.4 ± 0.2	6.7 ± 0.3	6.7 ± 0.4	6.6 ± 0.4	6.5 ± 0.3	6.5 ± 0.2	6.5 ± 0.1	6.3 ±0.1
YOH 40	6.0 ± 0.2	6.0 ± 0.1	6.2 ± 0.1	6.3 ± 0.2	6.4 ± 0.1	6.6 ± 0.2	6.4 ± 0.2	6.4 ± 0.1	6.2 ± 0.2	6.1 ±0.1
YOH 160	5.9 ± 0.1	6.0 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.3 ± 0.2	6.5 ± 0.2	6.5 ± 0.2	6.5 ± 0.2	6.5 ± 0.2	6.4 ±0.1
YOH 480	5.8 ± 0.2	6.1 ± 0.2	6.2 ± 0.2	6.2 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	6.0 ± 0.1	6.3 ± 0.2	6.4 ± 0.2	6.4 ±0.2
Urine specific gravity										
SAL	1.035 ± 0.003	1.035 ± 0.003	1.036 ± 0.003	1.037 ± 0.003§	1.039 ± 0.002§	1.037 ± 0.002	1.035 ± 0.002	1.037 ± 0.002	1.037 ± 0.002	1.035 ±0.003
XYL	1.043 ± 0.006	1.038 ± 0.004	1.016 ± 0.002†	1.007 ± 0.001†	1.011 ± 0.002†	1.018 ± 0.003†	1.030 ± 0.002	1.035 ± 0.003	1.036 ± 0.004	1.039 ±0.003
PRA	1.034 ± 0.003	1.032 ± 0.002	1.012 ± 0.003†	1.003 ± 0.001†	1.009 ± 0.002†	1.015 ± 0.004†	1.021 ± 0.003	1.024 ± 0.002	1.030 ± 0.004	1.032 ±0.003
ATI 40	1.031 ± 0.001	1.029 ± 0.002	1.022 ± 0.004	1.025 ± 0.005	1.019 ± 0.005	1.021 ± 0.004	1.026 ± 0.003	1.029 ± 0.002	1.033 ± 0.002	1.035 ±0.003
ATI 160	1.033 ± 0.002	1.030 ± 0.004	1.021 ± 0.005	1.027 ± 0.006	1.025 ± 0.004	1.024 ± 0.004	1.026 ± 0.005	1.031 ± 0.003	1.035 ± 0.002	1.036 ±0.001
ATI 480	1.045 ± 0.003	1.041 ± 0.005	1.033 ± 0.006	1.038 ± 0.005§	1.040 ± 0.005§	1.041 ± 0.005‡	1.041 ± 0.004	1.041 ± 0.004	1.039 ± 0.002	1.042 ±0.003
YOH 40	1.039 ± 0.004	1.032 ± 0.001	1.022 ± 0.004	1.008 ± 0.003†	1.010 ± 0.004†	1.026 ± 0.005	1.034 ± 0.005	1.041 ± 0.004	1.041 ± 0.005	1.045 ±0.005
YOH 160	1.035 ± 0.003	1.037 ± 0.003	1.023 ± 0.006	1.016 ± 0.005	1.017 ± 0.003	1.021 ± 0.004	1.028 ± 0.003	1.031 ± 0.003	1.035 ± 0.002	1.037 ±0.003
YOH 480	1.038 ± 0.002	1.036 ± 0.002	1.030 ± 0.002	1.037 ± 0.002§	1.034 ± 0.003§	1.035 ± 0.003	1.034 ± 0.003	1.036 ± 0.002	1.035 ± 0.002	1.033 ±0.002

Data are shown as the mean ± standard error.

† Value differs significantly ($p < 0.05$; † $p < 0.01$) from the baseline value (0 h). ‡§ Within a time point, value differed significantly (‡ $p < 0.05$; § $p < 0.01$) from the XYL group.

Table 4. Plasma and urine osmolality (mmol/kg), osmolar clearance (mL/kg/min), free water clearance (mL/kg/min), and glomerular filtration rate (mL/kg/min) in the same experiment

