Role of Renal Nerves and Vasopressin in Renal Responses to Acute Volume Expansion in Rats (43213)

KAUSHIK P. PATEL[1]

Department of Physiology and Pharmacology, School of Medicine, University of South Dakota, Vermillion, South Dakota 57069

Abstract. **This study was to determine whether the presence or absence of renal nerves and vasopressin altered the diuretic and natriuretic responses to acute volume expansion. Two forms of volume expansion were used: (i) inflation of a small balloon in the veno-atrial junction and (ii) an infusion of isotonic saline at a rate of 1 ml/min for a period of 15 min, approximately 7% of body weight. Balloon inflation produced a significant diuresis from both the intact and denervated kidneys but only produced a significant natriuresis from the intact kidney. Volume expansion (infusion of saline) produced a significant diuresis and natriuresis from both intact and denervated kidneys. Blocking the V_2 receptor for vasopressin with a V_2-specific receptor blocker $d(CH_2)_5[D\text{-}Ile^2,Val^4]AVP$ (40 μg/kg bolus dose followed by infusion of 4 μg/kg/min) did not alter the diuretic and natriuretic responses to volume expansion. However, the absence of renal nerves or the absence of actions of vasopressin produced a significant reduction in the capacity of the kidneys to increase the relative amount of diuresis or natriuresis, thus losing the control over output; i.e., absence of renal nerves only allowed 12-fold increase in diuresis to volume expansion compared with 25-fold in the intact state and absence of vasopressin only allowed 4.6-fold increase in diuresis to volume expansion compared with 25-fold in the intact state.**

Examining the "volume reflex" in terms of a control system trying to regulate fluid balance, the presence of either renal nerves or actions of vasopressin allows the volume regulating system a greater range in which to control the diuresis and natriuresis (making it possible to fine tune the output to much greater extent). [P.S.E.B.M. 1991, Vol 196]

There is ample evidence showing that volume expansion produces a diuresis and natriuresis (1, 2). However, the role of the renal nerves and vasopressin in these responses remains controversial. Volume expansion, either by stretching the veno-atrial junction or saline infusion, has been shown to produce renal sympatho-inhibition (3–5). Furthermore, renal denervation abolished the increased diuresis and natriuresis to stretching of the veno-atrial junction (5). In contrast, Kaufman and Stelfox (6) have demonstrated that veno-atrial stretch in Brattleboro rats (rats that lack endogenous vasopressin) produces diuresis and natriuresis regardless of the presence or absence of renal nerves. In addition, Kappagoda *et al.* (7) showed that destruction of the posterior pituitary in the dog did not prevent the diuresis elicited by distention of the pulmonary vein and left atrium. Thus, they have argued that although renal nerves and vasopressin may be important in the volume reflex (diuresis and natriuresis produced in response to volume expansion), they are not crucial in the operation of the volume reflex (7).

All of these studies examined the volume reflex with different stimuli (veno-atrial distention or saline infusion) or in different animal models (Brattleboro rats or Sprague-Dawley rats). The present study was conducted to elucidate the precise role of renal nerves and vasopressin in the volume reflex (diuresis and natriuresis to acute volume expansion) using both types of volume stimulus: (i) balloon inflation in the veno-atrial junction stimulating a select and limited group of volume receptors, or (ii) volume loading with saline presumably stimulating all of the volume receptors. In addition, with the use of V_2 receptor blocker, the va-

[1] To whom requests for reprints should be addressed at Department of Physiology and Pharmacology, School of Medicine, University of South Dakota, Vermillion, SD 57069.

Received September 4, 1990. [P.S.E.B.M. 1991, Vol 196]
Accepted December 5, 1990.

0037-9727/91/1964-0438$3.00/0

sopressin component of the volume reflex was eliminated. The advantage that this approach has over the use of Brattleboro rats is the possible inherent differences between the control strain and the Brattleboro rats (8). In addition, with the present design the control rats with vasopressin action intact can also be examined.

