Laboratory Studies

Hemodynamic Effects of Infusion Versus Bolus Administration of Atrial Natriuretic Factor

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SUMMARY The cardiovascular responses to intravenous bolus administration of several synthetic atrial natriuretic peptides were examined in conscious spontaneously hypertensive rats and compared with the hemodynamic effects of continuous infusions of the peptides. Rats were instrumented with pulsed Doppler flow probes to allow measurement of regional blood flow in the conscious, unrestrained hypertensive rat. Bolus administration of increasing doses (0.036-18 nmol/kg) of atriopeptin II, α-rat atrial natriuretic peptide, Wy-47,663, or α-human atrial natriuretic peptide caused short-lived, dose-dependent reductions in mean arterial pressure and renal vascular resistance. A marked but transient (10—40 seconds) increase in renal blood flow was observed after administration of the peptides. Mesenteric and hindquarter vasodilation also were observed after bolus injection of high doses of the atrial peptides. Infusion of α-rat atrial natriuretic peptide or Wy-47,663 (0.045–1.44 nmol/kg/min) resulted in sustained reductions in mean arterial pressure. The fall in arterial pressure was accompanied by significant reductions in regional blood flow in the renal, mesenteric, and hindquarter vascular beds. Dose-dependent increases in regional vascular resistances were observed in all three vascular beds during the peptide infusions. These data indicate that the hemodynamic responses to synthetic atrial peptides are greatly dependent on the mode of administration of the peptide in conscious spontaneously hypertensive rats. Stable, sustained responses were observed only during infusion steady state conditions. (Hypertension 8: 866–873, 1986)

KEY WORDS • regional blood flow • conscious spontaneously hypertensive rats • renal vascular resistance • renal vasodilation • atriopeptin

The family of synthetic atrial peptides, collectively referred to as atrial natriuretic factor (ANF), has been demonstrated to possess potent natriuretic and diuretic properties (for recent reviews, see References 1 through 3) and to inhibit the release of aldosterone, renin, and vasopressin. Atrial natriuretic factor has also been reported to exert a marked influence on cardiovascular function. The atrial peptides have been reported to reduce arterial pressure in many experimental animal models and humans; however, the depressor mechanism of action of ANF remains quite controversial. Initial observations suggested that ANF was a peripheral vasodilator, reducing arterial pressure by lowering total peripheral resistance. Indeed, several investigators have reported that ANF was a potent relaxant of isolated vascular smooth muscle. Also, ANF was observed to reduce arterial pressure and total peripheral resistance in conscious rats, suggesting that ANF might serve as an endogenous vasorelaxant. However, other investigators have failed to observe reductions in total peripheral resistance after administration of the atrial peptides in conscious and anesthetized rats. The contrary, total peripheral resistance was unchanged or increased in these studies. Interestingly, the depressor actions of ANF were mediated through dose-dependent reductions in cardiac output and stroke volume that correlated with reductions in central venous pressure and left atrial pressure.

The effects of ANF on regional hemodynamics are also controversial. Intrarenal or intravenous injection of the atrial peptides has been reported to increase total renal blood flow and reduce renal vascular resistance in dogs and rats. The vasodilator responses appear to be renal-selective, as no significant change in vascular resistance was observed in other vascular beds. However, infusion of the atrial peptides generally has not resulted in sustained increases in total renal blood flow. To the contrary, several studies have reported that renal blood flow is unchanged or
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reduced by ANF during infusion steady state conditions. However, two studies have reported sustained increases in renal blood flow during the infusion of ANF. Thus, the purpose of the present study was to compare hemodynamic responses to several synthetic atrial peptides administered by bolus or continuous infusion in conscious, instrumented spontaneously hypertensive rats (SHR), allowing the assessment of the contribution of mode of administration and alteration of ANF structure to the cardiovascular effects of the atrial peptides.

