Central and Peripheral Vasopressin Interact Differently With Sympathetic Nervous System and Renin-Angiotensin System in Renal Hypertensive Rabbits

Hiroo Kumagai, Hiromichi Suzuki, Masashi Ichikawa, Yasuo Matsumura, Masahito Jimbo, Munekazu Ryuzaaki, and Takao Saruta

This study was designed to elucidate how central and peripheral arginine vasopressin (AVP) interacts with the sympathetic nervous system and the renin-angiotensin system to maintain blood pressure in two-kidney, one-clip hypertensive rabbits. We recorded renal sympathetic nerve activity (RSNA) in the conscious state as an index of sympathetic nervous system function. The changes in mean arterial pressure, heart rate, and RSNA were recorded continuously for 60 minutes after intravenous administrations of captopril (2.5 mg/kg) and nicardpine (3.2 μg · kg⁻¹ · min⁻¹) in eight identical rabbits. Despite equivalent reductions in mean arterial pressure (10±1 mm Hg), the increase in RSNA was significantly larger with captopril than that with nicardpine, and the plasma concentration of AVP was elevated (from 100% to 255±24%) with captopril. Mean arterial pressure was reduced, and RSNA was increased by intravenous infusion of AVP antagonist d(CH₂)₅Tyr(Me)AVP (n=6), whereas vertebral artery infusion of the antagonist (n=6) did not change RSNA. During central and peripheral infusions of AVP antagonist, RSNA was exaggerated by blood pressure reduction with nicardpine as well as with captopril. Increases in RSNA induced by captopril and nicardpine were larger by central infusion of AVP antagonist than by intravenous infusion. The decrease in mean arterial pressure by captopril (30±4 mm Hg) in eight sinoaortic-denervated hypertensive rabbits was larger than that in hypertensive rabbits with intact baroreflex. These data suggest that compensatory activation of RSNA was revealed by central and peripheral attenuation of AVP and that the sympathetic nervous system became the most important mechanism for blood pressure maintenance in the absence of AVP. The interaction of AVP with the sympathetic nervous system may be independent of the state of the renin-angiotensin system, since the exaggeration of RSNA by AVP antagonist was qualitatively the same with nicardpine as with captopril. In conscious renal-hypertensive rabbits, AVP in the central nervous system played a substantial role when blood pressure was reduced, although it did not contribute to blood pressure maintenance in the basal condition. (Circulation Research 1993;72:1255–1265)

KEY WORDS • arginine vasopressin • baroreflex • interaction • pressor systems • renal sympathetic nerve activity • renin-angiotensin system • sinoaortic denervation • sympathetic nervous system

The interaction of arginine vasopressin (AVP) with the sympathetic nervous system (SNS) and with the renin-angiotensin system (RAS) in maintaining blood pressure (BP) of normotensive animals is well known.¹⁻³ Hasser et al⁴ found a close relation between AVP and the SNS in normotensive dogs. Exogenous AVP administered in the cerebroventricle stimulates the SNS,⁵ and AVP increases the SNS activity in salt-induced hypertensive models.⁶ In contrast, few studies have examined the role of endogenous AVP in regulating BP and the relation among AVP, the SNS, and the RAS in renal hypertension, although a redundant interaction between the RAS and SNS has been shown.⁷

Earlier studies used changes in mean arterial pressure (MAP) and heart rate (HR) in response to ganglionic blockade or 6-hydroxydopamine as indicators of SNS function when examining the interaction of the pressor systems.¹⁻⁴,⁷⁻¹⁰ Plasma concentration of norepinephrine has also been used as an index of SNS; however, it does not always reflect the actual state of the SNS, as Folkow et al¹¹ have cautioned.

We recorded renal sympathetic nerve activity (RSNA) in the conscious state to investigate the role of endogenous AVP and angiotensin II (Ang II) in modulating the baroreflex control of the SNS in two-kidney, one-clip hypertensive rabbits.¹² The purpose of the present study was to elucidate how endogenous AVP interacts with the SNS and the RAS in the regulation of BP of renal-hypertensive rabbits. Contrary to previous
that the peripheral AVP interact differently with the SNS. In this study, we assessed the relative contributions of AVP, the RAS, and the SNS during different hypotensive challenges, and we chose drug regimens to produce equivalent decreases in MAP but to make very different profiles for the contribution of the pressor systems.

