Effects of Atrial Natriuretic Factor on the Vasoconstrictor Actions of the Renin–Angiotensin System in Conscious Rats

Rodney W. Lappe, Joy A Todt, and Robert L. Wendt

Previous studies have indicated that the hypotensive effects of atrial natriuretic factor were enhanced in renin-dependent hypertensive rats, suggesting that the atrial peptides may antagonize the vasoconstrictor effects of the renin–angiotensin system. The present study was designed to define further the interaction between atrial natriuretic factor and the renin–angiotensin system by examining the hemodynamic effects of Wy-47,663, a synthetic human atrial natriuretic factor, in conscious normotensive rats, in renin-dependent (aortic-ligated) hypertensive rats, and in rats made hypertensive by chronic infusion of angiotensin II. Changes in renal and mesenteric blood flow were continuously monitored in the rats using pulsed Doppler flow probes chronically implanted in the animals one week prior to testing. Infusion of increasing doses of Wy-47,663 caused dose-dependent reductions in mean arterial pressure in all three groups of rats, but the depressor responses were significantly greater in renal hypertensive and angiotensin II-infused rats. Renal blood flow tended to increase during the infusion of the atrial peptide in the angiotensin II-treated rats, and renal vascular resistance fell significantly (−37 ± 6%). However, Wy-47,663 significantly reduced renal blood flow in the normotensive and renal hypertensive rats, while renal vascular resistance was increased (29 ± 6%) and unchanged (3 ± 9%), respectively. Mesenteric blood flow was reduced significantly, and mesenteric vascular resistance was increased markedly in all three groups of rats during infusion of the atrial peptide. In a separate group of renal hypertensive rats, the hemodynamic effects of complete blockade of the renin–angiotensin system were assessed by injection of an angiotensin II converting enzyme inhibitor (Wy-44,655). In contrast to the hemodynamic effects of Wy-47,663, inhibition of converting enzyme activity in the renal hypertensive rats significantly increased renal and mesenteric blood flow and reduced regional vascular resistance in both vascular beds. Since renal vasodilation and hypotension were observed during infusion of Wy-47,663 into rats whose hypertension was mediated purely through the pressor effects of angiotensin II, these data suggest that the atrial peptides are capable of suppressing the renal vasoconstrictor actions of angiotensin II in conscious rats. However, the data indicate further that overall hemodynamic responses to Wy-47,663 in the renal hypertensive rats were not mediated solely through antagonism of the vasoconstrictor actions of the renin–angiotensin system, as typified by converting enzyme inhibition. (Circulation Research 1987;61:134–140)

Many previous studies have suggested that atrial natriuretic factor (ANF) may antagonize the vasoconstrictor actions of the renin–angiotensin system. Several investigators have reported that the atrial peptides effectively relax aortic vascular smooth muscle precontracted with angiotensin II. Pretreatment of aortic smooth muscle with ANF also significantly suppressed the vasoconstrictor actions of angiotensin II in vitro. In isolated perfused kidneys with no vasoconstrictor tone, addition of ANF to the renal perfusate increased renal vascular resistance. When the isolated kidneys were precontracted with angiotensin II, addition of the ANF reduced vascular resistance, suggesting that the peptide antagonized the renal vasoconstrictor actions of angiotensin II. Similarly, infusion of the atrial peptides significantly attenuated angiotensin II-induced pressor responses in a dose-dependent fashion in pithed rats. In 1-kidney–1-clip hypertensive rats, a renin-dependent model of hypertension, the hypotensive responses to ANF were markedly potentiated as compared with responses in normotensive rats. The depressor actions of the atrial peptides in the 1-kidney–1-clip rats were mediated through significant reductions in total peripheral resistance. This differs from other non–renin-dependent models of hypertension in rats (SHR, steroid-salt) where falls in arterial pressure after infusion of ANF have been associated with reduced cardiac output and increased total peripheral resistance. The peripheral vasodilation observed in the renin-dependent hypertensive rats would tend to suggest that the atrial peptides may have antagonized the pressor actions of the renin–angiotensin system.