Materials and Methods

General Protocol. Male Sprague-Dawley rats (200–250 g) were placed in individual cages in a room with a controlled 12-hr light cycle and maintained at 20–22°C. Food and water were available *ad libitum*.

On the day of the experiment, rats were anesthetized with Inactin (0.1 g/kg ip). Body temperature was maintained between 36°C and 38°C via external warming by a heated stage. After tracheal intubation, the animals were allowed to breathe independently. The left femoral artery was cannulated with PE-50 polyethylene tubing and connected to a pressure transducer (Gould P23 ID) for the continuous recording of arterial pressure. Heart rate was triggered from the arterial pulse wave and recorded along with the arterial pressure on a Grass polygraph (model 7D). The left femoral vein was cannulated with PE-50 tubing and a constant infusion (20 μl/min) of isotonic saline was started.

Renal Denervation and Ureteral Cannulation. The kidneys were exposed through an abdominal incision and left renal denervation was performed by stripping the sheath and adventitia from the exposed left renal artery and vein. To destroy any remaining nerve fibers, the renal vessels were painted with 95% ethanol. Previously, these techniques have been shown to decrease renal norepinephrine concentration to less than 5% (9). In addition, the urine output from the denervated kidney was consistently greater than the contralateral intact kidney. It is recognized that such a preparation will have increased renal nerve activity in the intact kidney because of both Inactin anesthesia (10) and the reno-renal reflex (11). However, this effect is not expected to alter the renal sympatho-inhibition that should occur in response to volume expansion.

Subsequently, both ureters were cannulated with PE-10 tubing. Surgery was completed within 75 min and an additional 30-min stabilization period was allowed before the start of the first urine collection.

Experiment 1. *Renal responses to acute balloon inflation.* To test whether stimulation of specific volume receptors (in the veno-atrial junction) alters the diuretic and natriuretic response in the presence and absence of renal nerves, we examined the renal responses to balloon expansion at the veno-atrial junction in unilaterally denervated rats.A small inflatable balloon was introduced into the right superior vena caval-right atrial junction via the right jugular vein as previously reported by Kaufman and Stelfox (6) and Kaufman (12). Briefly, a small inflatable balloon was fashioned from a fine rubber membrane tied over the flared end of a piece of polyethylene binding (PE-50). This was passed into the right jugular vein and advanced down the right superior vena cava so that the tip lay at the vein-atrial junction. The peculiar anatomy of the rat, whereby blood from the left jugular vein enters the inferior vena cava via a left superior vena cava, enables one to inflate the balloon without interfering with venous return from the head. The balloons were inflated with 100 μl of isotonic saline. Hemodynamic parameters are reported to be unaltered due to this maneuver (12). Arterial pressure did not change significantly due to the introduction of the balloon into the veno-atrial junction or during the balloon inflation in the present experiment. The position of the balloon was confirmed at the end of the experiment.

The diuretic and natriuretic responses to acute balloon inflation were measured in all rats. Urine was collected in preweighed tubes from each of the kidneys separately and urine volume was measured gravimetrically. After two 15-min control collection periods, urine was collected during a period of acute balloon inflation (15 min). Subsequently, sodium (ion selective electrode, Beckman Ion Analyzer) of each of the urine samples was analyzed.

Experiment 2. *Renal responses to acute volume expansion.* To test whether stimulation of "all volume receptors" throughout the cardiopulmonary system alters the diuretic and natriuretic responses in the presence and absence of renal nerves, we examined the renal responses to volume expansion in unilaterally denervated rats.

The diuretic and natriuretic responses to acute volume expansion by the intravenous infusion of isotonic saline (1.0 ml/min) for 15 min were recorded for all rats. Urine was collected and analyzed as described in Experiment 1.

