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## The Role of Vasopressin in Reduced Renal Mass-Saline Hypertension\*

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**Abstract:** The cause of reduced renal mass (RRM)-saline hypertension is not clear. In our study, it was aimed to investigate the role of vasopressin (AVP) in this type of hypertension by using selective  $V_1$  antagonist of AVP. RRM was performed in 32 normotensive male Wistar rats under anesthesia in which we used a mixture of ketamine (130 mg/kg, i.m.) and chlorpromazine (1.3 mg/kg, i.m.). After a one-week of recovery period, all of the rats were given a low sodium diet and either distilled water (group 1) or 1% NaCl (group 2) as drinking water for 4 weeks. Five weeks after nephrectomy, indirect blood pressure (BP) and heart rate (HR) measurements were performed. Group 1 was normotensive but group 2 was hypertensive. One day later, AVP antagonist (10

$\mu\text{g}/\text{kg}$ ) or vehicle was intravenously injected to normotensive and hypotensive rats under pentobarbital sodium anesthesia. After injections, BP and HR values were recorded for 30 minutes. The antagonist caused decreases in the mean arterial pressures of both groups which were greater in the hypertensive group ( $p < 0.05$ ). In the normotensive group, HR was increased by the antagonist ( $p < 0.05$ ). No significant difference was present between the two groups with regard to plasma volume and plasma osmolality. Thus, it was concluded that the pressor effect of AVP may have been augmented in RRM-saline hypertension.

**Key Words:** Hypertension, vasopressin, nephrectomy, sodium intake.

### Introduction

Reduced renal mass-saline hypertension model is characterized with high peripheral vascular resistance and low plasma renin level (1,2). Eventhough some neural and humoral factors have been hold responsible to explain these findings, the exact physiological mechanisms have not been identified yet (1,3,4).

Blood pressure increases and decreases cause changes in the renal sympathetic nerve activity and heart rate levels. The quantities of these changes are altered by high  $\text{Na}^+$  diet via central cardiovascular control mechanism (5). These findings support the possible effect of high  $\text{Na}^+$  diet on the baroreceptor reflexes.

AVP interacts with the sympathetic nervous system, renin-angiotensin-aldosterone system (RAAS) and baroreceptor reflex mechanisms (6,7). Any change at the efficiency of these interacting variables may affect blood pressure as well as AVP's pressor response (4,8,9,10). In previous study, this interaction was discussed for DOCA-salt hypertensive rats and the hyper-

tension was explained with the impaired baroreflex responses that augmented AVP's pressor effect (11). Similar mechanisms may be involved in the RRM-saline hypertension model as well. If AVP is the only mediator that is involved in this hypertension, blocking the  $V_1$  receptor on vascular smooth muscle should reduce the blood pressure. The aim of this study was to investigate the role of AVP in RRM-saline hypertension, by using selective  $V_1$  antagonist.

### Material and Methods

Experiments were carried out on 32 male Wistar rats (DECAM, Adana, Turkey), weighing  $311.13 \pm 2.12$  g (mean  $\pm$  SEM). The rats were housed at constant room temperature ( $22^\circ\text{C}$ ) and given normal rat chow (containing 0.5 % NaCl and 22 % protein) and ordinary tap water. Light was switched on from 8 a.m. to 8 p.m. Initially, the rats were normotensive. Normotension was determined indirectly by measuring the systolic blood pressure by using the tail cuff method (rat tail BP monitor, Harvard Apparatus Ltd., Kent,