In addition to the effects on vascular smooth muscle, ANF may also influence the actions of the renin–angiotensin system by reducing plasma renin activity. Significant decreases in plasma renin activity and renin secretory rate have been reported in anesthetized dogs. Also, ANF has been observed to decrease renin release from rat kidney slices in vitro. Thus, ANF may attenuate the pressor effects of the renin–angiotensin system in vivo by reducing the formation of angiotensin I and angiotensin II.
In the present study, possible interactions between ANF and the renin-angiotensin system were investigated further by comparing the hemodynamic responses to graded infusions of a synthetic human atrial natriuretic peptide (102-126), Wy-47,663, in normotensive, renin-dependent hypertensive, and angiotensin II-dependent hypertensive conscious rats. To determine whether the cardiovascular effects of ANF in the renal hypertensive rats could be attributed solely to antagonism of the renin-angiotensin system, the hemodynamic responses to complete blockade of the renin-angiotensin system, as assessed by inhibition of angiotensin converting enzyme, were compared with the responses observed after an equidepressor infusion of Wy-47,663 in renin-dependent hypertensive rats.

Materials and Methods

Surgical Preparation

Male Sprague-Dawley rats (310-380 g) were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and received a presurgical injection of atropine (2 mg/kg i.p.) and penicillin (400,000 U i.m.). Through a midline incision, the right renal artery and superior mesenteric artery were cleared of connective tissue. Miniaturized pulsed Doppler flow probes were positioned around each artery and sutured into place. The flow probes were tunneled subcutaneously, exteriorized at the base of the skull, and soldered into a small receptacle. The receptacle was secured to the skull using jeweler’s screws and dental cement. A polyethylene cannula was also inserted into the abdominal aorta to allow infusion of drugs. The cannulae were filled with sterile isotonic saline and sealed when not in use.

After the instrumentation was complete, renal hypertension was produced in one group of rats by completely ligating the abdominal aorta. A 2-0 silk ligature was placed around the abdominal aorta at a point between the origins of the right and left renal artery but always below the superior mesenteric artery. The ligature was tied tight, completely occluding the abdominal aorta. In all rats, the tip of the aortic cannula lay above the ligation. In a second group of rats, the ligature was positioned around the aorta but was not tied. This group acted as a sham-ligated control. In a third group of rats, a cannula was inserted into the jugular vein, and angiotensin II was continuously infused at a rate of 75 ng/min via an osmotic minipump. All groups were allowed to recover and stabilize for 7 days after surgery.

After the recovery period, the conscious, unrestrained rats were examined in their home cage environment. All three groups of rats were connected to the pulsed Doppler flow meter (University of Iowa, Iowa City, Iowa) by a flexible, spring-guarded cable. The animals were allowed to equilibrate for a minimum of 30 minutes, after which baseline measurements of mean arterial pressure, heart rate, renal blood flow, and mesenteric blood flow were recorded. Increasing doses (0.125, 0.5, and 2.0 µg/kg/min) of Wy-47,663 (human ANF [102-126]) were infused intravenously. The atrial peptide was infused for a minimum of 30 minutes or until the hemodynamic parameters were stable at each dose. Changes in the hemodynamic parameters were recorded during infusion steady state.

To assess the vasconstrictor actions of the renin-angiotensin system in the renal hypertensive rats, the hemodynamic responses to a maximal dose of an angiotensin II converting enzyme inhibitor, Wy-44,655 (R.W. Lappe, unpublished data) were examined in a separate group of aortic-ligated rats. Wy-44,655 [(-)-(S)-1-[2-(25)-2-{[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino}-1-oxopropy]-1,2-dihydro-1H-indole-2-carboxylic acid, sodium salt] is a member of a series of converting enzyme inhibitors described by Kim et al. Seven days after ligation, baseline values were recorded, and Wy-44,655 (3 mg/kg) was injected intravenously into the conscious hypertensive rats. Injection of Wy-44,655 has previously been demonstrated to cause stable hemodynamic changes in conscious rats lasting for over an hour (R.W. Lappe, unpublished data). Since Wy-47,663 has a short half-life, a continuous infusion was necessary to produce sustained responses. The changes in MAP, heart rate, renal blood flow, and mesenteric blood flow were recorded after the parameters had stabilized (15-20 minutes postdose).

The data were represented as means ± SEM. Regional vascular resistance was calculated by dividing MAP by the regional blood flow. Intragroup changes in the cardiovascular parameters were compared with predose baseline values using a Student’s paired t test. Intergroup comparisons of the effects of Wy-47,663 in normotensive, renal hypertensive, and angiotensin II hypertensive rats were performed using a repeated measures one-way analysis of variance and a Student-Newman-Keuls nonpaired t test. Similar analyses were used to compare the hemodynamic responses to Wy-44,655 and Wy-47,663 in the renal hypertensive rats. Differences were considered to be statistically significant if p<0.05. All experiments in conscious rats were performed in accordance with institutional guidelines for the care and treatment of conscious animals.