Experiment 3. *Renal responses to acute volume expansion in the presence of the vasopressin V_2 antagonist.* To test whether vasopressin is involved in the diuretic and natriuretic responses to volume expansion, we examined the renal responses to volume expansion in unilaterally denervated rats pretreated with vasopressin V_2 receptor blocker $d(CH_2)_5[D\text{-}Ile^2,Val^4]AVP$ (40-μg/kg bolus dose followed by infusion of 4 μg/kg/min; Peninsula Lab Inc., Belmont, CA). Preliminary studies in our laboratory demonstrated that the diuresis dose-response curve for this antagonist plateaus at a dose of 10 μg/kg bolus injection for approximately 2 hr. Subsequently we used $d(CH_2)_5[D\text{-}Ile^2,Val^4]AVP$ at a dose of 40 μg/kg bolus injection followed by infusion of 4 μg/kg/min to ensure blockade of the V_2 receptors during the length of the present experiments. In the preliminary studies it was determined that the plateau phase of the diuresis was achieved 30 min after the

administration of the V_2 antagonist. Volume expansion was performed during the plateau phase of the diuresis curve, i.e., 30 min after the bolus dose. This dosage regimen is consistent with previous reports of the V_2 receptor antagonist activity (13–15). An infusion of 100 μl of isotonic saline/min was maintained during the vasopressin V_2 receptor blockade to compensate for fluid loss during the blockade.

The diuretic and natriuretic responses to acute volume expansion by the intravenous infusion of saline (1.0 ml/min) for 15 min was performed in all rats. Urine was collected and analyzed as described in Experiment 1.

All of the urine flow and sodium excretion data were expressed as microliters or microequivalents per min per gram kidney weights. The kidney weights were similar between groups as well as between the intact and denervated kidneys within groups.

Statistical Analysis. All data are reported as mean ± SE. A paired *t* test was used to detect differences between (i) control and balloon inflation and (ii) control and volume expansion in the intact kidneys and the denervated kidneys. A $P < 0.05$ was considered to indicate statistical significance.

Results

Mean Systemic Blood Pressure. Acute volume expansion or balloon inflation did not produce a significant change in mean arterial pressure (averaged over the total intervention period) in any of the groups. In addition, the mean arterial pressure did not change significantly after the administration of the V_2 receptor blocker in Experiment 3 (Table I).

Experiment 1. *Renal responses to acute balloon inflation.* Urine flow and sodium excretion before and during balloon inflation from intact and denervated kidneys are shown in Figure 1. Basal (control) urine flow and sodium excretion were greater from the denervated kidneys compared with those from the intact kidneys (Fig. 1). Balloon inflation produced a greater diuresis in both the intact and denervated kidneys (Fig. 1A). Although there was a significant increase in sodium excretion from the intact kidney, there was no significant change from the denervated kidney after balloon inflation (Fig. 1B).

Table I. Basal Mean Arterial Blood Pressure Before and After Either Balloon Inflation or Volume Expansion

	Arterial pressure (mm Hg)	
	Basal	Treatment
Balloon inflation ($n = 13$)	101 ± 3[a]	98 ± 2[a]
Volume expansion ($n = 6$)	125 ± 5	123 ± 5
Vasopressin antagonist + volume expansion ($n = 8$)	119[b] ± 6	113 ± 6	113 ± 5

[a] Values represent mean ± SE. No significant differences between basal and treatment.
[b] Indicates the pressure before the administration of vasopressin antagonist.