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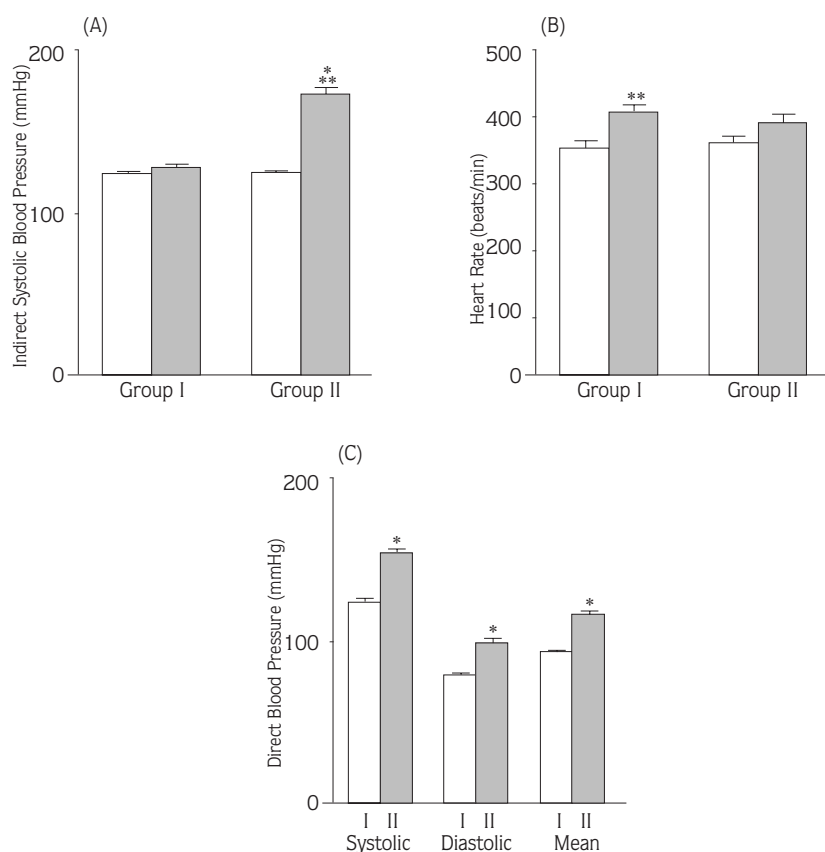


Figure 1. Indirect blood pressure (A) and heart rate (B) values at one day before (open bar) and five weeks after (dashed bar) the nephrectomy. C) Direct blood pressure values at five weeks after the nephrectomy. I: Group I (Normotensive), II: Group II (Hypertensive), indirect: with tail cuff method. Values are means  $\pm$  SEM. \* $p < 0.05$  v group I, \*\* $p < 0.05$  v the values before the nephrectomy.

England). The rats having systolic blood pressures of less than 130 mmHg were accepted as normotensive. Subtotal nephrectomy was carried out under anesthesia in which we used a mixture of ketamine (130 mg/kg, i.m.) and chlorpromazine (1.3 mg/kg, i.m.). Through a midline abdominal incision, the right kidney and approximately 50 percent of the left kidney (both poles) were removed. The removal of 50 percent of the left kidney was estimated with the assumption that both kidneys were equal in weight. Silk ties were used to excise the poles of the left kidney. A loop of 3-0 silk suture was placed around each pole of the kidney and then pulled tight. One week after nephrectomy, the rats were divided into two groups; group 1: the rats which were given rat chow containing low NaCl (0.02 %), and distilled water as drinking water (n=17), group 2: the rats which were given rat chow containing low NaCl and 1 % saline as drinking water (n=15). These different diets were given for 4 weeks.

Systolic blood pressures (SBP) and heart rates (HR) were measured indirectly at the end of the 5th week, after nephrectomy. On the next day of the measurements, tracheostomy (PE-240 tubing, Clay Ad-

