Short-term Effects of Angiotensin II Blockade on Renal Blood Flow and Sympathetic Activity in Awake Rats

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Abstract To investigate the effects of an angiotensin II type 1 receptor antagonist (CV-11974) on renal blood flow and renal sympathetic nerve activity compared with a calcium antagonist (nicardipine), we measured both parameters in conscious spontaneously hypertensive rats aged 13 to 15 weeks. One to 2 days after surgery, CV-11974 (n=9) and nicardipine (n=8) were intravenously administered to decrease arterial pressure in a similar time course and degree of hypotension. CV-11974 increased renal blood flow by 23±4% at the maximal fall in mean arterial pressure (−32±1 mm Hg), and renal nerve activity increased by 70±7%. The maximal increase in renal blood flow (+27±4%) was observed when mean pressure was reduced by approximately 20 mm Hg. The maximal reduction of renal vascular resistance (−33±5%) correlated significantly with pretreatment levels of plasma renin concentration (r=−.792). In contrast, nicardipine produced a progressive reduction of renal blood flow and marked increases in heart rate and renal nerve activity. Increases in heart rate and nerve activity were greater than those with CV-11974 treatment (P<.001). At the maximal fall in mean pressure (−32±1 mm Hg), renal blood flow decreased by 23±4%, which was significantly correlated with percent changes in renal nerve activity (+150±11%, r=−.744). Renal denervation in another set of rats (n=6) improved renal blood flow and renal vascular resistance responses to nicardipine. These results suggest that blockade of the angiotensin II type 1 receptor increases renal blood flow with less sympathetic activation, whereas calcium antagonism decreases renal blood flow with reflex-mediated exacerbation of heart rate and renal nerve activity. (Hypertension. 1994;24:445-450.)

Key Words • renal circulation • angiotensin II • sympathetic nervous system • pressoreceptors • nicardipine

The renal sympathetic nervous and renin-angiotensin systems are known to play important roles in the regulation of renal function.1-4 We demonstrated that a reduction in arterial pressure by hypotensive agents through direct vasodilating actions reflexly enhanced sympathetic nerve activities, which decreased renal blood flow (RBF) in conscious animals.5-7 Furthermore, it is now clear that the sympathetic nervous system and renin-angiotensin system interact.8,9 In this regard, it is important to understand the effects of antihypertensive drugs on renal hemodynamics and the sympathetic nervous system in relation to the renin-angiotensin system. Recently, losartan, a specific nonpeptide antagonist of the angiotensin II (Ang II) type 1 (AT₁) receptor,4 has proved to be a useful antihypertensive agent10 and valuable tool for investigating the functional roles of endogenous Ang II.11-13

In the present study, to clarify the effects of an AT₁ receptor antagonist on RBF, renal sympathetic nerve activity (RSNA), and the baroreceptor reflex, we compared a nonpeptide AT₁ receptor antagonist with a calcium antagonist by measuring both RBF and RSNA simultaneously in conscious, unrestrained spontaneously hypertensive rats (SHR). TCV-116 is a highly potent and long-acting novel AT₁ receptor antagonist.14,16 For the purposes of the present study, CV-11974,14,16 an active metabolite of TCV-116, and nicardipine,17 a dihydropyridine derivative calcium antagonist, were administered intravenously to decrease arterial pressure with a similar time course and degree of hypotension. The drugs were also compared in rats with renal denervation to evaluate the effects of reflex-mediated enhancement of RSNA on RBF.

Methods

Animal Preparation

Male 8-week-old SHR were purchased from Charles River Japan Inc and fed standard laboratory rat chow (Japan Clea) and tap water ad libitum. All procedures were in accordance with the guiding principles provided by The Animal Care and Use Committee, University of The Ryukyus.