Variable Group	Time after xylazine injection (h)									
	0	0.5	1	2	3	4	5	6	7	8
Urine osmolality										
SAL	1,543 ± 156	1,507 ± 153	1,518 ± 161‡	1,570 ± 146§	1,611 ± 135§	1,520 ± 82	1,433 ± 114	1,494 ± 112	1,459 ± 134	1,368 ± 145
XYL	1,808 ± 257	1,569 ± 189	631 ± 123†	236 ± 5†	464 ± 60†	845 ± 128†	1,426 ± 57	1,537 ± 87	1,480 ± 144	1,648 ± 109
PRA	1,623 ± 154	1,459 ± 115	631 ± 129†	206 ± 19†	489 ± 128†	863 ± 188*	1,112 ± 141	1,219 ± 106	1,476 ± 153	1,515 ± 140
ATI 40	1,371 ± 84	1,193 ± 76	944 ± 153	1,044 ± 205	828 ± 207	958 ± 177	1,183 ± 79	1,273 ± 84	1,345 ± 107	1,456 ± 118
ATI 160	1,509 ± 128	1,245 ± 168	921 ± 185	1,242 ± 224§	1,155 ± 187	1,076 ± 169	1,165 ± 188	1,387 ± 138	1,534 ± 118	1,540 ± 56
ATI 480	1,938 ± 152	1,636 ± 200	1,327 ± 203	1,593 ± 207§	1,784 ± 142§	1,759 ± 173‡	1,831 ± 124	1,804 ± 105	1,673 ± 70	1,781 ± 72
YOH 40	1,523 ± 108	1,360 ± 38	902 ± 160	367 ± 97†	457 ± 136†	1,082 ± 170	1,415 ± 187	1,617 ± 168	1,701 ± 215	1,833 ± 198
YOH 160	1,619 ± 125	1,564 ± 127	1,003 ± 260	768 ± 238	828 ± 144	1,022 ± 166	1,265 ± 132	1,319 ± 109	1,454 ± 140	1,489 ± 159
YOH 480	1,684 ± 102	1,515 ± 98	1,205 ± 71†	1,522 ± 109§	1,462 ± 92§	1,491 ± 87	1,394 ± 91	1,427 ± 102	1,333 ± 76	1,346 ± 92
Plasma osmolality										
SAL	318.6 ± 1.9	317.8 ± 1.7	317.6 ± 2.0	318.0 ± 2.2	316.8 ± 1.3‡	316.2 ± 1.0	317.0 ± 1.3	316.0 ± 1.1	317.4 ± 1.8	318.0 ± 1.2
XYL	319.2 ± 1.6	319.6 ± 1.1	323.0 ± 1.1	324.8 ± 1.7	326.6 ± 1.3	322.6 ± 1.5	322.6 ± 1.4	323.4 ± 1.5	323.0 ± 1.5	323.8 ± 1.3
PRA	317.2 ± 2.5	317.4 ± 2.8	319.4 ± 2.5	322.8 ± 2.5	323.8 ± 1.8	321.8 ± 2.6	321.6 ± 2.1	321.6 ± 2.1	321.6 ± 2.1	321.6 ± 2.0
ATI 40	318.0 ± 2.0	318.8 ± 2.2	321.2 ± 2.2	321.0 ± 1.9	320.4 ± 2.4	321.0 ± 2.7	321.8 ± 2.9	319.4 ± 3.0	320.6 ± 3.1	320.2 ± 3.1
ATI 160	316.6 ± 1.7	318.6 ± 2.2	317.6 ± 1.5	318.6 ± 1.9	317.6 ± 2.1	319.4 ± 1.7	318.2 ± 1.4	320.0 ± 2.1	319.6 ± 1.8	319.4 ± 1.4
ATI 480	318.4 ± 1.8	319.4 ± 2.1	323.2 ± 3.5	320.2 ± 3.2	319.0 ± 2.7	318.0 ± 2.1	320.6 ± 2.0	321.6 ± 1.6	321.4 ± 1.3	322.0 ± 2.0
YOH 40	317.6 ± 2.3	319.0 ± 1.5	319.4 ± 0.8	321.8 ± 1.9	319.4 ± 1.7	321.0 ± 1.7	321.8 ± 1.8	321.6 ± 2.0	320.2 ± 1.2	322.6 ± 1.1
YOH 160	315.4 ± 1.9	316.4 ± 1.1	316.4 ± 0.9	317.8 ± 1.2	318.2 ± 0.9	317.6 ± 0.5	317.2 ± 1.1	317.8 ± 1.2	317.4 ± 1.1	319.0 ± 1.9
YOH 480	317.2 ± 1.1	317.2 ± 1.2	321.2 ± 1.7	317.0 ± 0.7	316.6 ± 0.5‡	317.0 ± 1.3	317.6 ± 1.5	319.0 ± 1.3	319.6 ± 1.4	319.8 ± 2.0
Osmolar clearance										
SAL	0.065 ± 0.005	0.064 ± 0.006	0.061 ± 0.006	0.065 ± 0.007	0.064 ± 0.007	0.058 ± 0.009	0.052 ± 0.009	0.049 ± 0.008	0.048 ± 0.006	0.045 ± 0.004
XYL	0.055 ± 0.010	0.053 ± 0.008	0.059 ± 0.010	0.064 ± 0.008	0.060 ± 0.007	0.058 ± 0.009	0.049 ± 0.008	0.043 ± 0.003	0.038 ± 0.005	0.044 ± 0.004
PRA	0.050 ± 0.001	0.053 ± 0.003	0.066 ± 0.002	0.050 ± 0.005	0.056 ± 0.006	0.057 ± 0.009	0.048 ± 0.008	0.050 ± 0.008	0.049 ± 0.008	0.048 ± 0.007
ATI 40	0.049 ± 0.004	0.044 ± 0.009	0.059 ± 0.006	0.066 ± 0.006	0.056 ± 0.003	0.046 ± 0.007	0.048 ± 0.006	0.036 ± 0.003	0.037 ± 0.002	0.039 ± 0.001
ATI 160	0.059 ± 0.007	0.057 ± 0.008	0.064 ± 0.001	0.056 ± 0.006	0.052 ± 0.004	0.045 ± 0.003	0.046 ± 0.005	0.043 ± 0.002	0.043 ± 0.005	0.045 ± 0.002
ATI 480	0.066 ± 0.010	0.069 ± 0.006	0.072 ± 0.008	0.060 ± 0.006	0.061 ± 0.008	0.060 ± 0.004	0.060 ± 0.005	0.062 ± 0.009	0.056 ± 0.005	0.050 ± 0.004
YOH 40	0.044 ± 0.005	0.044 ± 0.004	0.061 ± 0.006	0.053 ± 0.006	0.037 ± 0.004	0.037 ± 0.002	0.038 ± 0.003	0.037 ± 0.002	0.040 ± 0.004	0.041 ± 0.004
YOH 160	0.062 ± 0.003	0.061 ± 0.006	0.062 ± 0.007	0.061 ± 0.007	0.066 ± 0.008	0.050 ± 0.005	0.049 ± 0.003	0.041 ± 0.004	0.043 ± 0.002	0.043 ± 0.005
YOH 480	0.062 ± 0.006	0.065 ± 0.005	0.057 ± 0.003	0.067 ± 0.009	0.064 ± 0.006	0.060 ± 0.004	0.051 ± 0.002	0.051 ± 0.004	0.047 ± 0.003	0.048 ± 0.004
Free water clearance										
SAL	- 0.051 ± 0.005	- 0.050 ± 0.006	- 0.048 ± 0.006	- 0.052 ± 0.007§	- 0.051 ± 0.007	- 0.046 ± 0.007	- 0.041 ± 0.008	- 0.039 ± 0.007	- 0.037 ± 0.005	- 0.035 ± 0.004
XYL	- 0.046 ± 0.010	- 0.042 ± 0.007	- 0.026 ± 0.009	0.024 ± 0.002*	- 0.014 ± 0.007	- 0.033 ± 0.008	- 0.038 ± 0.006	- 0.032 ± 0.003	- 0.029 ± 0.005	- 0.035 ± 0.003
PRA	- 0.039 ± 0.002	- 0.041 ± 0.002	- 0.022 ± 0.011	0.029 ± 0.005*	- 0.001 ± 0.017	- 0.028 ± 0.012	- 0.032 ± 0.004	- 0.036 ± 0.005	- 0.036 ± 0.005	- 0.037 ± 0.005
ATI 40	- 0.041 ± 0.003	- 0.031 ± 0.006	- 0.033 ± 0.005	- 0.032 ± 0.015‡	- 0.016 ± 0.014	- 0.025 ± 0.004	- 0.034 ± 0.004	- 0.027 ± 0.002	- 0.028 ± 0.002	- 0.031 ± 0.001
ATI 160	- 0.046 ± 0.006	- 0.042 ± 0.007	- 0.035 ± 0.007	- 0.040 ± 0.006§	- 0.036 ± 0.003	- 0.029 ± 0.004	- 0.030 ± 0.006	- 0.033 ± 0.002	- 0.034 ± 0.004	- 0.036 ± 0.002
ATI 480	- 0.055 ± 0.009	- 0.055 ± 0.007	- 0.052 ± 0.008	- 0.046 ± 0.005§	- 0.049 ± 0.006	- 0.048 ± 0.003	- 0.049 ± 0.004	- 0.051 ± 0.007	- 0.045 ± 0.004	- 0.041 ± 0.003
YOH 40	- 0.035 ± 0.005	- 0.034 ± 0.003	- 0.036 ± 0.007	0.008 ± 0.010*	0.003 ± 0.009*	- 0.024 ± 0.002	- 0.028 ± 0.002	- 0.029 ± 0.001	- 0.032 ± 0.003	- 0.034 ± 0.003
YOH 160	- 0.049 ± 0.003	- 0.049 ± 0.005	- 0.035 ± 0.009	- 0.014 ± 0.017	- 0.037 ± 0.009	- 0.031 ± 0.003	- 0.036 ± 0.003	- 0.031 ± 0.004	- 0.032 ± 0.003	- 0.033 ± 0.005
YOH 480	- 0.050 ± 0.005	- 0.051 ± 0.005	- 0.042 ± 0.002	- 0.053 ± 0.008§	- 0.050 ± 0.004	- 0.046 ± 0.002	- 0.039 ± 0.002	- 0.039 ± 0.004	- 0.036 ± 0.003	- 0.036 ± 0.003
Glomerular filtration rate										
SAL	2.42 ± 0.11	2.65 ± 0.14	2.54 ± 0.24	2.88 ± 0.34	2.71 ± 0.15	2.51 ± 0.24	2.21 ± 0.28	2.21 ± 0.19	2.24 ± 0.17	2.37 ± 0.27
XYL	2.34 ± 0.31	2.29 ± 0.13	2.46 ± 0.27	2.22 ± 0.19	1.84 ± 0.07	1.83 ± 0.13	1.72 ± 0.14	1.68 ± 0.09	1.57 ± 0.12	1.83 ± 0.08
PRA	2.21 ± 0.14	2.23 ± 0.20	2.29 ± 0.25	1.66 ± 0.16	2.00 ± 0.26	1.80 ± 0.22	1.64 ± 0.22	1.82 ± 0.15	1.80 ± 0.20	1.83 ± 0.17
ATI 40	1.91 ± 0.14	1.78 ± 0.23	2.27 ± 0.09	2.26 ± 0.10	2.27 ± 0.19	1.73 ± 0.23	1.92 ± 0.15	1.56 ± 0.12	1.77 ± 0.17	1.80 ± 0.09
ATI 160	2.25 ± 0.10	2.16 ± 0.17	2.43 ± 0.11	1.99 ± 0.13	1.87 ± 0.12	1.75 ± 0.14	1.71 ± 0.10	1.69 ± 0.09	1.72 ± 0.13	1.98 ± 0.16
ATI 480	2.63 ± 0.18	2.61 ± 0.10	2.41 ± 0.26	2.19 ± 0.13	2.11 ± 0.19	2.04 ± 0.06	2.06 ± 0.12	2.27 ± 0.19	2.21 ± 0.11	2.13 ± 0.09
YOH 40	2.18 ± 0.10	2.05 ± 0.19	2.66 ± 0.16	2.01 ± 0.11	1.60 ± 0.23	1.54 ± 0.12	1.53 ± 0.06	1.57 ± 0.04	1.71 ± 0.14	1.75 ± 0.19
YOH 160	2.49 ± 0.14	2.69 ± 0.15	2.24 ± 0.16	2.40 ± 0.31	2.09 ± 0.07	2.08 ± 0.12	2.04 ± 0.10	1.99 ± 0.08	2.25 ± 0.17	2.20 ± 0.19
YOH 480	2.15 ± 0.17	2.25 ± 0.17	2.23 ± 0.11	2.55 ± 0.22	2.35 ± 0.10	2.39 ± 0.10	2.18 ± 0.15	2.23 ± 0.07	2.18 ± 0.11	2.14 ± 0.12