ams, Parsipanny, NJ) and femoral artery and vein [PE-50 tubing, filled with heparinized saline (10 I.U./ml)] and bladder (PE-90 tubing) catheterizations were made under pentobarbital sodium anesthesia (50 mg/kg, i.p.). The catheter that was inserted into the femoral artery was connected to a Satham P23XL (Gould, Oxnard, CA) transducer to monitor BP and HR. Insensible fluid losses were compensated with 0.9 % NaCl via femoral vein with an infusion pump (Cole Parmer Instrument Company, IL) at a rate of 0.02 ml/min during the experiment. At the end of the stabilization period, which had lasted 30 minutes after completion of the surgical procedure, a blood of 0.1 ml/100 g body weight was withdrawn for determination of plasma osmolality. Volume replacement was made by an infusion of cells suspended with 0.9 % NaCl. Osmolality was determined by freezing point osmometry (Osmette, Precision Systems Inc., MA). Direct SBP, diastolic blood pressure (DBP) and HR recordings (Oscillograph, Model 7, Grass Instrument CO., MA) were made at the 15th minute after volume replacement. We used ( $\beta$ -mercapto- $\beta$ ,  $\beta$  Cyclopentamethylenepropionyl<sup>1</sup>, O-Me-Tyr<sup>2</sup>, Arg<sup>3</sup>)-Vasopressin

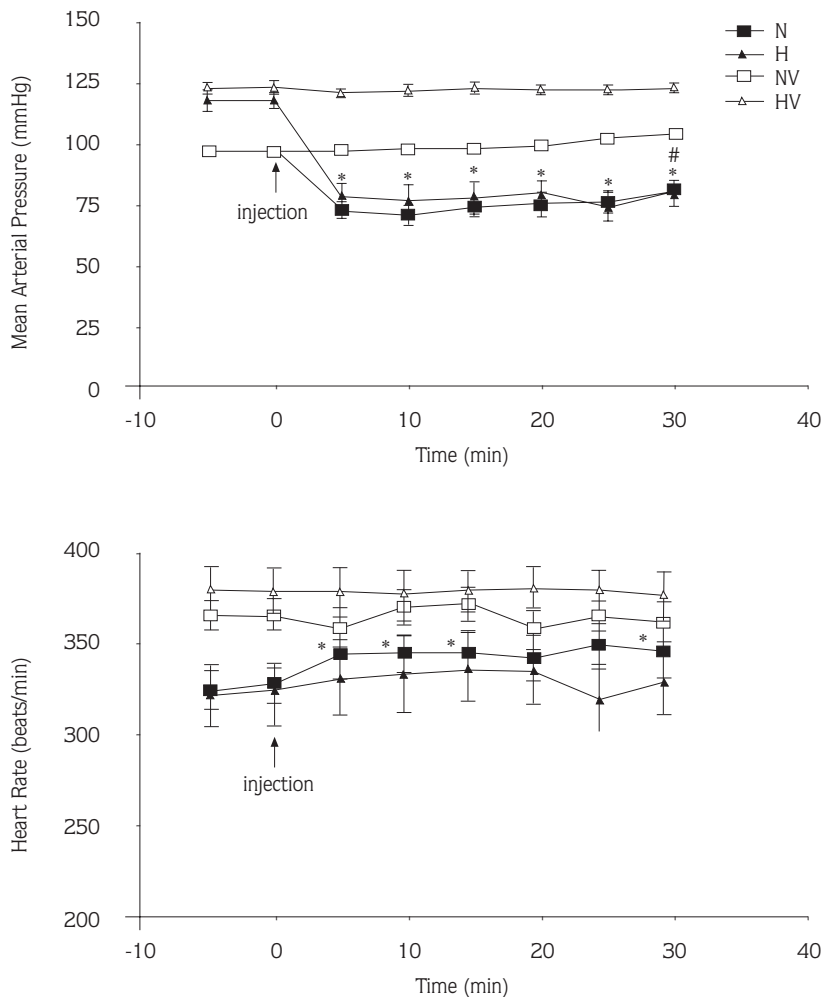


Figure 2. The effect of injection of AVP antagonist (10  $\mu\text{g}/\text{kg}$ ) or vehicle on mean arterial pressure and heart rate. N: Normotensive + antagonist, H: Hypertensive + antagonist, NV: Normotensive + vehicle, HV: Hypertensive + vehicle. Values are means  $\pm$  SEM. \* $p < 0.05$  v pre-injection value.