### Materials and Methods

#### General Preparations

Experiments were performed on 110 female Japanese White rabbits weighing 2.5–3.2 kg in accordance with the “Guiding Principles for Research Involving Animals and Humans” (Department of Health and Human Services, publication No. [NIH] 86-23). In surgery, anesthesia was induced with 30 mg/kg intravenous pentobarbital sodium and maintained with 6 mg·kg⁻¹·hr⁻¹ pentobarbital. Three days before applying a renal clip (described below), polyethylene catheters (PE-60, Clay Adams, Parsippany, N.J.) were placed in the left subclavian artery for measuring the arterial BP and in the external jugular veins for administrations of drugs. These catheters were exteriorized at the back of the neck. Measurements of arterial BP

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Normotension</th>
<th>Hypertension</th>
<th>SAD plus normotension</th>
<th>SAD plus hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>76±2</td>
<td>90±2*</td>
<td>77±5</td>
<td>98±4*†</td>
</tr>
<tr>
<td>HR (bpm)</td>
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<td>221±5</td>
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SAD, sinoaortic denervation; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute. Values are mean±SEM. *p<0.05 vs. rabbits with normotension. †p<0.05 vs. rabbits with SAD plus normotension.

studies that determined the relation of the pressor systems in the periphery, we examined the role of AVP in the central nervous system by infusing AVP antagonist into the vertebral artery. We advanced the hypothesis that, in renal hypertension, the SNS would be activated for BP maintenance when AVP is reduced and that the SNS would be exaggerated only when the RAS is attenuated. We also hypothesized that central and peripheral AVP interact differently with the SNS. In this study, we assessed the relative contributions of AVP, the RAS, and the SNS during different hypotensive challenges, and we chose drug regimens to produce equivalent decreases in MAP but to make very different profiles for the contribution of the pressor systems.

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(model AP-611G, Nihon Kohden Electronics Co., Tokyo) and HR (AT-601G, Nihon Kohden) were recorded for 60 minutes each day.

On day 11, another catheter for vertebral artery infusion of AVP antagonist (in protocol 2, below) was placed in the right subclavian artery. The tip of the catheter was placed at the origin of the vertebral artery according to the method of van Zweiten. The vertebral artery was chosen as the route of AVP antagonist infusion on the basis of an observation by Undesser et al. that AVP potentiated the baroreflex control of RSNA at the brainstem in conscious rabbits.

Production of Renal-Hypertensive and Sham-Clipped Rabbits

On day 0, a stainless-steel clip with a slit 0.5 mm wide was placed on the right renal artery through a flank incision. We used a dissecting microscope to avoid damaging the renal nerves. These animals were designated as renal-hypertensive rabbits. We also prepared sham-clipped rabbits in which the clip was placed close to the renal artery.

Recording and Quantification of RSNA

The method for recording and quantifying RSNA is described elsewhere. On day 11, via a retroperitoneal approach, the left renal artery and vein were exposed. Teflon-coated stainless-steel wire electrodes (0.002 in. i.d., 0.009 in. o.d., A-M Systems Inc., Everett, Wash.) were implanted around the renal nerves. The nerves and electrodes were fixed together with silicone gel (Silgel 604A and B, Wacker Chemie, Munich, FRG). The electrodes were then exteriorized at the back of the neck. After the electrodes were implanted, the rabbits were kept in standard cages and had free access to food and water.

On the days of the experiment, the RSNA was amplified with a differential amplifier (model AVB-10, Nihon Kohden) with a band-pass filter of 50–3,000 Hz. The amplified RSNA signal was rectified and integrated by a root-mean-square integrator (model EI-601G, Nihon Kohden) with a time constant of 28 msec. This signal was further filtered at a time constant of 2 seconds. We designated this signal as “mean RSNA” and used it for quantification. The pulse pressure, MAP, HR, original neurogram of RSNA, and mean RSNA were recorded simultaneously on a thermal array recorder (model RTA-1300, Nihon Kohden) and stored in a multichannel data recorder (model A-89, Sony Inc., Tokyo).

Sinoaortic Denervation

Fourteen or 15 days before the renal clipping or sham clipping, the bilateral carotid sinus nerves and aortic nerves were sectioned. Immediately after the section was performed, sinoaortic denervation (SAD) was confirmed by the absence of reflex bradycardia and tachycardia in response to 30 mm Hg elevations and reductions of MAP produced with infusions of phenylephrine and nitroglycerin. On day 0, the renal or sham clipping was performed in the SAD groups.