Data are shown as the mean ± standard error.

† Value differs significantly ($p < 0.05$; † $p < 0.01$) from the baseline value (0 h). ‡§ Within a time point, value differed significantly (‡ $p < 0.05$; § $p < 0.01$) from the XYL group.

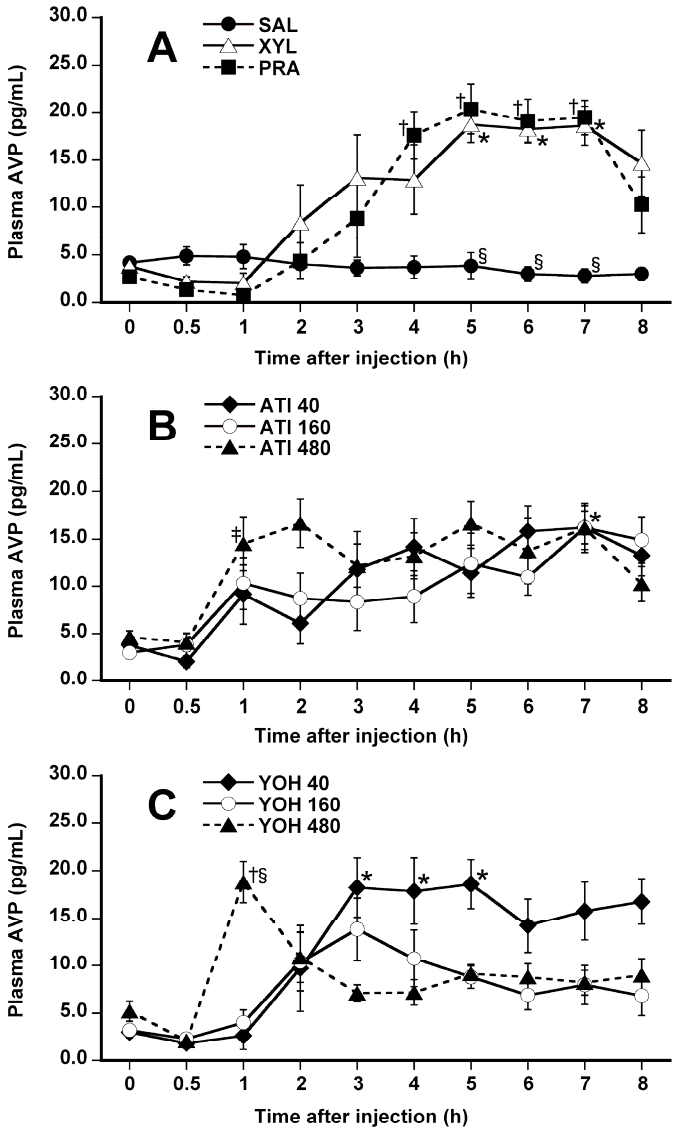

Fig. 14. Plasma AVP concentration (mean ± standard error) of five cats (A, B, and C) before and after injection of physiological saline or medetomidine. See Fig. 12 for the remainder of the key.

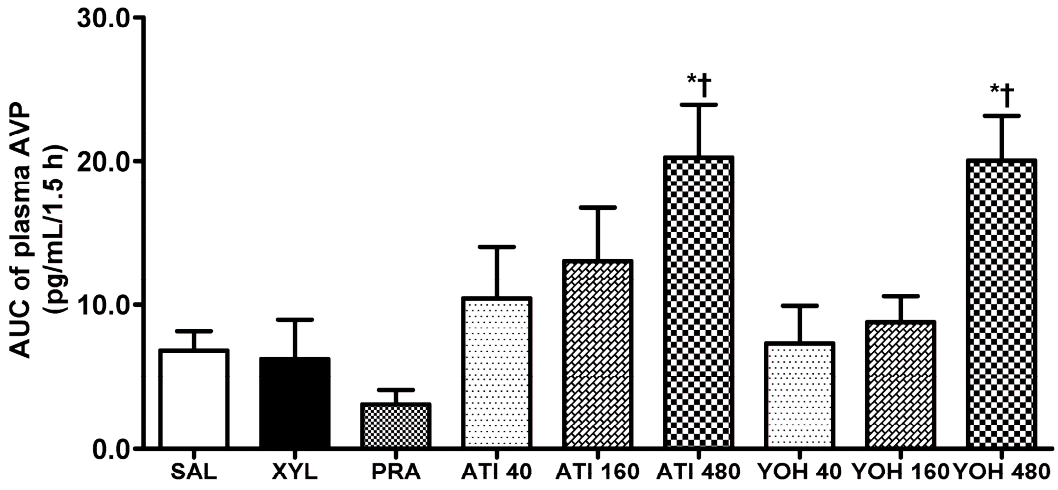

Fig. 15. Area under the curve (AUC) data for plasma AVP concentration for 5 cats after injection of the prazosin or various doses of atipamezole or yohimbine from 0.5 to 2 h. The symbols indicate a significant difference from the value for the SAL group (*, $P < 0.05$) or from the value for the XYL group (†, $P < 0.05$).