(Sigma, St. Louis, MO) as  $V_1$  antagonist of AVP. This peptide was dissolved in a solution that was composed of 5 ml of 0.9 % NaCl, 5 mg bovine serum albumin and 1.5  $\mu\text{l}$  acetic acid and adjusted to pH=6.4 with NaOH. The concentration of antagonist in this solution was 10  $\mu\text{g}/\text{ml}$ . Five weeks after nephrectomy, group 1 that remained normotensive was divided into two groups (group N,  $n=9$ ; group NV,  $n=8$ ) and group 2 which developed hypertension was separated into two groups (group H,  $n=7$ ; group HV,  $n=8$ ). AVP antagonist was injected into group N and group H as i.v. bolus at a dose of 10  $\mu\text{g}/\text{kg}$ . Vehicle injection was administered into group NV and group HV. SBP, DBP and HR were recorded for 30 minutes after injections. Plasma volume was measured by the dilution principle using the Evans Blue dye technique. For this purpose, 200  $\mu\text{l}$  Evans Blue (Merck, Darmstadt, Germany; 5 mg/ml dissolved in 0.9 % NaCl) was injected intravenously into group NV and group HV, and the tubing was flushed with 50  $\mu\text{l}$  0.9 % NaCl at the end of the

BP recording period of 30 minutes. Urine was collected for 10 minutes after the injection of Evans Blue and a blood sample was withdrawn at the end of the 10th minute. Plasma and urine samples were stored at  $-70^\circ\text{C}$  until analysis. Plasma and urine Evans Blue concentrations were measured with spectrophotometer (Schimadzu, UV-1201, Kyoto, Japan) at 605 nm.

Statistics: The results were expressed as the mean  $\pm$  SEM. Differences between groups were evaluated by Kruskal-Wallis ANOVA. Mann-Whitney U test was used to reveal significant differences. Wilcoxon matched pairs test for paired data was used to analyze the BP and HR changes induced by infusions and to assess other differences within groups. Results with  $p < 0.05$  were considered to be statistically significant.

## Results

SBP and HR values that were indirectly measured

Group/Time (min)	Decrease in MAP (Percentage of baseline MAP)						
	0	5	10	15	20	25	30
N (n=7)	-0.98±	-26.28±	-26.73±	-23.36±	-24.48±	-21.89±	-16.33±
H (n=7)	0.92	3.18*	4.93*	4.40*	3.81*	4.73*	3.61*
	0.66±	-33.60±	-35.63±	-34.74±	-34.04±	-37.13±	-33.65±
NV (n=8)	0.92	4.10*	4.46*	4.02*	3.24*	3.93*	2.60**
	0.22±	0.64±	0.85±	0.91±	1.98±	4.89±	6.45±
HV (n=8)	0.22	0.31	0.74	1.23	0.98	1.71	1.76
	0.34±	-1.70±	-0.72±	-0.38±	-0.67±	-0.85±	-0.85±
	0.34	1.35	1.22	1.35	1.51	1.30	1.28

N: Normotensive+antagonist, H: Hypertensive+antagonist, NV: Normotensive+vehicle, HV: Hypertensive+vehicle.  
 Values are means±SEM. \*\*p<0.05 v N, \*p<0.05 v NV or HV.

Table Change in Mean Arterial Pressure (MAP) After the Injection of AVP Antagonist.