Results

Baseline mean arterial pressure (MAP) was significantly elevated in the renal and angiotensin II hypertensive rats as compared with the normotensive control group of animals (Table 1). Renal hypertensive rats also tended to have slightly higher baseline MAP than the angiotensin II-treated rats, but these differences were not statistically significant. Baseline heart rates were similar in the normotensive and renal hypertensive rats but were significantly slower in the angiotensin II-treated group.

Infusion of Wy-47,663 caused significant reductions in MAP in all three groups of rats (Figure 1).
Table 1. Baseline Values for Mean Arterial Pressure (MAP) and Heart Rate in the Normotensive (NTR), Renal Hypertensive (RHR), and Angiotensive II-Infused (Ang II) Rats

<table>
<thead>
<tr>
<th>Rats</th>
<th>MAP (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTR (n = 8)</td>
<td>98 ± 2</td>
<td>345 ± 13</td>
</tr>
<tr>
<td>RHR (n = 7)</td>
<td>174 ± 3*</td>
<td>372 ± 15</td>
</tr>
<tr>
<td>Ang II (n = 7)</td>
<td>155 ± 4*</td>
<td>311 ± 19†</td>
</tr>
</tbody>
</table>

*p < 0.05, compared with NTR group, †p < 0.05, compared with NTR and RHR groups

normotensive rats, MAP was not significantly lowered from baseline levels until the highest infusion rate of the atrial peptide. Wy-47,663 markedly decreased MAP to a similar extent in the renal and angiotensin II-treated hypertensive rats. The depressor responses in the hypertensive rats were significantly greater than those observed in the normotensive rats. Heart rate responses were quite variable in the conscious rats during the infusion of Wy-47,663 (Figure 1). In normotensive and renal hypertensive rats, heart rate tended to decline, but no significant changes from baseline levels were observed. The tachycardia observed in angiotensin II-treated rats differed significantly from responses observed in the other groups of rats.

Renal vascular responses to Wy-47,663 also differed significantly among the three groups of conscious rats (Figure 2). Renal blood flow fell significantly in a dose-related fashion in the normotensive and renal hypertensive rats during the infusion of the atrial peptide. Renal vascular resistance was increased and unchanged in the normotensive and renal hypertensive rats, respectively. In contrast, Wy-47,663 failed to alter renal blood flow in the angiotensin II rats, although blood flow tended to increase during the infusion of the peptide. Wy-47,663 reduced renal vascular resistance in a dose-related manner in the angiotensin II rats. The renal responses in the angiotensin II rats were significantly different from those observed in the other groups of rats.

Unlike in the kidney, mesenteric blood flow markedly declined in all rats during the infusion of Wy-47,663 (Figure 3). Mesenteric vascular resistance in
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Figure 4. Effects of Wy-47,663 or converting enzyme inhibition (CEI) on mean arterial pressure and heart rate in conscious renal hypertensive rats. Δ, change.

Figure 5. Comparison of renal (left panels) and mesenteric (right panels) hemodynamic responses to Wy-47,663 or converting enzyme inhibition (CEI) in conscious renal hypertensive rats. Δ, percent change. *p < 0.05, compared with Wy-47,663-treated rats.

Discussion

The purpose of the present study was to compare the hemodynamic actions of ANF in two different models of angiotensin II-dependent hypertension. In the first group of rats, hypertension was produced by complete ligation of the abdominal aorta, which markedly increases the release of renin from the kidney below the ligature. Arterial pressure is quickly elevated, and the hypertension is sustained through the hyperactivity of the renin–angiotensin system. In the second group of rats, the kidneys were not manipulated to increase in all groups of conscious rats, but the greatest mesenteric vasoconstriction was observed in the normotensive rats. Similar increases in mesenteric vascular resistance were observed in the two hypertensive groups of rats.

To assess further the possible interactions between the renin–angiotensin system and the atrial peptides in the renal hypertensive rats, the hemodynamic responses to the highest infusion rate of Wy-47,663 (2 µg/kg/min) were compared with the effects of an equidressor dose of a converting enzyme inhibitor, Wy-44,655 (3 mg/kg). Similar baseline MAP (174 ± 3 vs. 165 ± 8 mm Hg) and heart rate (372 ± 15 vs. 357 ± 22 beats/min) values were observed in the ANF- and Wy-44,655-treated rats. A significant tachycardia was observed after converting enzyme inhibition in the renal hypertensive rats (Figure 4) while heart rate was unchanged in the ANF-treated group, even though MAP was decreased to a similar extent in rats receiving the atrial peptide (−46 ± 5 mm Hg) and Wy-44,655 (−38 ± 7 mm Hg).