Table 5. Plasma sodium,potassium, and chloride concentrations (mmol/L) and fractional clearance of sodium,potassium, and chloride (%) in the same experiment

Variable	Group	Time after xylazine injection (h)									
		0	0.5	1	2	3	4	5	6	7	8
Plasma sodium	SAL	154.2 ± 0.7	154.0 ± 0.6	154.4 ± 1.0	154.6 ± 0.8	154.4 ± 0.7	154.2 ± 0.7	154.8 ± 0.8	155.0 ± 0.7	155.0 ± 0.7	155.2 ± 0.7
	XYL	154.0 ± 0.8	152.2 ± 1.1	151.6 ± 1.0	153.0 ± 0.9	154.2 ± 0.7	154.2 ± 1.2	155.4 ± 0.8	155.0 ± 0.6	155.0 ± 0.7	154.8 ± 0.9
	PRA	153.2 ± 0.5	150.8 ± 0.9	151.2 ± 1.0	152.0 ± 1.0	153.4 ± 0.9	154.8 ± 0.5	155.0 ± 0.5	154.8 ± 0.7	154.2 ± 0.5	154.0 ± 0.3
	ATI 40	156.0 ± 0.9	153.0 ± 0.6	153.8 ± 0.9	153.6 ± 0.8	155.8 ± 1.2	156.0 ± 1.0	156.8 ± 0.8	155.8 ± 0.9	155.8 ± 1.0	156.0 ± 1.2
	ATI 160	154.2 ± 0.7	151.8 ± 0.4	153.8 ± 0.7	152.8 ± 0.8	153.0 ± 0.7	153.2 ± 0.4	153.8 ± 0.7	153.4 ± 0.6	154.2 ± 0.6	153.6 ± 0.7
	ATI 480	155.6 ± 1.7	152.8 ± 1.2	155.4 ± 1.3	156.6 ± 1.9	155.0 ± 1.1	154.8 ± 1.0	154.8 ± 1.4	155.0 ± 0.9	154.6 ± 0.7	154.8 ± 0.8
	YOH 40	153.2 ± 0.9	150.8 ± 1.1	151.2 ± 1.0	153.4 ± 0.6	153.8 ± 0.7	154.8 ± 0.8	154.0 ± 0.6	154.8 ± 0.9	154.4 ± 0.6	154.8 ± 0.6
	YOH 160	154.0 ± 0.6	152.2 ± 0.6	154.2 ± 0.7	154.2 ± 0.7	154.6 ± 1.2	155.2 ± 1.0	155.4 ± 1.1	155.6 ± 1.0	154.8 ± 1.1	155.6 ± 1.0
	YOH 480	154.2 ± 0.8	153.2 ± 0.9	155.8 ± 0.7	154.8 ± 0.6	155.0 ± 0.6	154.8 ± 1.0	154.8 ± 0.7	155.2 ± 0.7	155.6 ± 0.9	155.6 ± 0.9
Plasma potassium	SAL	3.72 ± 0.09	3.66 ± 0.08	3.66 ± 0.05	3.78 ± 0.13	3.90 ± 0.12	3.76 ± 0.12	3.82 ± 0.13	3.80 ± 0.14	3.78 ± 0.14	3.72 ± 0.13
	XYL	3.56 ± 0.09	3.66 ± 0.07	3.76 ± 0.05	3.74 ± 0.13	3.76 ± 0.13	3.74 ± 0.06	3.76 ± 0.10	3.84 ± 0.11	3.72 ± 0.13	3.86 ± 0.10
	PRA	3.76 ± 0.21	3.68 ± 0.11	3.60 ± 0.11	3.72 ± 0.16	3.52 ± 0.19	3.54 ± 0.17	3.56 ± 0.12	3.48 ± 0.21	3.54 ± 0.16	3.64 ± 0.09
	ATI 40	3.84 ± 0.18	3.80 ± 0.04	3.70 ± 0.20	3.82 ± 0.12	3.68 ± 0.07	3.72 ± 0.08	3.90 ± 0.19	3.74 ± 0.07	3.70 ± 0.06	3.76 ± 0.15
	ATI 160	3.68 ± 0.17	3.60 ± 0.09	3.44 ± 0.15	3.54 ± 0.09	3.66 ± 0.10	3.88 ± 0.15	3.76 ± 0.13	3.84 ± 0.16	3.88 ± 0.16	3.80 ± 0.19
	ATI 480	4.02 ± 0.14	3.84 ± 0.12	3.46 ± 0.19	3.76 ± 0.26	4.02 ± 0.18	4.00 ± 0.12	3.84 ± 0.10	3.80 ± 0.07	3.82 ± 0.06	3.78 ± 0.12
	YOH 40	3.66 ± 0.18	3.70 ± 0.15	3.82 ± 0.15	3.72 ± 0.15	3.74 ± 0.24	3.90 ± 0.17	3.82 ± 0.15	3.78 ± 0.11	3.84 ± 0.24	3.86 ± 0.23
	YOH 160	3.88 ± 0.11	3.64 ± 0.07	3.62 ± 0.13	3.70 ± 0.08	3.66 ± 0.17	3.72 ± 0.11	3.68 ± 0.12	3.74 ± 0.10	3.68 ± 0.07	3.76 ± 0.08
	YOH 480	3.76 ± 0.14	3.84 ± 0.06	3.42 ± 0.18	3.70 ± 0.17	3.94 ± 0.08	4.06 ± 0.20	3.86 ± 0.13	3.90 ± 0.14	4.06 ± 0.15	3.84 ± 0.18
Plasma chloride	SAL	117.8 ± 0.8	117.2 ± 0.7	117.6 ± 0.6	116.8 ± 0.3	118.0 ± 0.7	118.0 ± 0.3	118.2 ± 0.2	118.4 ± 0.2	118.8 ± 0.5	118.6 ± 0.5
	XYL	116.6 ± 1.8	115.2 ± 2.1	114.2 ± 2.4	115.8 ± 1.6	116.6 ± 1.8	116.6 ± 1.6	117.4 ± 1.9	117.8 ± 1.8	117.6 ± 1.6	118.4 ± 1.7
	PRA	118.0 ± 1.7	115.6 ± 2.0	115.8 ± 1.6	115.8 ± 2.4	117.8 ± 2.2	118.8 ± 1.8	119.4 ± 1.7	119.4 ± 1.8	118.6 ± 1.8	119.8 ± 1.4
	ATI 40	120.4 ± 0.9	118.6 ± 0.7	119.0 ± 0.5	118.4 ± 1.0	119.6 ± 0.9	120.6 ± 0.7	121.6 ± 0.2	120.6 ± 0.8	120.8 ± 0.8	121.2 ± 1.0
	ATI 160	117.6 ± 1.1	115.8 ± 1.0	117.2 ± 0.6	117.6 ± 0.7	118.0 ± 0.7	117.8 ± 0.9	118.4 ± 0.7	118.2 ± 0.7	119.0 ± 0.4	120.0 ± 0.9
	ATI 480	119.8 ± 1.5	117.0 ± 1.5	118.4 ± 1.1	119.8 ± 1.2	119.8 ± 1.0	119.0 ± 0.8	119.0 ± 0.7	118.8 ± 0.9	118.2 ± 0.6	119.4 ± 0.8
	YOH 40	117.6 ± 1.9	115.2 ± 2.1	115.6 ± 2.1	117.2 ± 1.7	118.0 ± 2.0	119.6 ± 2.1	119.0 ± 1.9	120.0 ± 1.5	119.6 ± 2.2	120.0 ± 1.7
	YOH 160	119.0 ± 0.7	117.2 ± 0.8	118.2 ± 0.9	118.6 ± 0.9	119.2 ± 1.2	120.2 ± 1.1	119.4 ± 1.2	120.4 ± 1.4	119.6 ± 1.3	120.6 ± 1.4