At 13 to 15 weeks of age, rats were anesthetized with pentobarbital sodium (50 mg/kg body wt IP and 10 to 15 mg/kg IV every hour as a supplemental dose). PE-10 catheters connected to PE-50 polyethylene tubing (Clay Adams) were inserted into the abdominal aorta via the femoral artery for measurement of arterial blood pressure and into the inferior vena cava via the femoral vein for administration of drugs. The right renal artery was exposed via a retroperitoneal approach, and under an operating microscope, a miniature pulsed-Doppler flow probe (DBF 1.0 gauge, Crystal Biotech) was placed around the artery close to the bifurcation from the aorta, with great care taken not to damage the renal nerves.18 The flow probe was then sutured in place with fine silk thread. After closure of the right flank incision, the left renal nerves...
were exposed through a retroperitoneal approach. A branch of the nerves was separated from surrounding fat and connective tissue and placed on a bipolar silver wire electrode (7855, A-M Systems). When an optimal neurogram was obtained, the nerve and electrode were embedded in a small amount of silicone gel (Sil-Gel 604, Wacker) and allowed to harden. Catheters and lead wires from the recording electrode and flow probe were exteriorized through the dorsal skin of the neck and fixed to the skin.

In another set of SHR, the right kidney was denervated by severing visible nerve fibers along the renal artery, stripping the adventitia, and painting the artery with 10% phenol in ethanol. After denervation was complete, a pulsed-Doppler flow probe was placed around the right renal artery, and a recording electrode was placed under the left renal nerve as mentioned above.

Recording Procedures

Arterial pressure was measured through a pressure transducer (P231D, Gould), and heart rate was monitored using a heart rate counter (AT601 G, Nihon Kohden) triggered by the arterial pressure pulse. Changes in blood flow velocity were measured as the Doppler shift in kilohertz by a pulsed-Doppler flow/dimension system (VF-I, Crystal Biotech). Assessment of blood flow velocity with the pulsed-Doppler system has shown to be directly and linearly related to volume flow.9 Changes in RBF were expressed as percent changes from the basal mean Doppler shift in kilohertz. Renal vascular resistance (RVR) was derived as RVR (mm Hg/kHz)=Mean Arterial Pressure (MAP)/Doppler Shift, and changes in RVR were expressed as percent changes from basal RVR. For measurement of RSNA, original renal nerve signals were amplified by a biophysical amplifier (DPA-100E, Dia Medical Systems) with a bandwidth frequency between 100 and 1000 Hz. The output from the amplifier was fed into a spike counter (DSE-325, Dia Medical System), which identified spikes exceeding a preselected level. The renal neurogram and Doppler shift of the flow signal along with the arterial pressure pulse were stored on a magnetic tape recorder (RD-130 TE, TEAC) for later analysis. The cutoff level of the spike counter was set to filter out the background noise that persisted after injection of phenylephrine hydrochloride (5 μg) or after rat death by overdose of pentobarbital. The number of nerve spikes per 1 or 2 seconds was continuously displayed on a chart recorder (RJG-4128, Nihon Kohden) together with pulsatile pressure and MAP, heart rate triggered by arterial pressure pulse, and RBF as the Doppler shift. Changes in RSNA were expressed as percent changes from basal spike counts.

Experimental Protocol

Rats were allowed at least 18 hours after surgery for recovery, which was considered adequate on resumption of regular eating, drinking, and grooming habits. Experiments were carried out with rats in a conscious and unrestrained state. Each rat was placed in a plastic bowl with a diameter and depth of 18 cm. After a stabilization period of at least 30 minutes, CV-11974 (0.1 to 0.2 mg/kg; n=9 in innervated rats, n=6 in denervated rats) or nicardipine hydrochloride (Yamanouchi; 7.5 to 20 μg/kg per minute; n=8 in innervated rats, n=6 in denervated rats) was administered intravenously in doses sufficient to lower MAP at least 20% from the pretreatment value. The nicardipine infusion rate was adjusted to lower arterial pressure in a time course and degree of hypotension similar to those during CV-11974 administration because nicardipine had been found to decrease arterial pressure more rapidly than CV-11974 in a preliminary study. If the renal neurogram had not deteriorated 2 days after surgery, the second drug was administered 24 hours from the previous treatment in one rat.