Marked differences were also observed in the renal and mesenteric vascular responses to the atrial peptide and converting enzyme inhibition (Figure 5). Renal and mesenteric blood flow were greatly increased after injection of Wy-44,655, while infusion of ANF significantly reduced regional blood flow in both vascular beds. Marked reductions in regional vascular resistance occurred in both vascular beds after inhibition of converting enzyme, while renal and mesenteric vascular resistance were unchanged and increased by infusion of Wy-47,663, respectively.
produce angiotensin II–dependent hypertension. Instead, angiotensin II was infused continuously via an osmotic pump, producing a sustained increase in mean arterial pressure mediated through the pressor actions of angiotensin II without impairing function in one kidney. Mean arterial pressure was increased to a similar extent in the renal hypertensive and angiotensin II-treated rats, and both groups were hypertensive for the same period of time (7 days). Any nonspecific vascular or cardiac changes in response to the elevated arterial pressure should have been similar in both groups of rats.

Infusion of Wy-47,663 markedly reduced arterial pressure in the renal hypertensive and angiotensin II-treated rats. The depressor responses to ANF in the hypertensive rats were much greater than those observed in normotensive rats. It is doubtful that the differences observed between groups can be attributed simply to differences in baseline arterial pressure. Previous studies have indicated that the depressor actions of ANF are also augmented in conscious spontaneously hypertensive rats as compared with normotensive controls. However, the fall in arterial pressure during the infusion of Wy-47,663 in the renal hypertensive and angiotensin II-treated rats in the present study was still greater than responses observed in the spontaneously hypertensive rats during infusion of identical doses of Wy-47,663, even though all three groups of rats had similar resting arterial pressures. In addition to elevated arterial pressures, other factors such as venous tone, cardiac filling pressure, and activity of the renin–angiotensin system must contribute to the enhanced hypertensive actions of the ANF in the angiotensin II–dependent hypertensive rats.

Other investigators have also observed that the depressor actions of the atrial peptides are enhanced in renin-dependent hypertensive rats. These data, in conjunction with findings that the atrial peptides inhibit the in vitro vasoconstrictor actions of angiotensin II, suggest that the atrial peptides might serve as a functional antagonist of the actions of the renin–angiotensin system in renal hypertensive rats, leading to vasodilation and an enhanced fall in arterial pressure. In support of this hypothesis, infusion of ANF in anesthetized 2 kidney–1 clip hypertensive rats reduced total peripheral resistance, while in anesthetized normotensive and steroid-salt hypertensive rats, total peripheral resistance was unchanged or increased. In the present study, no evidence for regional vasodilation in the renal or mesenteric vascular beds was observed in the conscious renal hypertensive rats during the infusion of ANF. In fact, mesenteric vascular resistance increased. It is possible that even with the observed responses in the renal and mesenteric vascular beds, total peripheral resistance may have decreased in the renal hypertensive rats due to marked vasodilation in other vascular beds. This would not seem very likely.

Also, if the cardiovascular actions of Wy-47,663 in the renal hypertensive rats were mediated solely through blockade of the renin–angiotensin system, then the regional hemodynamic responses to the atrial peptide and true antagonists of the renin–angiotensin system such as angiotensin receptor blockers or inhibitors of renin or converting enzyme activity, should be qualitatively similar. Even though similar hypertensive responses to Wy-47,663 and converting enzyme inhibition were observed in the renal hypertensive rats, renal and mesenteric vascular responses to these two agents were contradictory. These data suggest that the hemodynamic and depressor actions of Wy-47,663 were not mediated solely through blockade of the renin–angiotensin system. Rather, it appears that other factors (i.e., reductions in cardiac output) contribute to the ANF-induced fall in arterial pressure in conscious, aortic-ligated rats.