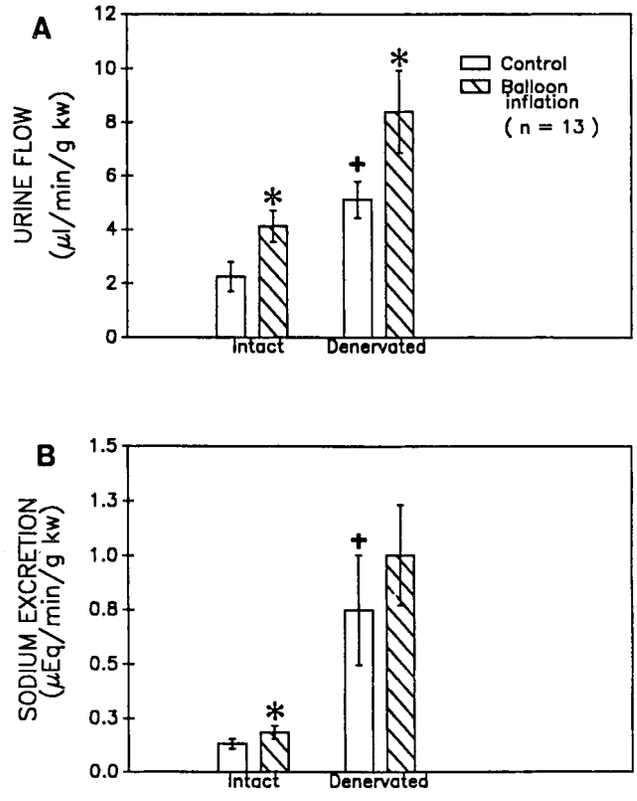

Figure 1. Urine flow (A) and sodium excretion (B) before and during balloon inflation from intact and denervated kidneys. Values represent mean value for each group ± SE. *$P < 0.05$ versus control, +$P < 0.05$ versus intact (control) ($n = 13$).

Experiment 2. *Renal responses to acute volume expansion.* Urine flow and sodium excretion before and during volume expansion from intact and denervated kidneys are shown in Figure 2. Basal (control) urine flow and sodium excretion were greater from the denervated kidneys compared with those from the intact kidneys (Fig. 2). Volume expansion produced a significantly greater diuresis and natriuresis in both the intact and denervated kidneys (Fig. 2). Note that the rise in urine flow from 1 to 25 μl/min in the intact kidneys is a 25-fold increase, whereas an increase from 5 to 60 μl/min in the denervated kidneys of control rats is approximately 12-fold, although the absolute increase is greater in the denervated kidney (55 μl/min) compared with the intact kidney (24 μl/min) (Fig. 2A). The "fold" changes as well as absolute changes were significantly different between the intact and denervated kidneys (by paired *t* test).

Experiment 3. *Renal responses to acute volume expansion in the presence of the vasopressin V_2 antag-*

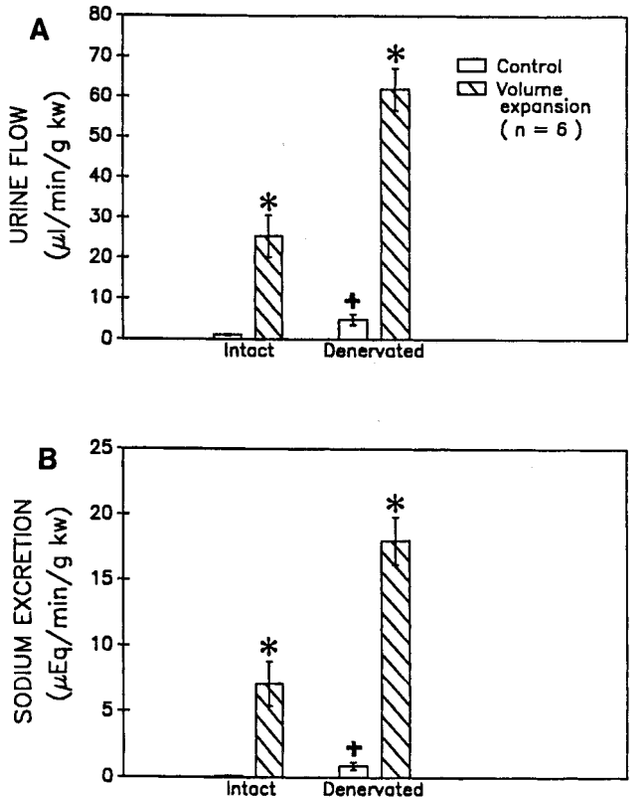

Figure 2. Urine flow (A) and sodium excretion (B) before and during volume expansion from intact and denervated kidneys. Values represent mean value for each group ± SE. *$P < 0.05$ versus control. +$P < 0.05$ versus intact (control) ($n = 6$).