Arterial blood (0.3 mL) was taken for measurement of plasma renin concentration (PRC) before administration of drugs in rats with the innervated kidneys. PRC was determined by radioimmunoassay of Ang I.20 Angiotensigen was prepared from the plasma of nephrectomized rats by the method of Haas et al.21

Statistical Analysis

Values are expressed as mean±SEM. Data were analyzed by either Student's t test or repeated-measures ANOVA where appropriate. Linear regression was calculated by the least-squares method. A value of P<.05 was considered statistically significant.

Results

Rats With Innervated Kidneys

Basal levels of MAP and heart rate were not different between the two groups. The AT1 receptor antagonist CV-11974 decreased arterial pressure in association with an increase in RSNA, whereas RBF was increased as shown in Fig 1. In contrast, the calcium antagonist nicardipine decreased RBF with a pronounced increase in RSNA (Fig 2). Fig 3 shows the time course of changes in MAP, heart rate, RSNA, and RBF.
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CV-11974 decreased MAP maximally from 140±4 to 108±4 mm Hg (0.1 mg/kg of CV-11974, one rat; 0.2 mg/kg, eight rats), increased heart rate from 379±14 to 427±17 beats per minute (bpm), and enhanced RSNA by 70±7% from the pretreatment level (Fig 4). At the point of maximal increase in RBF, RSNA was higher by 50±7% than the pretreatment level. There was no significant correlation between percent changes in RSNA and RBF at any MAP level.

Nicardipine decreased MAP from 139±3 to 107±3 mm Hg in a time course similar to that during CV-11974 treatment (Fig 3), increased heart rate from 355±10 to 460±10 bpm, and augmented RSNA by 150±11% (Fig 4). Increases in heart rate and RSNA caused by nicardipine infusion were significantly greater than those with CV-11974 treatment (F =70.75 and F=126.30, respectively; P<.001). Parallel to changes in MAP, heart rate, and RSNA, RBF gradually decreased (Figs 3 and 5). RBF began to decline when MAP fell by a mean of 7±1 mm Hg associated with enhancement of RSNA by 55±4%. Thereafter, RBF progressively decreased: −19±3% at 20% reduction of MAP and −23±4% at the nadir of MAP, where there was a significant correlation between the percent changes in RBF and RSNA (r = −.744, P<.05). At the maximal decrease in RBF (MAP, −31±1 mm Hg from the preinfusion value), the percent decrease in RBF (−25±3%) also significantly correlated with the percent increase in RSNA (147±14%; r=−.857, P<.01). Although RVR was reduced until MAP fell by 20 mm Hg, it was not altered from preinfusion levels during further reduction of MAP (Fig 5). Percent changes in RSNA and RVR were significantly correlated at maximal decreases in MAP and RBF (r= .716 and .839, respectively).

As shown in Fig 4, there were linear relations with steep slopes between changes in heart rate and RSNA and decreases in MAP up to 20 mm Hg reduction. The slope of heart rate changes during CV-11974 treatment was significantly less steep than during nicardipine treatment (−1.92±0.44 and −4.15±0.67 bpm/mm Hg, respectively; P<.02). The slope of RSNA changes during CV-11974 was also significantly less steep than during nicardipine (−2.20±0.31 and −5.20±0.67 %/mm Hg, respectively; P<.001).

PRC levels before drug treatment were not different between the CV-11974 group (9.18±1.70 nmol/L per hour, n=7) and nicardipine group (10.6±2.08 nmol/L per hour, n=7). Although a correlation between PRC and the maximal percent increase in RBF did not attain statistical significance (r= .698, P<.1), there was a negative correlation between PRC and maximal changes in
RBF RVR
-40 -30 -20 -10
r
-»— mcaridine
—•— CV-11974
decrease in MAP
-20 -10

Fig 5. Line plots show percent changes in renal blood flow (RBF) (left) and renal vascular resistance (RVR) (right) in relation to decreases in mean arterial pressure (MAP) from levels before treatment with CV-11974 (n=9) and nicardipine (n=8). a indicates point of MAP nadir; b, maximal increase in RBF; and c, RBF nadir.