Drugs

Doses of antihypertensive agents had been determined to induce a 10 mm Hg reduction of MAP in conscious renal-hypertensive rabbits with intact baroreflex in our previous studies. Nicardipine, a calcium channel blocker that produces direct vasodilation, was infused at a dose of 3.2 μg·kg⁻¹·min⁻¹ (diluted in saline) for 30 minutes and at 1.8 μg·kg⁻¹·min⁻¹ for a subsequent 30 minutes at a rate of 42 μl/min with a microliter syringe pump (Harvard Apparatus, South Natick, Mass.). This dose and infusion rate had been determined to simulate the depressor response of captopril. Captopril, an angiotensin I converting enzyme inhibitor, was injected at a dose of 2.5 mg/kg. Immediately after the injection, saline was infused at a rate of 42 μl/min to match the other drug regimens. Clonidine, a centrally acting agent to attenuate the sympathetic outflow, was administered at 5 μg/kg by bolus injection, followed by a 0.5 μg·kg⁻¹·min⁻¹ infusion (diluted in saline) at a rate of 42 μl/min. This dose and infusion rate of clonidine affect the central sympathetic outflow without causing peripheral resetting of the arterial baroreceptors. Vehicle (saline) was infused at a rate of 42 μl/min.

Protocol 1: Effects of Captopril and Nicardipine in Rabbits With Intact Aortic Baroreflex

The relative contributions of AVP, the SNS, and the RAS during different hypotensive challenges were assessed in conscious renal-hypertensive (n=8) and normotensive (n=6) rabbits with intact baroreflex. The responses of MAP, RSNA, and HR to vehicle, captopril, and nicardipine were recorded continuously for 60 minutes. Mean RSNA just before drug administration was defined as 100%. Captopril and nicardipine were given in identical rabbits on different days (day 14 or 15), and the order of administration was determined in a randomized manner. On day 15, the examination with vehicle was performed at 4 hours before beginning the examination with captopril or nicardipine.

Protocol 2: Effects of Captopril and Nicardipine During Vertebral Artery Infusion of AVP Antagonist

The purpose of this protocol was to examine how AVP interacts with the SNS to maintain BP in the central nervous system. The responses of MAP, HR, and RSNA during vertebral artery infusion of AVP antagonist d(CH2)5Tyr(Me)AVP (7 ng·kg⁻¹·min⁻¹, diluted in saline, 42 μl/min, Sigma) were recorded continuously for 30 minutes in six hypertensive and six normotensive rabbits. This dose of AVP antagonist had been determined to eliminate the pressor effect of 2.0 milliunits·kg⁻¹·min⁻¹ i.v. AVP in our preliminary study. The responses to vehicle, captopril, and nicardipine during the vertebral artery infusion of AVP antagonist were recorded for the subsequent 60 minutes. RSNA before the AVP antagonist infusion was defined as 100%. AVP antagonist plus vehicle, AVP antagonist plus captopril, and AVP antagonist plus nicardipine were given in the same rabbits on different days (days 14–16).

Protocol 3: Effects of Captopril and Nicardipine During Intravenous Infusion of AVP Antagonist

This protocol was performed to contrast the difference in central and peripheral effects of endogenous AVP on the SNS in modulation of BP in conscious renal-hypertensive rabbits (n=8). MAP, HR, and RSNA during intravenous infusion of AVP antagonist (7 ng·kg⁻¹·min⁻¹) were recorded in the same manner as in protocol 2. The responses to vehicle, captopril, and
nicardipine during the intravenous infusion of AVP antagonist were recorded for a subsequent 60 minutes. AVP antagonist plus vehicle, AVP antagonist plus captopril, and AVP antagonist plus nicardipine were given in the same rabbits on different days (days 14–16).

**Protocol 4: Effects of Captopril and Nicardipine in SAD Rabbits**

The purpose of this protocol was to examine whether the afferent limb of the baroreflex is necessary for AVP to support BP. The responses of MAP, HR, and RSNA to the drugs were recorded for 60 minutes in eight hypertensive and six normotensive rabbits with SAD. RSNA before the drug administration was defined as 100%.