	YOH 480	118.2 ± 1.0	117.0 ± 1.0	118.4 ± 1.1	118.8 ± 1.4	118.6 ± 1.1	119.6 ± 1.3	119.2 ± 1.2	119.4 ± 1.4	120.2 ± 1.6	121.0 ± 1.3
Fractional clearance of sodium	SAL	0.31 ± 0.05	0.28 ± 0.04	0.29 ± 0.03	0.27 ± 0.02	0.26 ± 0.03‡	0.25 ± 0.04	0.27 ± 0.07	0.28 ± 0.06	0.29 ± 0.06	0.31 ± 0.07
	XYL	0.26 ± 0.07	0.26 ± 0.05	0.51 ± 0.11	0.76 ± 0.22	1.31 ± 0.31	1.42 ± 0.49	0.87 ± 0.21	0.73 ± 0.21	0.62 ± 0.16	0.62 ± 0.16
	PRA	0.39 ± 0.15	0.31 ± 0.11	0.71 ± 0.22	0.67 ± 0.18	0.94 ± 0.20	1.37 ± 0.37	1.06 ± 0.34	0.95 ± 0.19	0.70 ± 0.19	0.65 ± 0.14
	ATI 40	0.29 ± 0.05	0.31 ± 0.04	0.55 ± 0.08	0.77 ± 0.12	0.77 ± 0.18	1.06 ± 0.36	0.89 ± 0.27	0.76 ± 0.27	0.56 ± 0.15	0.54 ± 0.09
	ATI 160	0.26 ± 0.07	0.30 ± 0.06	0.52 ± 0.10	0.69 ± 0.09	0.79 ± 0.17	0.66 ± 0.10	0.73 ± 0.12	0.74 ± 0.12	0.63 ± 0.17	0.39 ± 0.09
	ATI 480	0.34 ± 0.11	0.35 ± 0.08	0.67 ± 0.19	0.95 ± 0.36	0.85 ± 0.21	0.91 ± 0.21	0.90 ± 0.21	0.78 ± 0.17	0.65 ± 0.17	0.50 ± 0.19
	YOH 40	0.11 ± 0.02	0.17 ± 0.03	0.25 ± 0.03	0.43 ± 0.06	0.35 ± 0.09	0.66 ± 0.16*	0.61 ± 0.15	0.48 ± 0.11	0.39 ± 0.09	0.37 ± 0.11
	YOH 160	0.23 ± 0.03	0.23 ± 0.03	0.47 ± 0.08	0.57 ± 0.11	1.16 ± 0.22†	0.75 ± 0.13	0.69 ± 0.13	0.49 ± 0.10	0.36 ± 0.06	0.32 ± 0.05
	YOH 480	0.25 ± 0.03	0.29 ± 0.05	0.39 ± 0.06	0.44 ± 0.10	0.50 ± 0.14	0.38 ± 0.08	0.52 ± 0.11	0.49 ± 0.11	0.41 ± 0.11	0.42 ± 0.09
Fractional clearance of potassium	SAL	16.5 ± 1.0	14.8 ± 0.9	16.5 ± 2.0	15.0 ± 1.8	13.8 ± 1.4	14.7 ± 1.2	14.0 ± 0.9	14.6 ± 1.6	12.8 ± 1.4	11.3 ± 1.4
	XYL	13.5 ± 2.8	12.6 ± 2.7	12.9 ± 1.7	14.5 ± 0.9	17.7 ± 3.1	27.5 ± 7.4	26.7 ± 4.3	22.3 ± 2.9	21.5 ± 2.5	19.9 ± 4.4
	PRA	9.7 ± 1.0	9.8 ± 1.7	21.7 ± 3.4	16.7 ± 2.5	18.3 ± 2.6	17.6 ± 1.7	25.4 ± 5.6*	26.0 ± 3.0*	24.3 ± 2.0	25.3 ± 2.7*
	ATI 40	10.7 ± 1.9	10.7 ± 2.0	19.3 ± 3.2	16.2 ± 2.4	12.0 ± 1.2	13.4 ± 1.4	14.9 ± 1.6	15.8 ± 1.3	15.7 ± 2.0	17.9 ± 1.4
	ATI 160	11.6 ± 1.2	11.0 ± 1.4	13.8 ± 1.2	16.5 ± 2.8	13.0 ± 1.2	10.4 ± 1.2‡	13.3 ± 0.9	19.1 ± 2.3	18.4 ± 0.9	18.8 ± 2.8
	ATI 480	10.0 ± 2.8	12.1 ± 2.1	17.2 ± 3.2	14.8 ± 3.2	11.6 ± 1.7	11.7 ± 1.4‡	14.3 ± 1.5	17.7 ± 2.0	19.3 ± 2.1	18.9 ± 3.5
	YOH 40	7.4 ± 0.8	9.5 ± 1.1	11.6 ± 1.6	15.5 ± 2.6	12.4 ± 1.3	14.9 ± 1.3	17.7 ± 3.6	17.3 ± 1.6	13.8 ± 2.0	19.1 ± 2.6*
	YOH 160	13.3 ± 1.5	12.8 ± 1.2	20.2 ± 2.6	18.2 ± 1.8	19.8 ± 4.0	14.7 ± 2.6	16.8 ± 2.7	13.5 ± 2.5	12.2 ± 2.2	11.5 ± 1.7
	YOH 480	12.5 ± 2.9	13.8 ± 2.4	14.4 ± 1.5	13.4 ± 1.3	12.1 ± 1.5	10.9 ± 1.3‡	12.2 ± 2.2	12.4 ± 2.5	9.0 ± 1.6§	10.3 ± 2.0
Fractional clearance of chloride	SAL	0.73 ± 0.11	0.60 ± 0.11	0.57 ± 0.10	0.59 ± 0.12	0.58 ± 0.13	0.55 ± 0.14	0.59 ± 0.17	0.56 ± 0.15	0.52 ± 0.13	0.48 ± 0.13
	XYL	0.41 ± 0.14	0.30 ± 0.05	0.37 ± 0.04	0.51 ± 0.16	1.06 ± 0.28	1.55 ± 0.59	0.92 ± 0.20	0.66 ± 0.16	0.57 ± 0.13	0.54 ± 0.11
	PRA	0.68 ± 0.22	0.50 ± 0.15	0.56 ± 0.15	0.57 ± 0.14	0.82 ± 0.21	1.30 ± 0.34	1.07 ± 0.34	0.98 ± 0.16	0.67 ± 0.15	0.52 ± 0.10
	ATI 40	0.68 ± 0.17	0.49 ± 0.08	0.55 ± 0.10	0.64 ± 0.11	0.68 ± 0.18	1.15 ± 0.45	0.99 ± 0.34	0.78 ± 0.24	0.59 ± 0.14	0.50 ± 0.09
	ATI 160	0.73 ± 0.14	0.51 ± 0.09	0.69 ± 0.14	0.77 ± 0.11	0.83 ± 0.19	0.57 ± 0.09	0.66 ± 0.13	0.57 ± 0.09	0.60 ± 0.16	0.44 ± 0.11
	ATI 480	0.85 ± 0.26	0.55 ± 0.12	0.91 ± 0.26	1.24 ± 0.51	0.93 ± 0.22	0.86 ± 0.29	0.81 ± 0.31	0.70 ± 0.26	0.53 ± 0.20	0.50 ± 0.21
	YOH 40	0.40 ± 0.09	0.29 ± 0.06	0.23 ± 0.04	0.40 ± 0.04	0.32 ± 0.09	0.79 ± 0.24	0.69 ± 0.22	0.53 ± 0.15	0.37 ± 0.08	0.35 ± 0.09
	YOH 160	0.80 ± 0.11	0.43 ± 0.07	0.46 ± 0.10	0.63 ± 0.15	1.39 ± 0.25	1.10 ± 0.16	0.99 ± 0.21	0.60 ± 0.12	0.48 ± 0.08	0.51 ± 0.10
	YOH 480	0.83 ± 0.09	0.48 ± 0.08	0.48 ± 0.06	0.65 ± 0.11	0.84 ± 0.25	0.59 ± 0.13	0.57 ± 0.14	0.45 ± 0.06	0.39 ± 0.06	0.43 ± 0.07