RVR (−33±3%; r=−.792, P<.05) in the CV-11974 group. No significant correlation was found between PRC and these parameters in the nicardipine group.

Rats With Denervated Kidney
In the SHR group whose right kidney had been denervated, RSNA responses recorded from the left renal nerve to intravenous CV-11974 or nicardipine were comparable to responses observed in SHR with intact renal nerves, suggesting a degree of reflex-mediated activation of sympathetic outflow similar to that in intact SHR. CV-11974 increased activities of the left renal nerve by 55±7% and 62±9% at the 25 mm Hg reduction and maximal reduction of MAP (−33±1 mm Hg), respectively. RBF and RVR responded to CV-11974 in a manner similar to the responses in SHR with intact renal nerves (Figs 5 and 6). With nicardipine infusion RSNA increased by 125±12% at the 25 mm Hg reduction and by 133±12% at the nadir of MAP (−32±2 mm Hg). However, RBF increased by 4±1% at the 5 mm Hg reduction of MAP and began to decline when MAP fell by a mean of 20±3 mm Hg associated with a 116±15% enhancement of activity of the left renal nerve. The response curve of RBF was profoundly different from that in innervated rats treated with nicardipine (F=45.1, P<.001; Figs 5 and 6). RVR progressively decreased, and its percent change was −20±3% at the nadir of MAP. The decrease in RVR, however, was still smaller than that during CV-11974 treatment (Fig 6).

Discussion
In the present study, opposite effects on RBF of two drugs were demonstrated. CV-11974, an active metabolite of TCV-116 (a novel AT1 receptor antagonist),14,15 increased RBF in association with less sympathetic activation. In contrast, nicardipine,17 a dihydropyridine derivative calcium antagonist, decreased RBF despite a time course and degree of hypotension similar to those elicited during treatment with the Ang II antagonist.
During CV-11974 treatment an increase in RBF despite a progressive reduction of MAP may be mainly attributed to a direct effect of AT₁ receptor blockade on the renal vasculature, because Ang II plays a pivotal role in the regulation of renal hemodynamics. Actually, a significant correlation was found between pretreatment levels of PRC and the minimal decrease in RVR. Nicardipine initiated a reduction of RBF when MAP fell by only a mean of 7 mm Hg and decreased RBF progressively parallel to reductions of MAP. Several mechanisms as well as a direct effect of Ang II blockade on the renal vasculature might be responsible for the difference in RBF and RVR responses to the drugs.

First, effects of reflexly enhanced RSNA on the renal vasculature should be considered. In the current study, percent increases in RSNA during nicardipine treatment were significantly greater than those during CV-11974 treatment and correlated inversely with percent changes in RBF, in accord with our previous studies on manidipine, another dihydropyridine derivative calcium antagonist. Furthermore, denervation of the renal nerve markedly ameliorated RBF and RVR responses during nicardipine treatment. Our laboratory also demonstrated that clonidine, a central α₂-adrenergic receptor agonist, restored the decrease in RBF induced by manidipine pretreatment in association with suppression of enhanced RSNA. These observations suggest that an enhancement of RSNA over a critical level may surpass the vasodilator effects of nicardipine in the innervated kidney. However, the ameliorated RBF and RVR responses with renal denervation during nicardipine treatment were not comparable to those of both parameters during CV-11974 treatment. This indicates that the vasodilator effect of the AT₁ receptor antagonist was greater than that of the calcium antagonist in the renal circulation in the present experimental conditions and that the effect of Ang II on the vasculature might not be altered by the calcium antagonist.