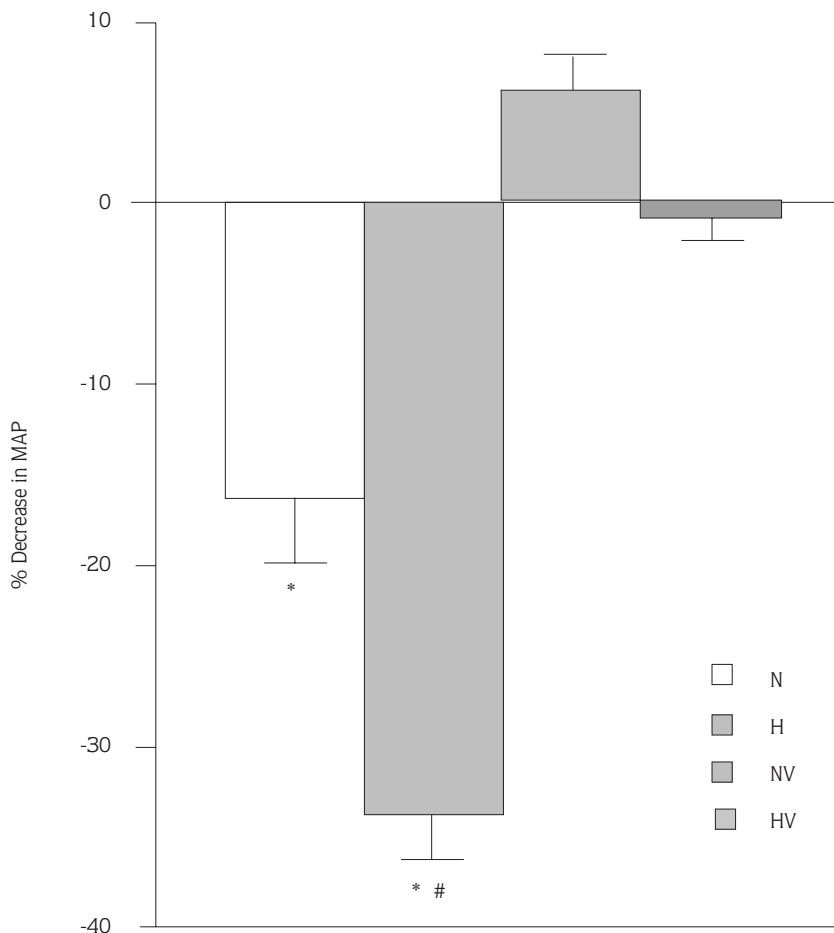


Figure 3. The amount of decrease in mean arterial pressure at the 30th minute after AVP antagonist (10 µg/kg) or vehicle injection. N: Normotensive+antagonist, H: Hypertensive + vehicle. \*p<0.05 v group NV or group HV. # p<0.05 v group N.

at one day before and 5 weeks after nephrectomy are shown in Figure 1. At the end of the 5th week, indirect SBP and direct SBP and DBP values in group 2 were greater than the values in group 1 (p<0.001, Figure 1). Direct BP and HR recordings which were measured 5 minutes before injection of AVP antagonist or vehicle were taken as baseline values. Five weeks after nephrectomy, the increase in indirect HR

was statistically significant only in group 1 (p<0.01). No significant differences were determined between normotensive and hypertensive groups, with regard to plasma osmolality (group 1: 313.25±7.05 mosm/kg, group 2: 318.86±8.11) and plasma volume (group NV: 32.21±1.15 ml/kg, group HV: 32.44±1.59)

V<sub>1</sub> antagonist of AVP caused significant decreases in the MAP of both group N and group H (p<0.05)

but vehicle did not change the MAP in group NV or in group HV during the 5-30 minutes after the injection (Figure 2). The decrease ratios were calculated as percent of baseline mean arterial pressure (MAP). MAP tended to decrease more in group H than in group N after the injection (Table). At the 30th minute of injection, difference between the decrease ratios of the mean arterial pressures in N and H groups was statistically significant ( $p < 0.05$ , Figure 3). The antagonist increased HR significantly only in group N ( $p < 0.05$ , Figure 2).

## Discussion

Since the  $V_1$  antagonist of AVP caused decreases in the mean arterial pressures of both groups, the pressor effect of AVP may be important to maintain BP level under anesthesia. When any component of the pressor system like the sympathetic nervous system, RAAS or vasopressin was blocked, the possible decrease in MAP is compensated by the other two systems (12-16). There are studies about depression of the sympathetic nervous system (17) and attenuation of cardiovascular reflexes (7) by pentobarbital sodium. In our study, the cause of augmented pressor effect of AVP may be due to a decreased sympathetic efficiency.