**Protocol 5: Effects of Captopril and Nicardipine During Intravenous Infusion of AVP Antagonist in SAD Rabbits**

This protocol was performed to examine the contribution of peripheral AVP in maintaining the cardiovascular regulation in SAD rabbits with renal hypertension. The responses of MAP, HR, and RSNA to intravenous infusion of AVP antagonist were recorded for 30 minutes in six renal-hypertensive rabbits with SAD. The responses to vehicle, captopril, and nicardipine during intravenous infusion of AVP antagonist were recorded for a subsequent 60 minutes. RSNA before the AVP antagonist infusion was defined as 100%.

**Protocol 6: Effects of Captopril and Nicardipine During Clonidine Infusion**

The purpose of this protocol was to examine whether endogenous AVP could respond to the attenuation of the central sympathetic outflow. The responses of MAP, RSNA, and HR to clonidine (5 µg/kg by bolus injection followed by 0.5 µg·kg⁻¹·min⁻¹ infusion¹⁹ at 42 µl/min) were recorded continuously for 30 minutes in eight hypertensive and six normotensive rabbits. The responses to vehicle, captopril, and nicardipine during the clonidine infusion were recorded for a subsequent 60 minutes. RSNA before clonidine administration was defined as 100%. Clonidine plus vehicle, clonidine plus captopril, and clonidine plus nicardipine were given in the same rabbits on different days (days 14–16).

**Plasma Concentrations of Neurohormones**

To examine the effects of humoral factors on hemodynamics and neural element, additional renal-hypertensive and normotensive rabbits were prepared. Drugs were administered as in protocols 1, 4, and 6 in eight renal-clipped and six sham-clipped rabbits for each protocol. A 5-ml blood sample was taken 30 minutes after administration of the drug to determine the plasma concentrations of Ang II, AVP, and norepinephrine. The plasma concentrations of Ang II and AVP were determined by radioimmunoassay²⁰,²¹ and that of norepinephrine by high-performance liquid chromatography.²² The intraassay coefficients of variation for Ang II, AVP, and NE were 8.4%, 4.4%, and 4.1%, respectively. The interassay coefficients of variation were 9.9%, 7.6%, and 7.4%, respectively.

**Statistical Analysis**

Data are presented as mean±SEM. We compared the basal MAP and basal HR with MAP, RSNA, and HR in response to the antihypertensive drugs and percent values of concentration of neurohormones with one-way analysis of variance with repeated measures.²³ These comparisons were followed by Scheffe’s F test for multiple-comparison procedures. Differences with p<0.05 were considered significant.

**Results**

**Basal Values of MAP and HR**

Fourteen days after the clipping, the MAP of renal-clipped rabbits was significantly higher than that of sham-clipped rabbits (Table 1). The difference in basal MAP between normotensive and hypertensive rabbits was maintained after SAD. Although the MAP in SAD rabbits with renal hypertension was slightly higher than that of hypertensive rabbits with intact baroreflex, the difference was not significant.

**Protocol 1: Effects of Captopril and Nicardipine in Rabbits With Intact Arterial Baroreflex**

Since a nadir in the BP response to captopril was observed 30 minutes after the injection and the mode of nicardipine infusion was arranged to simulate the depressor sequence of captopril, the maximum responses of RSNA and HR were obtained at 30 minutes. In renal-hypertensive rabbits, MAP was reduced by 10±1 and 11±1 mm Hg with captopril and nicardipine, respectively (Figure 1A and Table 2). The increase in RSNA induced by captopril (+68±4%) was significantly greater than that induced by nicardipine (+31±6%). The increase in HR was larger with nicardipine.

**Table 2. Mean Arterial Pressure, Renal Sympathetic Nerve Activity, Heart Rate, and Plasma Concentration of Arginine Vasopressin in Renal-Hypertensive Rabbits**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>1: Intact baroreflex</th>
<th>2: AVPX (VA) infusion</th>
<th>3: AVPX (iv) infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>CAP</td>
<td>NIC</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>90±2</td>
<td>80±1*</td>
<td>79±2*</td>
</tr>
<tr>
<td>RSNA (%)</td>
<td>100</td>
<td>168±4*†</td>
<td>131±6*</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>221±5</td>
<td>241±8*†</td>
<td>265±*</td>
</tr>
<tr>
<td>AVP (pg/ml)</td>
<td>3.3±0.4</td>
<td>8.4±0.3*†</td>
<td>4.1±0.4</td>
</tr>
</tbody>
</table>

CAP, captopril; NIC, nicardipine; AVP, arginine vasopressin antagonist; VA, vertebral artery; iv, intravenous; SAD, sinoaortic denervation; MAP, mean arterial pressure; RSNA, renal sympathetic nerve activity; HR, heart rate; bpm, beats per minute; AVP, plasma concentration of arginine vasopressin; ND, not determined. Values are mean±SEM.