Data are shown as the mean ± standard error.

† Value differs significantly ($p < 0.05$; † $p < 0.01$) from the baseline value (0 h). ‡§ Within a time point, value differed significantly (‡ $p < 0.05$; § $p < 0.01$) from the XYL group.

Discussion

The results of this study indicate that both atipamezole and yohimbine, but not prazosin, have antagonistic effects on xylazine-induced diuresis in healthy cats. These effects are consistent with the results of studies on the antagonistic action of atipamezole or yohimbine on xylazine- or medetomidine-induced diuresis in dogs [46, 47] and rats [5, 26, 27, 28, 29]. Prazosin has inconsistent results for decrease in the elevated urinary output caused by some α_2-adrenoceptor agonists in rats [1, 28, 41]. Our results indicated that the xylazine-induced diuretic effect in cats is not mediated by the α_1-adrenoceptor because it was not antagonized by prazosin. In the present study, yohimbine dose-dependently inhibited xylazine-induced diuresis, in contrast to atipamezole, at the tested doses. Atipamezole is known to be a highly selective and specific antagonist compared with yohimbine for centrally and peripherally located α_2-adrenoceptors [56]. Thus, the difference in responses to atipamezole and yohimbine may have been attributable to the differences in α_2-adrenoceptor selectivity, and high-dose of atipamezole may have shown ceiling effect. Atipamezole has an imidazoline structure in contrast to yohimbine, but we could not assess the influence of these action on xylazine-induced diuresis in this study.

In the present study, urine pH significantly increased in the XYL and PRA groups. These results differed from our previous findings that showed that xylazine did not significantly increase urine pH in cats [30]. This difference may be because of the fact that the cats in the previous study were administered fluids prior to xylazine treatment.

The changes in urine specific gravity and osmolality corresponded to the increase in urine volume after xylazine administration and antidiuretic action of atipamezole and yohimbine. In addition, higher doses of atipamezole and yohimbine potently reversed the xylazine-induced

changes and tended to accelerate recovery from the decreases in urine specific gravity and osmolality.

Osmolar clearance in the present study did not significantly change in any of the groups. In contrast, there was an almost perfect correlation between the increase in free water clearance and the increase in urine volume after xylazine administration. A previous study on rats proposed that xylazine or clonidine caused increase in both osmolar clearance and free water clearance [16, 17, 28]. Moreover, the clonidine-induced increase in free water clearance is blocked by prazosin [16]. However, our results in cats revealed that the diuretic effect of xylazine was caused only by an increase in free water clearance, which was blocked by atipamezole and yohimbine, α_2-adrenoceptor antagonists, but not by prazosin, α_1-adrenoceptor antagonist. Therefore, our results suggest that the mechanism of xylazine-induced diuresis in cats differs from that in rats.

In the present study, the plasma AVP significantly increased in the XYL group after the diuretic effect had returned to the baseline. Plasma AVP secretion is controlled by osmotic stimulus and also cardiovascular reflexes that respond to decrease blood pressure and/or blood volume. In addition, AVP is considerably more sensitive to small changes in osmolality than to similar percentage changes in blood volume [13]. Our result showed that plasma osmolality significantly increased at 3 h after peak diuresis in the XYL group. Therefore, stimulation of plasma AVP release after diuresis may cause by osmoreceptor-AVP feedback system. Both atipamezole and yohimbine tended to prevent the increase in plasma AVP concentrations after xylazine-induced diuretic effect. Furthermore, the highest dose of both treatments caused a transient but significant increase in the AUC value for plasma AVP from 0.5 to 2 h. One possible explanation for this change is that the mean values of plasma osmolality increased, but not significantly, at 1 h in the ATI 480 and YOH 480 groups, because a change in plasma osmolality

of only 1 % is sufficient to increase plasma AVP concentrations [13]. A second explanation is that an overdose of α_2-adrenoceptor antagonists may lead to hypotension. A previous study in dogs under sedation by medetomidine combined with midazolam and opioids revealed that mean arterial pressure significantly decrease after atipamezole administration [33]. Although the precise mechanism of the increase in plasma AVP after the administration of higher doses of atipamezole and yohimbine is unknown, this increase may be involved in the anti-diuretic action of only high doses of both agents, for xylazine-induced diuresis in cats.