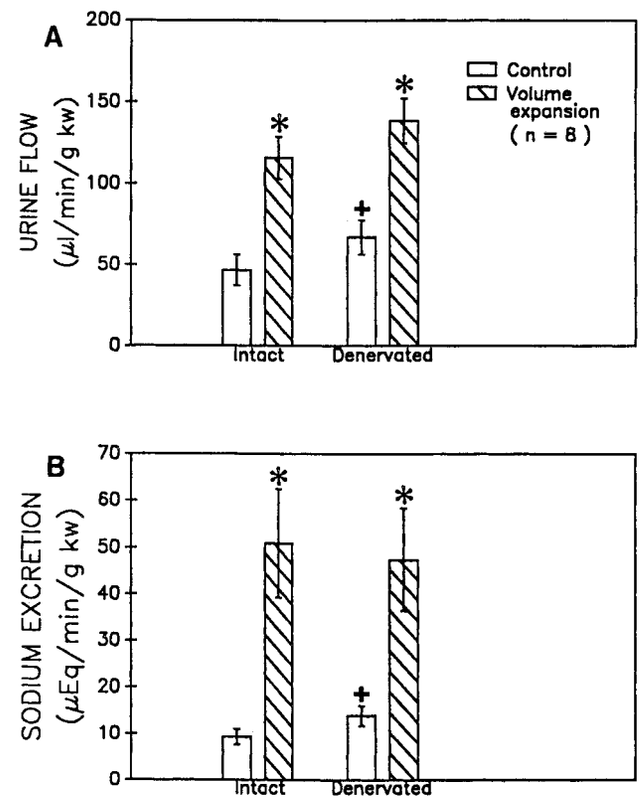

Figure 3. Urine flow (A) and sodium excretion (B) before and during volume expansion from intact and denervated kidneys of rats treated with an vasopressin V_2 receptor blocker. Values represent mean value for each group ± SE. *$P < 0.05$ versus control. +$P < 0.05$ versus intact (control) ($n = 8$).

onist. Basal (control) urine flow and sodium excretion were greater in this experiment compared with those in Experiment 2 because of V_2 receptor blockade (Fig. 3). Basal urine flow and sodium excretion were greater from the denervated kidneys compared with those from the intact kidneys (Fig. 3). Volume expansion produced a significantly greater diuresis and natriuresis in both the intact and denervated kidneys (Fig. 3). Note that the rise in urine flow from 1 to 25 μl/min in the intact kidneys is a 25-fold increase (Experiment 2, vasopressin action intact; Fig. 2A), whereas an increase from 25 to 115 μl/min in the intact kidneys of rats with V_2 receptor blockade is approximately 4.6-fold (Fig. 3A), although the absolute increase in volume is greater in the V_2 receptor-blocked rats (90 μl/min) compared with that in the rats without V_2 receptor blockade (24 μl/min). The fold changes as well as absolute changes were significantly different between the intact kidneys and intact kidneys treated with V_2 receptor blocker (by paired *t* test).

Discussion

These experiments demonstrate that stimulation of a few discrete atrial volume receptors at the veno-atrial junction or all volume receptors due to volume expansion does produce a diuresis and natriuresis. Furthermore, this systematic study shows that there was increased diuresis and natriuresis in response to volume expansion in the absence of renal nerves and/or blockade of V_2 receptors for vasopressin. It should be noted that the absence of renal nerves and/or blockade of V_2 receptors for vasopressin produced a reduction in the capacity of the kidney to increase urine flow and sodium excretion (relative to basal output) for a given volume load.