* p<0.05 vs. vehicle of protocol 1.
† p<0.05 vs. NIC in each protocol.
Table 2. Continued

Table 3 shows the plasma concentrations of Ang II, AVP, and NE. The concentrations of all neurohormones in hypertensive rabbits with intact baroreflex were significantly higher than those in normotensive rabbits (Table 3). We compared the percent values of these concentrations in rabbits with intact baroreflex; each concentration with vehicle was defined as 100% (Figure 2). In renal-hypertensive rabbits, the percent value of Ang II was 56±6% after captopril injection (Figure 2A), a value that was significantly smaller than that produced by vehicle or nicardipine. In contrast, AVP was markedly increased to 255±24% by captopril. With nicardipine infusion, the percent values of Ang II and NE were higher than those with vehicle.

In normotensive rabbits (Figure 1B), neither captopril nor nicardipine changed BP, RSNA, or HR. There were no significant differences in concentrations of neurohormones after drug administration (Figure 2B).

Protocol 2: Effects of Captopril and Nicardipine During Vertebral Arterial Infusion of AVP Antagonist

Figure 3A demonstrates that vertebral artery infusion of AVP antagonist did not significantly change either MAP, RSNA, or HR. By contrast, during the central infusion of AVP antagonist, reduction in MAP by captopril was associated with significant increases in RSNA (+112±6%) and HR. Reduction in MAP by nicardipine was also associated with significant increases in RSNA (+84±5%) and HR. Both values of RSNA during the vertebral artery infusion of AVP antagonist (Figure 3A) were significantly (p<0.01) greater than those without the antagonist (Figure 1A). During the central infusion of AVP antagonist, the increase in RSNA by captopril was significantly larger than that by nicardipine.

Protocol 3: Effects of Captopril and Nicardipine During Intravenous Infusion of AVP Antagonist

As shown in Figure 4, MAP was significantly reduced, and RSNA was increased by 34±5% with intravenous AVP antagonist in conscious renal-hypertensive rabbits. Captopril and nicardipine further reduced MAPs during intravenous AVP antagonist infusion; the significant reductions in MAP were associated with significant increases in RSNA (+82±5% and +64±6%, respectively). The increases in RSNA with captopril and nicardipine during intravenous infusion of AVP antagonist were significantly (p<0.05) smaller than those during vertebral artery infusion of the antagonist.

Protocol 4: Effects of Captopril and Nicardipine in SAD Rabbits

In SAD rabbits with renal hypertension, captopril and nicardipine significantly reduced MAP (Figure 5A and Table 2). The decrease in MAP with captopril in SAD rabbits with hypertension (30±4 mm Hg) was significantly larger than that in hypertensive rabbits with intact baroreflex (10±1 mm Hg). No changes in RSNA and HR were observed despite the significant decreases in MAP. In SAD rabbits with normotension, the decreases in MAP induced by captopril and nicardipine were 18±3 and 8±3 mm Hg, respectively (Figure 5B).

In SAD rabbits with renal hypertension, captopril did not decrease the plasma concentration of Ang II (Figure 6A). Ang II was significantly increased with nicardipine as compared with the vehicle.

Protocol 5: Effects of Captopril and Nicardipine During Intravenous Infusion of AVP Antagonist in SAD Rabbits

In SAD rabbits with renal hypertension, MAP was significantly reduced from 102±5 to 84±4 mm Hg by

Table 3. Plasma Concentration of Neurohormones During Vehicle Infusion

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Renal hypertension</th>
<th>Normotension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ang II</td>
<td>AVP</td>
</tr>
<tr>
<td>Intact baroreflex</td>
<td>52±3*</td>
<td>3.3±0.4*</td>
</tr>
<tr>
<td>SAD</td>
<td>40±2*</td>
<td>4.2±0.5*†</td>
</tr>
<tr>
<td>With clonidine</td>
<td>31±3*†</td>
<td>0.3±0.1†</td>
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</table>

Ang II, angiotensin II; AVP, arginine vasopressin; NE, norepinephrine; SAD, sinoaortic denervation. Values are mean±SEM.