Second, concerning interactions between RSNA and Ang II in the renal vascular beds, Pelayo and Blantz demonstrated that an increase in afferent arteriolar resistance induced by a moderate electrical stimulation of the renal nerve was eliminated by approximately 75% with Ang II blockade by treatment with an Ang II analogue or Ang I–converting enzyme inhibitor in anesthetized rats. They attributed this lesser alteration in arteriolar resistance to the modification of post synaptic mechanisms rather than to effects of Ang II on norepinephrine reuptake and/or release. In the present study, CV-11974 enhanced RSNA to a smaller extent and prior renal denervation exerted very little effect on the RBF and RVR responses to the drug. Collectively, these findings imply that much of the renal nerve efferent activity, at least in moderate activation, might be either mediated by or dependent on Ang II. Since both a reduction of renal perfusion pressure and an increase in RSNA are known to increase renin release, resultant high levels of Ang II might decrease RBF directly and through an interaction with renal sympathetic stimulation during nicardipine treatment but not during CV-11974 treatment.

Third, the hemodynamic effects of the drugs might be different in affecting the balance of cardiac output and systemic vascular resistance. However, it has been reported that the AT₁ receptor antagonist losartan did not change cardiac output in anesthetized SHR. Although studies on cardiac output with intravenous administration of nicardipine in SHR have not been available, even oral dihydropyridines are known to increase cardiac output, at least in the acute phase. Therefore, the effects of the two drugs on cardiac output may not explain the difference in RBF and RVR responses in the present study. The AT₁ receptor antagonist appears to have more primary intrarenal effects regardless of the systemic responses, since renal effects preceded systemic effects manifested by a decrease in arterial pressure in most rats tested.

Although the time course and degree of hypotension induced by the two drugs were similar, increases in RSNA and heart rate were significantly less during CV-11974 than during nicardipine treatment. This blunted sympathetic activation was also confirmed when compared with data obtained in our previous studies on manidipine and sodium nitroprusside conducted with experimental conditions similar to those of the present study. Interactions between the renin-angiotensin system, the sympathetic nervous system, and the baroreceptor reflexes have been well documented. However, the effects of Ang II on sympathetic nerve activity have been controversial. Guo and Abboud proposed a central action of Ang II to modulate the reflex control of lumbar sympathetic nerve activity from the finding that intravenous administration of Ang II decreased the nerve activity less compared with phenylephrine in anesthetized rabbits. In contrast, Matsumura et al found that an intravenous infusion of Ang II and phenylephrine produced the same reduction in RSNA in conscious rabbits.

Conversely, the present study clearly demonstrated that a specific antagonist of the AT₁ receptor blunted reflexly mediated activation of sympathetic outflow to the kidney and possibly to the heart in the face of hypotension equivalent to nicardipine-induced hypotension. Response curves relating changes in RSNA and heart rate to decreases in MAP were located to the left of the curves found during nicardipine treatment. The slopes of the linear portion of the curves between a 5 and 20 mm Hg reduction of MAP during CV-11974 treatment were less steep compared with those during nicardipine treatment. Although these findings suggest a shift of the set point and a suppression of the sensitivity of the baroreflex function by CV-11974 treatment, the results in the present study were obtained during hypotension induced by CV-11974 or nicardipine. To clarify the mechanisms by which CV-11974 modifies baroreflex control, further studies will be needed. Concerning the effects of the AT₁ receptor antagonist on the reflex control of heart rate, losartan has been reported to shift the baroreceptor reflex curve relating heart rate to arterial pressure to the left without changing the sensitivity in conscious rabbits.

In conclusion, intravenous administration of CV-11974, an AT₁ receptor antagonist, increased RBF with less sympathetic activation despite a marked decrease in arterial pressure, whereas nicardipine, a calcium antagonist, produced a progressive reduction of RBF with reflex-mediated exacerbation of RSNA and heart rate. The results suggest that blockade of Ang II receptors in the kidney and blunted sympathetic activation in the
face of hypotension may contribute to the lack of deteriorating effects of the AT1 receptor antagonist on the renal circulation.

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