The AUC value for plasma AVP from 0.5 to 2 h did not significantly change in the XYL group compared with the SAL group. Furthermore, there was no association between the total urine volume and the AUC of plasma AVP from 0.5 to 2 h in any of the groups. These results suggest that the xylazine-induced diuretic effect in the present study may have been independent of the changes in plasma AVP concentration. An in vitro study [22] revealed that α_2-adrenoceptor agonists (dexmedetomidine and clonidine) inhibited AVP-stimulated osmotic water permeability, which was reversed by α_2-adrenoceptor antagonists (yohimbine and atipamezole), except prazosin, in the rat collecting duct. Therefore, the antagonistic effects of atipamezole and yohimbine on xylazine-induced diuresis observed in this study may have been attributed to the changes in water permeability of α_2-adrenoceptors in the collecting duct.

Plasma sodium and chloride concentrations did not significantly change in any of groups in this study. We have previously shown that plasma sodium concentration significantly increased after IM administration of high xylazine doses (8 mg/kg) in cats [30]. A previous study has also reported that plasma potassium significantly increased after IM administration of 2 mg/kg xylazine and higher doses of atipamezole and yohimbine prevented the xylazine-induced increase in plasma potassium concentration in dogs [46]. Our result showed that, in contrast to

dogs, plasma potassium concentration did not increase in cats. Therefore, it is suggested that plasma ionic regulation to maintain an appropriate plasma potassium concentration is well controlled in cats compared with dogs.

Fractional clearance of sodium and potassium significantly increased after diuresis had peaked following xylazine administration, suggesting that this event is a rebound phenomenon. High doses of both atipamezole and yohimbine prevented the xylazine-induced increases in fractional clearance of sodium and potassium in the present study. These results indicate that the anti-diuretic actions of atipamezole and yohimbine contributed to reduce the xylazine-induced changes in fractional clearances of sodium and potassium. Fractional electrolyte excretion tests have been used for evaluation of renal dysfunction, particularly tubule impairments, in veterinary nephrology [23]. Therefore, when xylazine is administered in cats, the influence of xylazine on the interpretation of urinalysis should be considered, even if α_2-adrenoceptor antagonists are used.

In the present study, 3 different doses of both atipamezole and yohimbine were evaluated for determining the effective dose in antagonizing xylazine-induced diuresis in normal cats. Administration of 2 mg/kg xylazine approximately increased urine volume to 7 times in the peak diuresis, and subsequently caused dehydration in cats. Although this adverse effect of xylazine is independent of changes in glomerular filtration rate, we need to consider for antagonizing the diuresis, especially in cats with urinary tract obstruction, dehydration, or hypovolemia. A moderate dose of atipamezole (160 μg/kg) for 2 mg/kg xylazine can be clinically recommended because this dose produces an anti-diuretic effect without causing frequent behavioral side effects and hormonal changes. High doses of atipamezole and yohimbine (480 μg/kg) are certain to counteract the diuretic action of xylazine, but these doses not recommended normally, because cats were induced abnormal behaviors such as excitement, vocalization, salivation, or defecation.

On the other hand, there are limitations in this study, because the sample size is small and the cats were heterogeneous. In addition, we could not measure the arterial blood pressure and arterial blood gas analysis in this study. More studies with the use of larger populations would be necessary to determine the clinical influence of diuresis via α_2-adrenoceptors in cats.

In conclusion, both atipamezole and yohimbine showed profound anti-diuretic effects for xylazine-induced diuresis but prazosin did not. Although, in contrast to yohimbine, atipamezole did not dose-dependently inhibit diuretic action it had a greater inhibitory effect compared with yohimbine at our tested doses. The xylazine-induced diuretic effect in cats may be mediated by α_2-adrenoceptors but not by α_1-adrenoceptors. Increases in plasma AVP concentration after administration of high doses of both atipamezole and yohimbine may be involved in the anti-diuretic actions of both agents for xylazine-induced diuresis in cats. Both drugs can be used as antagonists against xylazine-induced diuresis in healthy cats.

General Conclusion

In chapter 1, both medetomidine and xylazine increased urine production for up to 5 h after injection. At the evaluated doses, xylazine induced a dose-dependent diuretic response, whereas medetomidine induced a diuretic response that was not dose dependent. Urine specific gravity and osmolality decreased in a dose-dependent manner for both treatments. Free water clearance increased for up to 5 h after injection, whereas GFR, osmolar clearance, plasma osmolality, and electrolyte concentrations did not change significantly. The AUC for plasma AVP concentration after administration of medetomidine, in contrast to that after administration of xylazine, decreased in a dose-dependent manner; however, this was not related to diuresis. The present study further revealed that changes in plasma AVP concentrations, GFR, and osmolar clearance are not part of the diuretic effects of either drug in cats. Both medetomidine and xylazine induced profound diuresis in cats by decreasing the reabsorption of water in the kidneys. The diuretic effect of medetomidine, including the change in AVP concentration, differed from that of xylazine. Other factors, such as the difference in α_2-and α_1-adrenoceptor selectivity or imidazoline receptor selectivity on the renal system, may be involved in the diuretic mechanism. Care must be used in administration of both drugs to cats with urinary tract obstruction, hypovolemia, or dehydration.

In chapter 2, both atipamezole and yohimbine showed profound anti-diuretic effects for medetomidine-induced diuresis but prazosin did not. The antidiuretic effect of atipamezole was more potent than that of yohimbine, but was not dose dependent, in contrast to the effect of yohimbine at the tested doses. Both atipamezole and yohimbine reversed medetomidine-induced

decreases in both urine specific gravity and osmolality, and increases in plasma osmolality and free-water clearance. Antidiuresis of either atipamezole or yohimbine was not related to the AUC for plasma AVP concentration, although the highest dose of both atipamezole and yohimbine initially and temporarily increased plasma AVP concentrations, suggesting that this may partly influence the antidiuretic effects of both agents. The diuretic effect of medetomidine in cats may be mediated by α_2-adrenoceptors, but not α_1-adrenoceptors. Administration of 40 μg/kg medetomidine yielded a potent diuresis, and subsequently caused dehydration in cats. It is recommended that medetomidine-induced diuresis should be antagonized, especially in cats with urinary tract obstruction, dehydration, or hypovolemia. Atipamezole and yohimbine can be used as antagonistic agents against medetomidine-induced diuresis in healthy cats.

In chapter 3, both atipamezole and yohimbine showed profound anti-diuretic effects for xylazine-induced diuresis but prazosin did not. Although, in contrast to yohimbine, atipamezole did not dose-dependently inhibit diuretic action it had a greater inhibitory effect compared with yohimbine at our tested doses. Both atipamezole and yohimbine reversed xylazine-induced decreases in both urine specific gravity and osmolality, and the increase in free water clearance. Glomerular filtration rate, osmolar clearance, and plasma electrolyte concentrations were not significantly altered. Antidiuresis of either atipamezole or yohimbine was not related to the AUC for plasma AVP concentration, although the highest dose of both atipamezole and yohimbine increased plasma AVP concentrations initially and temporarily, suggesting that this may in part influence antidiuretic effects of both agents. Diuretic effect of xylazine in cats may be mediated by α_2-adrenoceptors but not α_1-adrenoceptors. A moderate dose of atipamezole can be clinically recommended because this dose produces an anti-diuretic effect without causing frequent behavioral side effects and hormonal changes. High doses of atipamezole and yohimbine are

certain to counteract the diuretic action of xylazine, but cats were induced abnormal behaviors. Both drugs can be used as antagonists against xylazine-induced diuresis in healthy cats.