* p<0.05 vs. respective values for normotensive rabbits.
† p<0.05 vs. respective values for rabbits with intact baroreflex.
A. Renal Hypertensive

B. Normotensive

FIGURE 2. Bar graphs showing percent values (mean±SEM) of plasma concentrations of angiotensin II (ANG II), arginine vasopressin (AVP), and norepinephrine (NE) with vehicle (V), captopril (CAP), and nicardipine (NIC) in hypertensive and normotensive rabbits with intact baroreflex. Each concentration with V is defined as 100%. CAP decreased ANG II and increased AVP, whereas NIC increased ANG II in renal-hypertensive rabbits. *p<0.05 vs. V; †p<0.05 vs. NIC.

FIGURE 3. Bar graphs showing maximum responses (mean±SEM) of mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA), and heart rate (HR) with vertebral artery (VA) infusion of arginine vasopressin antagonist d(CH$_2$)$_5$Tyr(Me)AVP (AVPX) and the responses to captopril (CAP) and nicardipine (NIC) during the central infusion of AVPX in conscious renal-hypertensive (n=6) and normotensive (n=6) rabbits. NS, not significant. RSNA is expressed as a change from the baseline activity recorded before AVPX infusion. Increases in RSNA produced by CAP and NIC during VA infusion of AVPX were significantly larger than the increases in RSNA without AVPX (Figure 1A). *p<0.05.
intravenous AVP antagonist infusion. The reduction in MAP was significantly larger than that with intravenous AVP antagonist in renal-hypertensive rabbits with intact baroreflex (protocol 3). Captopril and nicardipine further lowered the MAP to 69±3 and 73±4 mm Hg, respectively. HR and RSNA were not changed by the significant MAP reduction with intravenous AVP antagonist.

![Bar graphs showing maximum responses (mean±SEM) of mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA), and heart rate (HR) with intravenous infusion of arginine vasopressin antagonist d(CH2)5Tyr(Me)AVP (AVPX) and the responses to captopril (CAP) and nicardipine (NIC) during intravenous infusion of AVPX in eight renal-hypertensive rabbits. NS, not significant. RSNA is expressed as a change from the baseline activity recorded before AVPX infusion. Contrary to no responses to vertebral artery infusion of AVPX, MAP was reduced and RSNA was increased by intravenous infusion of AVPX by itself. *p<0.05.](image)

**Figure 4.** Bar graphs showing maximum responses (mean±SEM) of mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA), and heart rate (HR) with intravenous infusion of arginine vasopressin antagonist d(CH2)5Tyr(Me)AVP (AVPX) and the responses to captopril (CAP) and nicardipine (NIC) during intravenous infusion of AVPX in eight renal-hypertensive rabbits. NS, not significant. RSNA is expressed as a change from the baseline activity recorded before AVPX infusion. Contrary to no responses to vertebral artery infusion of AVPX, MAP was reduced and RSNA was increased by intravenous infusion of AVPX by itself. *p<0.05.

**Protocol 6: Effects of Captopril and Nicardipine During Clonidine Infusion**

After a sharp increase, MAP gradually decreased by 10±1 mm Hg 30 minutes after the initiation of clonidine in renal-hypertensive rabbits (Figure 7A). By adding captopril and nicardipine, MAP was reduced by 25±2 and 20±2 mm Hg, respectively. Thirty minutes after the initiation of clonidine, RSNA and HR were significantly reduced by 75±4% and 22±2 beats per minute, respectively. RSNA was elevated to +26±4% by injecting captopril and to −18±3% by injecting nicardipine. During clonidine infusion, the increase in RSNA by captopril (+101±6%) was significantly larger than that by nicardipine (+57±5%). Contrary to the difference in HR responses without clonidine, HR did not differ between captopril and nicardipine during clonidine infusion. In normotensive rabbits, MAP was decreased by 8±1 mm Hg, and RSNA and HR were reduced with clonidine (Figure 7B). MAP was decreased with captopril by 18±2 mm Hg, which was not different from that with nicardipine.

In renal-hypertensive rabbits, clonidine infusion resulted in suppression of basal plasma levels of Ang II, AVP, and norepinephrine (Table 3). The percent values of neurohormones during clonidine infusion are shown in Figure 8. Captopril, compared with clonidine alone, significantly reduced Ang II. Even in the presence of clonidine, captopril significantly elevated plasma AVP (194±15%) and norepinephrine (190±12%). With nicardipine, Ang II was significantly increased.

