FULL PAPER *Internal Medicine*

Antagonistic Effects of Atipamezole, Yohimbine and Prazosin on Medetomidine-Induced Diuresis in Healthy Cats

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(Received 2 August 2013/Accepted 24 September 2013/Published online in J-STAGE 8 October 2013)

ABSTRACT. This 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 hr later. Volume, pH and specific gravity of urine; plasma arginine vasopressin (AVP) level; 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 area under the curve for AVP level, although the highest dose of both atipamezole and yohimbine initially and temporarily increased plasma AVP levels, 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.

KEY WORDS: α-adrenoceptor antagonists, arginine vasopressin, diuresis, feline, medetomidine.

doi: 10.1292/jvms.13-0398; *J. Vet. Med. Sci.* 76(2): 173–182, 2014

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 [13]. Pharmacologically, the α_2-adrenoceptors are classified into three 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 [22], and the α_2/α_1 receptor-binding selectivity ratio of medetomidine is 1,620:1 [31]. In addition, medetomidine contains an imidazole ring that has an affinity for imidazoline receptors [17].

Medetomidine has been shown to induce diuresis in rats [18] and dogs [3, 19, 24, 26]. Recently, our study showed that both medetomidine and xylazine induced profound diuresis in cats by decreasing water reabsorption in the kidneys [16]. 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 [16].

The α_2-adrenoceptor antagonists, atipamezole and yohimbine, have been clinically used to reverse the sedative and analgesic effects of α_2-adrenoceptor agonists [13, 20]. The α_2/α_1 adrenoceptor selectivity ratios of atipamezole and yohimbine are 8,526:1 and 40:1, respectively [30]. 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 [20]. Prazosin has a selectivity of 1,000:1 for the α_1/α_2 adrenoceptor [7]. In contrast, atipamezole, similar to medetomidine, has an imidazoline ring structure, whereas yohimbine and prazosin are non-imidazoline agents [28]. 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

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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 (three sexually intact males, one neutered male and one sexually intact female) aged from 1–2 years and weighing from 2.5–4.0 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. Intramuscular (IM) administrations of saline solution (0.9% NaCl; 0.1 m*l*/kg) as the 1st treatment at the beginning of the experiment and 2nd intravenous (IV) dose of saline solution (0.1 m*l*/kg) were given 0.5 hr 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 m*l*/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.) or yohimbine hydrochloride (40, 160 or 480 μg/kg; Sigma-Aldrich Japan) were administered. Prazosin hydrochloride was dissolved in distilled water at a concentration of 1.6 mg/m*l*, and yohimbine hydrochloride was dissolved in distilled water at a concentration of 5.0 mg/m*l*. 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 hr before the start of each experiment. After collecting the samples at 8 hr 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 [10]. 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 hr after injection) from each cat. Blood samples (2.5 m*l*) and urine samples were collected from the central venous and urinary catheters, respectively. An aliquot of 2.0 m*l* from each blood sample was mixed with ethylenediaminetetraacetic acid for measurement of plasma arginine vasopressin (AVP) levels, and the remaining 0.5 m*l* 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 levels, osmolality and electrolyte (sodium, potassium and chloride) levels and plasma AVP levels were measured using previously described procedures [24]. 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 glomerular filtration rate (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 4, San Diego, CA, U.S.A.). 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 area under the curve (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. 1A). The peak diuresis occurred 2 hr after injection. Both ATI and YOH treatments reversed the increase in the urine volume induced by medetomidine (Fig. 1B and 1C). The total urine volume from 0.5 to 5 hr increased significantly in the MED and PRA groups compared with the SAL group (Fig. 2A).