The converting enzyme inhibitor (Wy-44,655) used in the present study is a novel agent similar in structure and activity to enalapril. Wy-44,655 is a potent orally active antihypertensive agent, which significantly lowers arterial pressure in spontaneously hypertensive and renal hypertensive rats after doses as low as 0.3 mg/kg. The compound has a prolonged duration of action. Like other converting enzyme inhibitors, Wy-44,655 increases renal blood flow and reduces renal vascular resistance in conscious normotensive and spontaneously hypertensive rats (R.W. Lappe, unpublished observations). The compound is also a potent inhibitor of converting enzyme activity in humans. In all experiments, Wy-44,655 produced stable hemodynamic changes that persisted for more than 45 minutes after intravenous injection. In the present study, Wy-44,655 markedly reduced arterial pressure and renal and mesenteric vascular resistance in the renal hypertensive rats. Since other converting enzyme inhibitors elicit identical hemodynamic responses, the cardiovascular effects of Wy-44,655 are not unique to this particular compound. Rather, the responses to Wy-44,655 are indicative of total blockade of the renin–angiotensin system through converting enzyme inhibition in the conscious rats.

The discrepancies between the present study and a previous one may relate to the animal models examined. While both are high-renin models of hypertension, aortic ligation hypertension has a more rapid onset and is generally more malignant than 2 kidney–1 clip hypertension in rats. Anesthesia may contribute significantly to the differences between the two studies. In the present study, rats were conscious and unrestrained with normal baroreflex function, while the 2 kidney–1 clip rats were anesthetized, a state in which reflex compensatory mechanisms are compromised.

Renal vascular responses to Wy-47,663 differed significantly between the renal hypertensive and angiotensin II–treated rats, even though similar reductions in mean arterial pressure were observed in both groups of rats during infusion of Wy-47,663. In the angiotensin II–treated rats, renal vascular resistance fell in a dose-dependent fashion. Renal blood flow tended to increase but did not achieve statistical significance, possibly because of the marked reductions in mean arterial pressure. Interestingly, the renal vasodilator responses to Wy-47,663 were sustained...
throughout the infusion period. Transient reductions in renal vascular resistance have previously been observed in conscious normotensive and spontaneously hypertensive rats during infusion or bolus injection of Wy-47,663,14,21,25 but the renal vasodilatation was never sustained. These data suggest that the amount of angiotensin II-induced vasoconstriction present in the kidney may affect renal vascular responses to ANF.3,10,25 When angiotensin II-mediated renal vasoconstrictor tone is high, the vasodilator actions of ANF may become apparent. Thus, under certain conditions, ANF appears to antagonize the renal vasoconstrictor effects of angiotensin II.

No evidence of renal vasodilatation was observed in the renal hypertensive rats during the infusion of the atrial peptide. Marked reductions in arterial pressure and renal vascular resistance were observed after blockade of the renin–angiotensin system with a converting enzyme inhibitor. These data indicate that resting renal vascular resistance was elevated because of the vasoconstrictor effects of angiotensin II in the renal hypertensive rats. Still, renal vascular resistance was unchanged, and renal blood flow fell during the infusion of Wy-47,663. These data indicate that hyperactivity of the renin–angiotensin system, as was present in the renal hypertensive rats, is not necessarily predictive of the renal hemodynamic effects of the atrial peptides.

The explanation for the discrepancies between the renal vascular responses to ANF in the angiotensin II-treated and renal hypertensive rats is currently unknown. It is possible that ANF did antagonize the renal vasoconstrictor actions of angiotensin II in the renal hypertensive rats. The expected fall in renal vascular resistance may have been masked by compensatory renal vasoconstrictor mechanisms. This may involve the intrarenal release of pressor substances, such as vasoconstrictor prostaglandins. Reflex increases in renal sympathetic tone, in response to the fall in arterial pressure, may also contribute to the maintenance of renal vascular resistance during infusion of the atrial peptides.21 It is impossible to completely rule out the renal vasodilator effects of ANF in the renal hypertensive rats until the contribution of compensatory mechanisms to the renal hemodynamic responses to ANF has been defined.

No indication of mesenteric vasodilatation was observed in the normotensive or hypertensive rats during the infusion of ANF even though baseline mesenteric vascular resistance was elevated in the hypertensive rats. Again, this may reflect reflex vasoconstriction masking the vasorelaxant action of ANF in the mesenteric vascular bed. It is also possible that the atrial peptides may have little or no vasodilator effect on mesenteric resistance vessels. In support of the latter hypothesis, ANF failed to relax 250 μm mesenteric arterioles in vitro.26,27 ANF was a potent vasorelaxant in similar preparations of renal arcuate arteries.26,28 Renal, but not mesenteric, vasodilatation was observed after bolus injection of low doses of ANF in conscious dogs and rats.23 However, after high doses of ANF, mesenteric vascular resistance was reduced,23 suggesting that the vasorelaxant effects of the atrial peptides may be organ selective, with the renal effects predominating at low doses of ANF.

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