In conclusion, both medetomidine and xylazine induced profound diuresis in cats and these diuretic effects can be reversed effectively by both atipamezole and yohimbine but not prazosin. This study provided the clinical meaning of the diuresis of α_2-adrenoceptor agonists and their antagonism by antagonists from a clinical biochemical and hormonal changes in cats and improvement of the mechanism of the diuretic effect.

Abstract

The α_2-adrenoceptor agonists medetomidine and xylazine are used in veterinary medicine to induce reliable sedation and analgesia. Although both drugs are used similarly in practice, medetomidine is a more potent, selective, and specific α_2-adrenoceptor agonist than is xylazine. In addition, in contrast to xylazine, medetomidine contains an imidazole ring that has an affinity for imidazoline receptors. The α_2-adrenoceptor agonists can induce diuresis in several species. It has been suggested that several factors are involved in the mechanism of this diuresis. However, the exact mechanism of the diuretic effect of α_2-adrenoceptor agonists is still unknown, and may differ depending on the particular animal species. Given this effect, medetomidine and xylazine should be used with discretion in hypovolemic or dehydrated animals and avoided in those with urinary tract obstruction. In such cases, α-adrenoceptor antagonists may be used to reverse the diuretic actions. The α_2-adrenoceptor antagonists atipamezole and yohimbine have been clinically used to reverse the sedative and analgesic effects of α_2-adrenoceptor agonists. Prazosin is known to be α_1-adrenoceptor antagonist. In contrast, atipamezole has an imidazoline ring structure, whereas yohimbine and prazosin are non-imidazoline agents. These differences among atipamezole, yohimbine and prazosin may influence their antagonistic effects in the effects of α_2-adrenoceptor agonists. However, there are no published reports described the diuretic effects of medetomidine or xylazine and regarding the effects of α-adrenoceptor antagonists on medetomidine and xylazine-induced diuresis in cats.

In chapter 1, the study was aimed to investigate dose-related diuretic effects of medetomidine hydrochloride and xylazine hydrochloride in healthy cats. Five sexually intact cats were used

randomly in each of 11 treatment groups. Cats were treated by intramuscularly administration of saline (0.9% NaCl) solution (control treatment), medetomidine (20, 40, 80, 160, and 320 μg/kg), and xylazine (0.5, 1, 2, 4, and 8 mg/kg). Urine and blood samples were collected 9 times over a 24 h period. Variables measured were urine volume, pH, and specific gravity; plasma arginine vasopressin (AVP) concentration; and creatinine and electrolyte concentrations as well as osmolality in both urine and plasma. Both medetomidine and xylazine increased urine production for up to 5 h after injection. Xylazine had a dose-dependent diuretic effect, but medetomidine did not. Urine specific gravity and osmolality decreased in a dose-dependent manner for both treatments. Free water clearance increased for up to 5 h after injection, whereas glomerular filtration rate (GFR), osmolar clearance, plasma osmolality, and electrolyte concentrations did not change significantly. The area under the curve (AUC) for AVP concentration decreased in a dose-dependent manner for medetomidine but not for xylazine; however, this was not related to diuresis. Both medetomidine and xylazine induced profound diuresis in cats by decreasing the reabsorption of water in the kidneys. The diuretic effect of medetomidine, including the change in AVP concentration, differed from that of xylazine. Care must be used in administration of both drugs to cats with urinary tract obstruction, hypovolemia, or dehydration.

In chapter 2, the study aimed to investigate and compare the antagonistic effects of atipamezole, yohimbine, and prazosin on medetomidine-induced diuresis in healthy cats. Five cats were repeatedly used in each of the 9 groups. One group was not medicated. Cats in the other groups received 40 μg/kg medetomidine intramuscularly, and saline (as the control), 160 μg/kg prazosin, or 40, 160, or 480 μg/kg atipamezole or yohimbine intravenously 0.5 h later. Volume, pH, and specific gravity of urine; AVP concentration; and creatinine, osmolality, and

electrolyte levels in both urine and plasma were measured. Both atipamezole and yohimbine, but not prazosin, antagonized medetomidine-induced diuresis. The antidiuretic effect of atipamezole was more potent than that of yohimbine, but was not dose dependent, in contrast to the effect of yohimbine at the tested doses. Both atipamezole and yohimbine reversed medetomidine-induced decreases in both urine specific gravity and osmolality, and increases in plasma osmolality and free-water clearance. Antidiuresis of either atipamezole or yohimbine was not related to the AUC for AVP concentration, although the highest dose of both atipamezole and yohimbine initially and temporarily increased plasma AVP concentrations, suggesting that this may partly influence the antidiuretic effects of both agents. The diuretic effect of medetomidine in cats may be mediated by α_2-adrenoceptors, but not α_1-adrenoceptors. Atipamezole and yohimbine can be used as antagonistic agents against medetomidine-induced diuresis in healthy cats.

In chapter 3, the study aimed to investigate and compare the antagonistic effects of atipamezole, yohimbine, and prazosin on xylazine-induced diuresis in healthy cats. Five cats were repeatedly used in each of the 9 groups. One group was not medicated. Cats in the other groups received 2 mg/kg xylazine intramuscularly, and saline (as the control), 160 μg/kg prazosin, or 40, 160, or 480 μg/kg atipamezole or yohimbine intravenously 0.5 h later. Urine and blood samples were collected 10 times over 8 h. Urine volume, pH, and specific gravity; plasma AVP concentration; and creatinine, osmolality, and electrolyte values in both urine and plasma were measured. Both atipamezole and yohimbine antagonized xylazine-induced diuresis but prazosin did not. Antidiuretic effect of atipamezole was more potent than that of yohimbine but not dose-dependent, in contrast to the effect of yohimbine at the tested doses. Both atipamezole and yohimbine reversed xylazine-induced decreases in both urine specific gravity and osmolality, and the increase in free water clearance. GFR, osmolar clearance, and plasma electrolyte

concentrations were not significantly altered. Antidiuresis of either atipamezole or yohimbine was not related to the AUC for AVP concentration, although the highest dose of both atipamezole and yohimbine increased plasma AVP concentrations initially and temporarily, suggesting that this may in part influence antidiuretic effects of both agents. Diuretic effect of xylazine in cats may be mediated by α_2-adrenoceptors but not α_1-adrenoceptors. Atipamezole and yohimbine can be used as antagonistic agents against xylazine-induced diuresis in healthy cats.

In conclusion, both medetomidine and xylazine induced profound diuresis in cats and these diuretic effects can be reversed effectively by both atipamezole and yohimbine but not prazosin. This study provided the clinical meaning of the diuresis of α_2-adrenoceptor agonists and their antagonism by antagonists from a clinical biochemical and hormonal changes in cats.

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