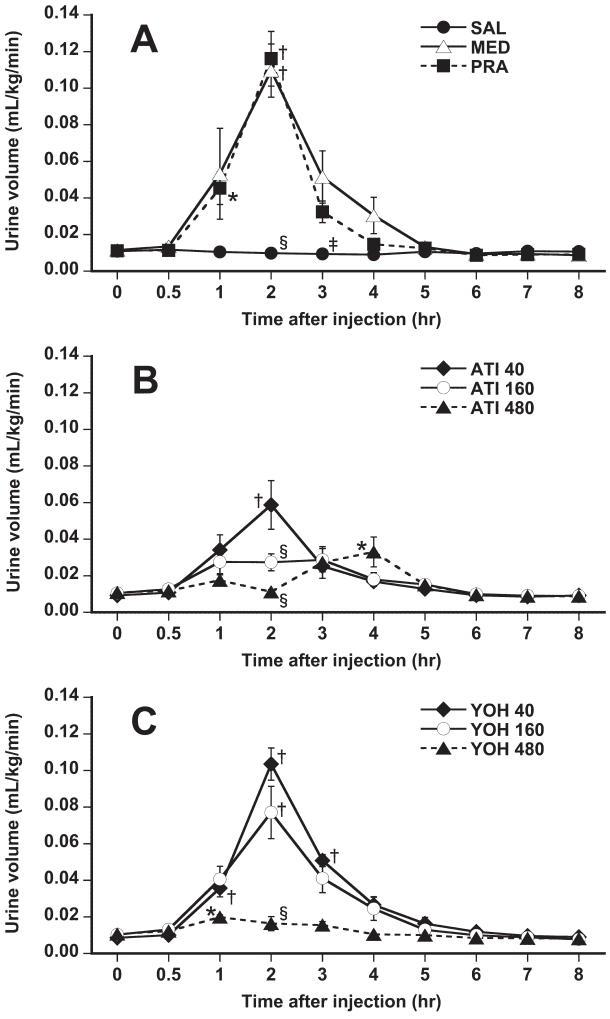

Fig. 1. Urine volume (mean ± SE) of five cats before and after injection of A) physiological saline (SAL), medetomidine followed by saline (MED), prazosin (PRA); B) atipamezole (ATI); C) yohimbine (YOH), the last two drugs in doses of 40, 160 and 480 μg/kg. Time of 1st injection was designated as time 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.

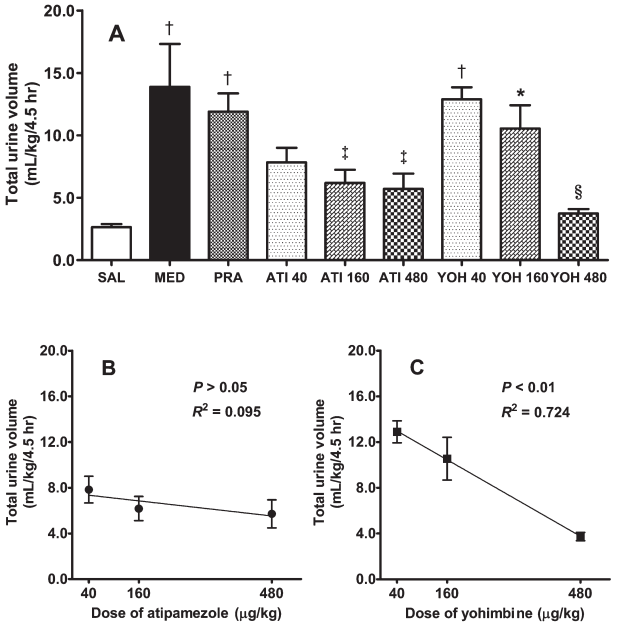

Fig. 2. Total urine volume (mean ± sSE) of five cats at 0.5 to 5 hr post-injection of prazosin or various doses of atipamezole or yohimbine (A). Simple linear regression analysis of the total urine volume of five cats at 0.5 to 5 hr after injection of various atipamezole doses (B) or yohimbine (C). See Fig. 1 for the remainder of the key.

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 hr after injection was significant in the YOH groups, but not in the ATI groups (Fig. 2B and 2C), 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 hr 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 hr after treatment compared with the baseline values. The mean values at 2 to 3 hr 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. 3A). All doses of ATI and YOH 480 treatments significantly inhibited the medetomidine-induced increase in free-water clearance (Fig. 3B and 3C). Osmolar clearance and GFR did not significantly change in any group.

Plasma AVP levels increased significantly in the MED and PRA groups compared with the baseline values after peak diuresis (Fig. 4A). The mean value at 3 hr after treatment in

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 hr 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

Biochemical variable and treatment group	Time after initial injection (hr)									
	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 ± SE. *† Value differs significantly (* $P<0.05$; † $P<0.01$) from the baseline value (0 hr). ‡§ Within a time point, value differed significantly (‡ $P<0.05$; § $P<0.01$) from the MED group.