Previous studies by Kaufman and Stelfox (6) have shown that renal denervation in conscious Brattleboro rats does not affect the diuresis and natriuresis produced by inflation of a balloon at the veno-atrial junction. The results of the present studies are not in disagreement with these observations, since we also observed an increased diuresis and natriuresis to volume expansion in rats treated with vasopressin V_2 receptor antagonist. However, the comparison between rats with and without vasopressin in the present study shows that there was a 25-fold increase in diuresis from intact kidneys of rats with vasopressin action intact compared with a 4.6-fold increase from rats with V_2 receptor blockade. These results suggest that vasopressin may be important in the gain of the volume reflex. Similarly, an absence of renal nerves produced an attenuated

response from the denervated kidney since volume expansion in the intact kidney produced a 25-fold increase in diuresis whereas the similar volume expansion produced a 12-fold increase in diuresis from the denervated kidneys. These data show that the absence of either the antidiuretic activity of vasopressin or loss of renal nerve activity reduces the capacity of the kidneys to conserve fluid and electrolytes, thus losing the gain of the control mechanism over output. Examining the volume reflex in terms of a control system trying to regulate fluid balance, the presence of either renal nerves or actions of vasopressin allows the volume-regulating system a greater range in which to control the diuresis and natriuresis (making it possible to fine tune the output from the kidney more precisely, to a given volume load). This concept is also consistent with the overall goal of the kidneys to regulate fluid balance by controlling the output. These data show that both renal nerves and vasopressin may allow the kidney to have a better control (i.e., higher gain of the input-output relationship) of the capacity to conserve over a wider range of outputs. Such an enhancement of the capabilities of the kidney multiplies its power to regulate the fluid balance much more precisely.

An alternate explanation of these data could be that in the absence of either renal nerves or the antidiuretic actions of vasopressin, although the basal urine flow was increased, the maximum urine flow possible is a constant. Therefore the "fold changes" in urine flow (expressed relative to the basal flow rates) were smaller. However, this is not quite true since the maximum urine flow was 60 μl/min in the absence of renal nerves but it went up to 115 μl/min in the absence of the antidiuretic action of vasopressin. Second, even if we assume that there is a maximum urine flow for each circumstance (i.e., renal denervation or V_2 receptor blockade) then it would suggest that the renal response curve (volume expansion versus diuresis, sigmoid curve) was at a plateau phase and, therefore, the slope of the curve (i.e., the gain of the stimulus-response curve-volume expansion versus diuresis) was substantially reduced. Therefore the reduced capacity of the kidneys to conserve fluid and electrolytes in the present study can be attributed to a reduced gain of the volume reflex control system.

Consistent with the observations in the present study, Kopp *et al.* (5) have demonstrated that right atrial balloon inflation results in a decrease in efferent sympathetic activity. In addition, in a limited group of rats ($n = 3$) they observed that renal denervation abolished the diuresis and natriuresis to right atrial balloon inflation. They concluded that diuresis and natriuresis to atrial balloon inflation was due to renal sympathoinhibition. Since the renal responses (i.e., diuresis and natriuresis) to balloon inflation are relatively small compared with volume expansion, results from such limited data can lead to possible errors in conclusions. In the present study, there was an increase in diuresis and natriuresis to atrial balloon inflation stimulus in spite of renal denervation, although the increase in natriuresis to balloon inflation from the denervated kidney did not reach statistical significance ($n = 13$). The purpose of this study (Experiment 1) was primarily to examine whether renal nerves are totally responsible for the diuretic and natriuretic responses to balloon inflation. The results indicate that renal nerves are partially involved in the diuretic and natriuretic responses to balloon inflation. One of the reasons for the difficulty of interpretation of data is the relatively small diuretic and natriuretic responses to balloon inflation compared with volume expansion. It should be noted that volume expansion (stimulation of all volume receptors) is a more potent stimulus compared with the right atrial balloon inflation (discrete group of volume receptors located in the veno-atrial junction).