**Discussion**

In the present study, we used RSNA recorded in the conscious state as an index of SNS function. Despite equivalent depressor effects, the responses of RSNA and AVP to captopril were different from those to nicardipine. After captopril injection, the increase in RSNA was greater, and plasma concentration of AVP was significantly increased (Figures 1A and 2). We speculate that these results were in part attributed to compensation for the attenuated RAS.

Therefore, we hypothesized that if AVP had been blocked, RSNA would be increased only by captopril but not by nicardipine. However, when AVP antagonist was infused in either the vertebral artery or the peripheral vein, RSNA was exaggerated in a nonspecific manner whether the RAS was attenuated or stimulated (Figures 3A and 4). These results show that when action of AVP was attenuated, the SNS could become the most important mechanism for BP support in conscious renal-hypertensive rabbits. Moreover, the interaction between AVP and the SNS seemed to be independent of the state of the SNS, since the exaggeration of RSNA induced by BP reduction with nicardipine was qualitatively the same as with captopril.

Intravenous AVP antagonist reduced MAP and increased RSNA, whereas vertebral artery infusion of AVP antagonist did not. During the central infusion of AVP antagonist, RSNA was exaggerated by BP reduction with captopril and nicardipine. The increases in RSNA to captopril and nicardipine were significantly larger during central attenuation of AVP than those with peripheral attenuation of AVP, despite the smaller magnitude of BP reduction. We presume that the
compensatory activation of the SNS by AVP antagonist is more prominent in the central nervous system. On the other hand, in conscious renal-hypertensive rabbits, activation of the SNS by attenuation of central AVP was revealed only when BP was reduced. These results suggest that AVP in the central nervous system did not contribute to the BP maintenance in the basal condition but that it played a substantial role when systemic BP was lowered. One of the reasons that intravenous AVP antagonist by itself activated RSNA in renal hypertension may be the stimulation of the vagal afferent and the aortic nerve by circulating AVP.

The results showing that captopril and nicardipine increased RSNA much more during AVP antagonist infusion than in the presence of AVP demonstrate that the role of the SNS in BP maintenance was critical when the effect of AVP was attenuated. The direct recording of RSNA thus revealed an important interaction between endogenous AVP and the SNS for BP support, despite the opposite state of the RAS. As far as we know, few studies have shown the relation of AVP with the SNS in hypertensive animals by recording the RSNA. In normotensive dogs, Hassel et al. reported that AVP inhibited the SNS by measuring the BP changes to vagal cold block. In conscious hypertensive rabbits, we found that the SNS was activated by central and intravenous infusion of AVP antagonist. The present result is different from the BP support mechanism of salt-induced hypertension, in which AVP activates the SNS. Our result also contrasts with the observation that intracerebroventricular administration of AVP stimulates sympathetic outflow.

In renal-hypertensive rabbits with SAD, the reduction in MAP by captopril was significantly larger than that in hypertensive rabbits with intact baroreflex, and the depressor effect was greater with captopril than with nicardipine. Both results may indicate that the RAS works as a compensatory mechanism to maintain BP when the baroreflex control of the SNS is eliminated. Therefore, the current data showed a close interaction between the RAS and the baroreflex in renal hypertension. In SAD rabbits with renal hypertension that were given captopril (Figure 5), the intrarenal baroreceptor mechanism would be expected to operate powerfully on renin release, since the MAP was reduced by 30 mm Hg and the afferent limb of the baroreflex was interrupted. Partially blocking the sympathetic outflow with clonidine did not abolish the enhanced response of RSNA and the increase in AVP made by captopril. AVP secretion was stimulated when both the SNS and RAS

![Figure 5](http://circres.ahajournals.org/)

**FIGURE 5.** Bar graphs showing maximum responses (mean±SEM) of mean arterial pressure (MAP) with vehicle (V), captopril (CAP), and nicardipine (NIC) in sinoaortic-denervated (SAD) rabbits with renal hypertension (n=8) and SAD rabbits with normotension (n=6). NS, not significant. The depressor response to CAP in SAD rabbits with renal hypertension was significantly greater than that in hypertensive rabbits with intact baroreflex.

*p<0.05.

![Figure 6](http://circres.ahajournals.org/)

**FIGURE 6.** Bar graphs showing percent values (mean±SEM) of plasma concentrations of angiotensin II (ANG II), arginine vasopressin (AVP), and norepinephrine (NE) with vehicle (V), captopril (CAP), and nicardipine (NIC) in sinoaortic denervated (SAD) rabbits with renal hypertension rabbits (panel A) and SAD rabbits with normotension (panel B). Each concentration with V is defined as 100%.