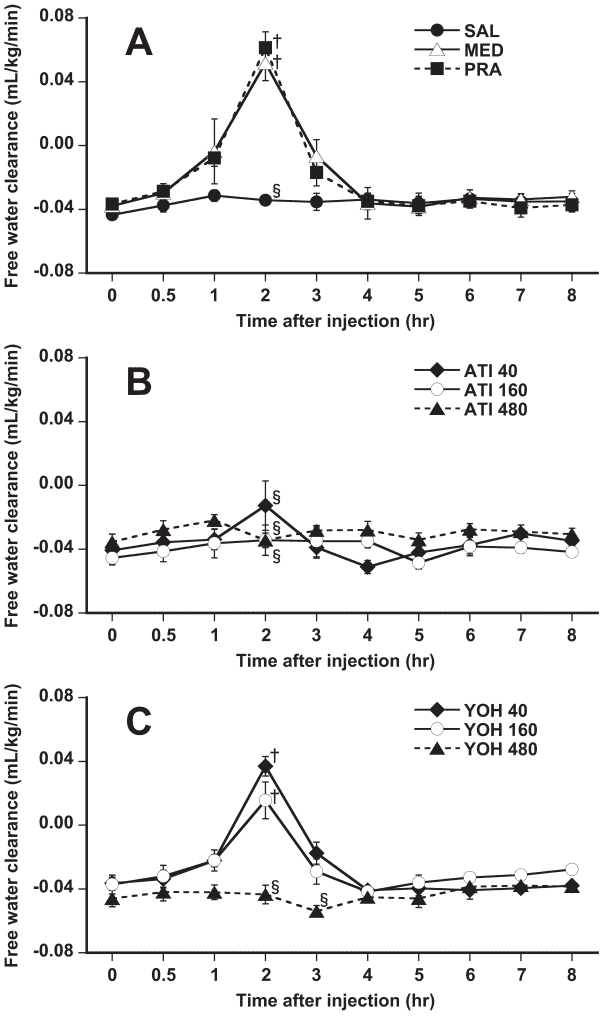

Fig. 3. Free-water clearance (mean ± SE) of five cats (A, B and C) before and after injection of physiological saline or medetomidine. See Fig. 1 for the remainder of the key.

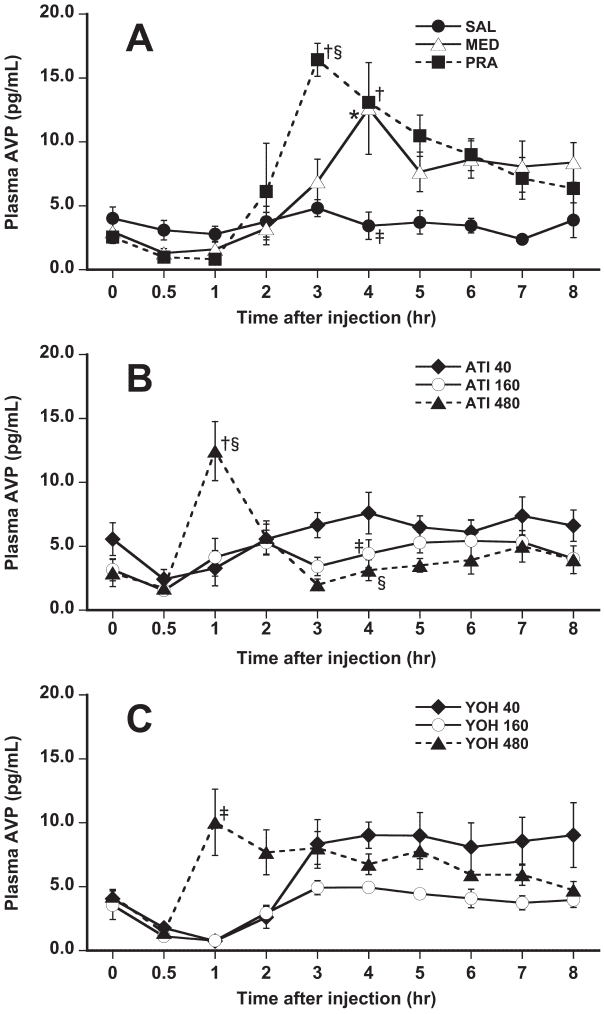

Fig. 4. Plasma AVP level (mean ± SE) of five cats (A, B and C) before and after injection of physiological saline or medetomidine. See Fig. 1 for the remainder of the key.