It had also been observed in previous studies that a high sodium diet may lead to hypertension in the rats which were restricted their renal excretion ability (1). Pressor effect of AVP might have been increased in RRM-saline hypertension model, because AVP antagonist tended to cause more decrease in MAP of the hypertensive group than the normotensive group. In another study, it has been suggested that AVP is not a major agent in nephrectomy-salt hypertension (18). Our finding is contrary to this result. The discrepancy may be due to different salt-loading periods.

Augmented pressor effect of AVP may be due to manifestation of its vasoconstrictor effect when it is considered that AVP leads to a decrease of cardiac output (7,19). In previous studies, it was reported that a high salt diet may change the sensitivity of aortic (20) and cardiopulmonary (21) reflexes in Dahl salt-resistant rats. Hence, investigation of baroreflex mechanism may contribute to clear the cause of augmented pressor effect of AVP in RRM-saline hypertension when AVP-baroreflex is considered (7).

Though an increase in HR was determined at the 5th week after the nephrectomy in the normotensive group, in the hypertensive group no significant increase in HR was observed. This difference may be due to the effect of a low sodium diet on HR when considered desensitization of arterial baroreceptors because of a low sodium diet (15). The tendency of heart rates to increase in the normotensive group after AVP antagonist injection is an expected result because of the effect of AVP to produce bradycardia (7). On the other hand, in the hypertensive group, since no significant increase in HR was observed after the injection, the neural mechanisms including baroreflexes might have been impaired. Actually, there is evidence that a high sodium diet attenuates the inhibitor response in HR against an increase in BP (5). A possible change in the sensitivity of baroreflex mechanisms, which mediate buffering of vasoconstrictor effect of AVP by decreasing the cardiac output, may contribute to augmented pressor effect of AVP.

It has been determined that endogenous-digitalis-like factor (EDLF) was released (1) and plasma renin levels were decreased in nephrectomy-salt hypertension. It has been reported that digitalis may augment the release of norepinephrine from the sympathetic nervous system terminals (3). Pressor doses of angiotensin II attenuate the pressor effect of AVP (22), whereas norepinephrine can enhance this effect (7). For these reasons, the pressor effect of AVP may have also been augmented by EDLF and low renin.

Since no significant difference was observed between the normotensive and hypertensive groups with regard to plasma volume, this parameter may not be direct determining factor in RRM-saline hypertension. This result is in line with the conclusions that include the independence of blood pressure increase from the volume expansion in salt-loading hypertension in anephric rats (23,24). We found no significant difference between the normotensive and the hypertensive groups regarding to plasma osmolality. This finding is compatible with the result revealing that the sodium ion might be a potent stimulator of ADH in addition to its osmolar action (23)

In summary, AVP may partly be responsible for RRM-saline hypertension, but it would be appropriate to investigate the efficiency of the mechanisms that mediate to buffer the vasoconstrictor effect of AVP, in order to clarify the cause of augmented pressor effect of AVP in RRM-saline hypertension.