*p<0.05 vs. V.
were attenuated (Figure 8A), whereas AVP did not respond to clonidine and nicardipine, suggesting that this neuropeptide could contribute to compensation for the attenuated RAS when the central sympathetic outflow was partially blocked. In contrast, in hypertensive rabbits with SAD, plasma AVP was not increased with captopril despite a marked BP reduction (Figure 6A). These findings may demonstrate that the intact afferent limb of the arterial baroreflex was necessary for AVP to operate as an important mechanism for BP support in renal hypertension. In SAD rabbits with renal hypertension, intravenous infusion of AVP antagonist significantly reduced MAP, and the reduction was larger than that with intravenous infusion of the antagonist in hypertensive rabbits with intact baroreflex. These data imply that endogenous AVP played an important role in maintenance of BP when the regulation of the SNS was impaired. Therefore, we demonstrate a mutual interaction between AVP and the SNS in conscious renal-hypertensive rabbits, since the SNS became crucial when the action of AVP was attenuated.

Other mechanisms accounting for the larger RSNA increase with captopril can be considered. Reduced endogenous Ang II, captopril itself, or both may have potentiated the arterial baroreflex control of RSNA. We have reported that captopril, but not nicardipine, increased the sensitivity of baroreflex control of RSNA in conscious renal-hypertensive rabbits. Cardiopulmonary reflex was also involved in mechanisms of the larger RSNA with captopril, since this reflex is blunted by captopril and potentiated by calcium channel blocker. Cardiac output is supposed to increase to compensate for BP reduction with nicardipine, whereas it is not increased with captopril. This difference may be another reason for the greater RSNA with captopril.

Since depressor effects produced by clonidine were equivalent in normotensive and hypertensive rabbits, it is unlikely that the SNS was more activated in our renal-hypertensive rabbits at this stage than in normotensive animals. This is different from pressor mechanisms in the renal-hypertensive models of Zimmerman et al and Faber and Brody, wherein the SNS was significantly activated. In our hypertensive rabbits, it is possible that Ang II peripherally intensified the cardiovascular response to the efferent sympathetic nerve activity, as Ferrario and McCubbin have indicated.
In normotensive rabbits, clonidine significantly decreased MAP, and captopril and nicardipine caused equivalent BP reductions in the presence of clonidine (Figure 7B), both of which mean that the SNS was the primary system for BP support. However, since captopril increased RSNA from that with clonidine alone and nicardipine did not, the RAS may partly supported BP in conjunction with the SNS when the central sympathetic outflow was attenuated. In this regard, our results are not compatible with a previous report that AVP became a more important pressor system than the RAS after autonomic blockade. An earlier study by Hasser and Bishop showed that in SAD animals the role of the RAS is greater because the accentuated SNS enhances the RAS. This enhancement may explain the mechanism whereby captopril significantly reduced BP in the normotensive animals with SAD.

In hypertensive rabbits, the increase in HR was larger with nicardipine than with captopril despite the smaller increase in RSNA. Nicardipine may decrease vagal nerve activity more than does captopril. This speculation is based on a report that Ang II reduces the vagal activity. We also presume that vagal nerve activity was not so attenuated or even facilitated with captopril. The difference in HR responses between captopril and nicardipine was not observed during clonidine infusion. Levy and Zieske showed that the slope of the curve between the vagal tone and changes in HR becomes null when the vagal tone is high. One of the mechanisms by which the increase in HR was not so large with nicardipine in the presence of clonidine was the stimulation of the prevailing vagal tone by clonidine alone.

In summary, the principal finding of the present study is that compensatory activation of RSNA was revealed by central and peripheral attenuation of AVP and that the SNS became the most important mechanism for BP maintenance in the absence of AVP. The interaction of AVP with the SNS seemed to be independent of the state of the RAS, since the exaggeration of RSNA by AVP antagonist was qualitatively the same with nicardipine as with captopril. In conscious renal-hypertensive rabbits, AVP in the central nervous system played a substantial role when BP was reduced, although it did not contribute to BP maintenance in the basal condition.

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H Kumagai, H Suzuki, M Ichikawa, Y Matsumura, M Jimbo, M Ryuzaki and T Saruta

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