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 levels, and a significant difference was detected at 4 hr after treatment in the ATI 160 and ATI 480 groups (Fig. 4B and 4C). Plasma AVP levels in the ATI 480 and YOH 480 groups increased significantly at 1 hr post-treatment compared with the SAL group. The AUC value for plasma AVP from 0.5 to 2 hr post-treatment increased significantly in the ATI 480 and YOH 480 groups compared with the MED group (Fig. 5). There were no correlations between the total urine volume and the AUC of plasma AVP from 0.5 to 2 hr after treatment in any group.

Plasma sodium levels 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 hr after treatment in the MED group. Plasma potassium and chloride levels did not significantly change

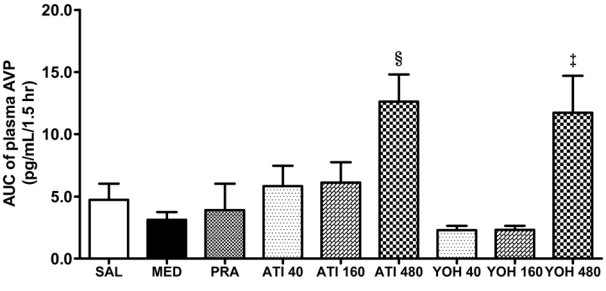

Fig. 5. Area under the curve (AUC) data for plasma AVP level (mean ± SE) of five cats after injection of the prazosin or various doses of atipamezole or yohimbine at 0.5 to 2 hr. The symbols indicate a significant difference from the value of the MED group (‡ $P<0.05$; § $P<0.01$).

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

Biochemical variable and treatment group	Time after initial injection (hr)									
	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	154.4 ± 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	154.0 ± 0.6	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 ± 0.2	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 ± 0.11
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 ± 0.14	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 ± 1.2	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

Table 2. (continued)

Biochemical variable and treatment group	Time after initial injection (hr)									
	0	0.5	1	2	3	4	5	6	7	8
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 ± 0.12	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 ± SE. *† Value differs significantly (*$P<0.05$; † $P<0.01$) from the baseline value (0 hr). ‡§ Within a time point, value differed significantly (‡ $P<0.05$; § $P<0.01$) from the MED group

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

DISCUSSION

Clinically, the recommended label dose of medetomidine as a sedative in cats is 40 to 80 μg/kg IM in cats [22]. 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 [27], and high dose was decided based on a previous study [29]. 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 [25, 26]. Prazosin has been known to cause an inconsistent decrease in the elevated urinary output caused by clonidine, guanabenz, guanfacine [1, 21] and xylazine [15] administration in rats, and it is known to be a highly selective α_1-adrenoceptor antagonist [7]. 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 [30]. In addition, atipamezole has an imidazoline ring structure in contrast to yohimbine [17]. 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 [16]. 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 [15]. 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 [8]. 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 levels 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 hr 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 levels did not significantly decrease after medetomidine administration in cats [16] and dogs [24]. 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 levels and was independent of the changes in vasopressin activity in rats [9]. Therefore, the medetomidine-induced diuretic effect in the present study may have been independent of the changes in plasma AVP levels. Plasma AVP accelerates free-water reabsorption through its action on vasopressin V2 receptors in the distal nephron and subsequently promotes cyclic

adenosine monophosphate (cAMP)-dependent trafficking of aquaporin-2 water channels to the luminal membrane of principal cells [23]. 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 [5, 6]. 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 [6]. 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 level at 3 hr 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 [14]. In this study, the mean values of plasma osmolality increased significantly at 3 hr 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 [25]. Therefore, the difference in plasma AVP levels between the MED and PRA groups at 3 hr 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 levels following the medetomidine-induced diuretic effect. Furthermore, the highest dose of both treatments caused a transient, but significant, increase in plasma AVP levels. 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 [13]. 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 hr 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 [11]. 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 level 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 levels did not significantly increase after IM administration of 40 μg/kg medetomidine in cats [16]. 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 level. These results were consistent with those of a previous study in dogs [26]. In the present study, plasma potassium and chloride levels did not significantly change in any group. A previous study reported that plasma potassium and chloride levels 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 levels in dogs [26]. In the present study, our result showed that, in contrast to dogs, plasma potassium levels did not increase in cats. Therefore, we propose that plasma ionic regulation to maintain appropriate plasma potassium and chloride levels 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 [12]. 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 levels 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.

ACKNOWLEDGMENTS. This study was supported in part by a Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science (to Y. Hikasa) and by a Tottori University President Discretion Grant (to Y. Hikasa).

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