## References

1. Kaoru Y, Goto A, Hui C, Yagi N, Nagoshi H, Sasabe M, Sugimoto T: Role of ouabain-like compound in rats with reduced renal mass-saline hypertension. *Am J Physiol* 266: H1357-H1362, 1994.
2. Blaustein MP, Hamlyn JM: Sodium transport, inhibition cell calcium and hypertension. *Am J Med* 77(4A): 45-59, 1984.
3. Haddy F: Humoral factors in hypertension. *News Physiol Sci* (4): 202-205, 1989.
4. Ryuzaki M, Suzuki H, Kumagai K, Kumagai H, Ichikawa M, Matsumura Y, Saruta T: Role of vasopressin in salt-induced hypertension in baroreceptor-denervated uninephrectomized rabbits. *Hypertension* 17:1085-1091, 1991.
5. Huang BS, Leenen FHH: Dietary Na, age, and baroreflex control of heart rate and renal sympathetic nerve activity in rats. *Am J Physiol* 262 (Heart Circ Physiol 31): H1441-H1448, 1992.
6. László FA, László F Jr, Wied DD: Pharmacology and clinical perspectives of vasopressin antagonists. *Pharmacol Rev* 43: 73-108, 1991.
7. Share L: Role of vasopressin in cardiovascular regulation. *Physiol Rev* 68: 1248-1284, 1988.
8. Cowley AW: Vasopressin and cardiovascular regulation. In: *Cardiovascular Physiology IV* edited by A.C.Guyton and J.H. Hall, Baltimore, MD. University Park, 1982, vol.26, p.189-242 (Int. Rev. Physiol. Ser.)
9. Huang BS, Frans HHL: Dietary Na and baroreflex modulation of blood pressure and RSNA in normotensive vs spontaneously hypertensive rats. *Am J Physiol* (Heart Circ Physiol 35): H496-H502, 1994.
10. Datar S, Chiu KYE, McNeill JR: Reflexes fail to reduce pressor activity of vasopressin in spontaneous hypertension. *Am J Physiol* 248 (Heart Circ Physiol 17): H49-H54, 1985.
11. Matsuguchi H, Schmid PG: Pressor response to vasopressin and impaired baroreflex function in DOC-salt hypertension. *Am J Physiol* 242 (Heart Circ Physiol 11): H44-H49, 1982.
12. Paller MS, Linas SL: Role of angiotensin II,  $\alpha$ -adrenergic system, and arginine vasopressin on arterial pressure in rat. *Am J Physiol* 24 (Heart Circ Physiol 15): H25-H30, 1984.
13. Osborn Jr JW, Meredith MS, Cowley Jr AV: Hemodynamic effects of vasopressin compared with angiotensin II in conscious rats. *J Physiol* 252 (Heart Circ Physiol 21): H628-H637, 1987.
14. Carp H, Vadhera R, Jayaram A, Garvey D: Endogenous vasopressin and renin-angiotensin systems support blood pressure after epidural block in humans. *Anesthesiology* 5:1000-1007, 1994.
15. Thibonnier M: Vasopressin and blood pressure. *Kidney Int* 34: S-52-S-56, 1988.
16. Webb RL, Osborn Jr, Cowley Jr AW: Cardiovascular actions of vasopressin: baroreflex modulation in the conscious rat. *Am J Physiol* 251 (Heart Circ Physiol 20): H1244-H1251, 1986.
17. Zimpfer M, Manders WT, Barger AC, Vatner SF: Pentobarbital alters compensatory neural and humoral mechanisms in response to hemorrhage. *Am J Physiol* 243 (Heart Circ Physiol 12): H713-H721, 1982.
18. Lee-Kwon WJ, Share L, Crofton JT, Shade RE: Vasopressin in the rat with partial nephrectomy – salt hypertension. *Clin Exp Hypertens* 3: 281-297, 1981.
19. Abboud FM, Floras JS, Aylward PE, Guo GB, Gupta BN, Schmid PG: Role of vasopressin in cardiovascular and blood pressure regulation. *Blood Vess* 27: 106-115, 1990.
20. Ferrari AU, Mark AL: Sensitization of aortic baroreceptors by high salt diet in Dahl salt-resistant rats. *Hypertension* 10: 55-60, 1987.
21. Victor RG, Morgan DA, Thoren P, Mark AL: High salt diet sensitizes cardiopulmonary baroreflexes in Dahl salt-resistant rats. *Hypertension* 8: II-21-II-27, 1986.
22. Burnier M, Brunner HR: Pressor responses of rats to vasopressin: effect of sodium, angiotensin, and catecholamines. *Am J Physiol* 244 (Heart Circ Physiol 13): H253-H258, 1983.
23. Hatzinikolaou P, Gavras H, Brunner HR, Gavras I: Sodium-induced elevation of blood pressure in the anephric state. *Science* 209:935-936, 1980.
24. Hatzinikolaou P, Gavras H, Brunner HR, Gavras I: Role of vasopressin, catecholamines, and plasma volume in hypertonic saline-induced hypertension. *Am J Physiol* 240 (Heart Circ Physiol 9): H827-H831, 1981.