At the same time it is recognized that both right atrial balloon inflation and volume expansion do increase diuresis and natriuresis regardless of the presence or absence of renal nerves or the action of vasopressin (6). This fact can allude to the possibility of humoral factor(s) other than vasopressin. One likely candidate is atrial natriuretic factor. With its rapid release and onset of action, it has been implicated in the renal responses to volume reflex (16). Both atrial distention (17) and volume expansion (18) have been demonstrated to elevate the circulating levels of atrial natriuretic factor. Until a specific antagonist for ANF is obtained, this possibility will remain to be examined.

This study demonstrated that the *relative* diuresis and natriuresis produced in the absence of either renal nerves or antidiuretic action of vasopressin was smaller than in their presence. Thus, we conclude that absence of either renal nerve activity or the antidiuretic actions of vasopressin produces a loss of fine control of diuresis and natriuresis by the kidney to a given volume load.

This work was supported by NIH Grant HL39046. The technical assistance of Tony Miller is greatly appreciated.

1. Gauer OH, Henry JP. Circulatory basis of fluid volume control. Physiol Rev **43**:423–481, 1963.
2. Linden RJ, Kappagoda CT. Atrial Receptors. Cambridge: Cambridge University Press, 1982.
3. DiBona GF. The functions of the renal nerves. Rev Physiol Biochem Pharmacol **94**:76–157, 1982.
4. Dibona GF, Sawin LL. Renal nerve activity in conscious rats during volume expansion and depletion. Am J Physiol **248**:F15–F23, 1985.
5. Kopp UC, Smith LA, Dibona GF. Natriuresis produced by right atrial balloon inflation is mediated by renal nerves [Abstract]. Fed Proc **46**:1249, 1987.
6. Kaufman S, Stelfox J. Atrial stretch-induced diuresis in Brattleboro rats. Am J Physiol **252**:R503–R506, 1987.

7. Kappagoda CT, Linden RJ, Snow HM, Whitaker EM. Effect of destruction of the posterior pituitary on the diuresis from the left atrial receptors. J Physiol Lond **244**:757–770, 1975.
8. Valtin H. The discovery of the brattleboro rats, recommended nomenculture, and the question of proper controls. Ann NY Acad Sci **394**:1–9, 1982.
9. Kline RL, Mercer PF. Functional reinnervation and development of supersensitivity to norepinephrine after renal denervation. Am J Physiol **247**:R353–R358, 1980.
10. Krayacich J, Kline RL, Mercer PF. Supersensitivity to NE alters renal function of chronically denervated rat kidneys. Am J Physiol **252**:F856–F864, 1987.
11. DiBona G, Rios LL. Renal nerves in compensatory renal response to contralateral renal denervation. Am J Physiol **238**:F26–F30, 1980.
12. Kaufman S. Chronic, nonocclusive, and maintenance-free central venous cannula in the rat. Am J Physiol **239**:R123–F125, 1980.
13. Kinter LB, Huffman WF, Stassen FL. Antagonists of the antidiuretic activity of vasopressin. Am J Physiol **254**:F165–F177, 1988.
14. Manning M, Nawrocka E, Misicka A, Olma A, Klis WA, Seto J, Sawyer WH. Potent and Selective antagonist of antidiuretic responses to arginine-vasopressin based on modifications of [1(β-Mercapto-β,β-pentamethylenepropionic acid, 2-D-isolucine, 4-valine] arginine-vasopressin at position 4. J Med Chem **27**:423–429, 1984.
15. Tang AH, Ho PM. A specific antagonist of vasopressin produced plasma hyperosmolality and reduced ischemic-induced cerebral edema in rats. Life Sci **43**:399–403, 1988.
16. Cantin M, Genest J. The heart and atrial natriuretic factor. J Endocrine Rev **6**:107–127, 1985.
17. Ledsome JR, Wilson N, Corneya CA, Rankin AJ. Release of atrial natriuretic peptide by atrial distension. Can J Physiol Pharmacol **63**:739–742, 1985.
18. Kohno M, Clegg KB, Sambi MP. Effect of volume change on circulating immunoreactive atrial natriuretic factor in rats. Hypertension **10**:171–175, 1987.