Materials and Methods

Male SHR obtained from Taconic Farms (weight, 300–330 g) were chronically instrumented with miniature pulsed Doppler flow probes to allow continuous measurement of regional blood flow in the conscious rat. In brief, rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and placed on a heated surgical pad. Through a midline abdominal incision, Doppler flow probes were carefully placed around the right renal artery, superior mesenteric artery, and left iliac artery and secured with a silk suture. The probe wires were tunneled subcutaneously, exteriorized at the base of the skull, and soldered into a small receptacle. The receptive was cemented to the skull using jeweler’s screws and dental acrylic. Polyethylene cannulas (PE-10) were inserted into the descending aorta and inferior vena cava to allow measurement of mean arterial pressure (MAP) and to facilitate the administration of the atrial peptides, respectively. Cannulas were secured to the abdominal wall, tunneled subcutaneously, and exteriorized in the midscapular region. The cannulas were filled with heparinized saline (100 U/ml) and sealed when not in use. After operation, the SHR were allowed to recover for a minimum of 4 days before hemodynamic responses were examined. The rats lost weight after surgery, but body weight had returned to presurgical levels before the animals were tested.

On the day of the experiment, the SHR were randomly divided into two groups. In one group of rats, after baseline hemodynamic measurements were recorded, increasing intravenous bolus doses (0.036–18 nmol/kg) of a synthetic ANF were administered. Maximal changes in MAP, heart rate, and regional blood flow were recorded after each dose. The hemodynamic parameters were allowed to return to baseline levels (15–20 minutes) before subsequent doses were administered. Changes in the hemodynamic parameters were determined using predose baseline values recorded immediately before injection of the peptides. Hemodynamic responses to vehicle (saline) administration were recorded in each rat before dosing with ANF. Several synthetic atrial natriuretic peptides were examined in the bolus protocol, including atriopeptin II (103–127), $\alpha$-rat atrial natriuretic polypeptide (99–126), Wy-47,663 (human ANF [102–126]), and $\alpha$-human atrial natriuretic polypeptide (99–126).

Baseline parameters were measured in a second group of rats. Increasing doses of an atrial peptide (0.045–1.44 nmol/kg/min) or vehicle (0.625–10 $\mu$l/min) were infused intravenously. The doses were increased at 15-minute intervals. Changes in the hemodynamic parameters were recorded at each dose level after a steady state condition was achieved. Wy-47,663 and $\alpha$-rat atrial natriuretic polypeptide were examined according to this protocol.

In all experiments, absolute changes in MAP and heart rate were recorded. Changes in regional blood flow were expressed as the percent change from pre-dose baseline. Regional vascular resistance was calculated by dividing MAP by the regional blood flow. Percent changes in vascular resistance were also calculated. All of the synthetic atrial peptides used in the present study were obtained from BaChem (Torrance, CA, USA) and were reported to be greater than 97% pure by high-performance liquid chromatography analysis. All experiments in the present study were performed in compliance with institutional guidelines for treatment of conscious animals.

Baseline MAP and heart rate averaged 164 ± 7 mm Hg and 338 ± 16 beats/min, respectively, in rats receiving bolus administration of the atrial peptides. Similar baseline values for MAP (161 ± 6 mm Hg) and heart rate (328 ± 15 beats/min) were observed in SHR that were infused with the peptide. Also, baseline regional blood flows in renal (6.2 ± 0.6 vs 6.3 ± 0.7 kHz), hindquarter (3.7 ± 0.5 vs 4.4 ± 0.8 kHz), and mesenteric (8.2 ± 1.6 vs 9.2 ± 1.1 kHz) vascular beds were not significantly different in bolus-treated or infusion-treated SHR, respectively.

Bolus administration of the atrial peptides caused a modest, but dose-dependent, reduction in MAP in conscious SHR (Figure 1). Significant falls in MAP were observed primarily after higher (1.8 nmol/kg) doses. The depressor responses to rat and human peptide sequences were not statistically different. Significant tachycardia responses were also recorded after injection of higher doses of ANF.

Injection of the synthetic atrial peptides also had marked effects on renal hemodynamics, as illustrated in Figure 2. An immediate, transient (20–40 seconds) increase in renal blood flow was observed after injection of ANF. Higher bolus doses did not significantly prolong the renal response to ANF. The increase in renal blood flow was accompanied by a significant fall in renal vascular resistance (Figure 3). These responses were dose-dependent. Intergroup comparisons failed to indicate any significant differences in the renal responses to rat or human sequences. Renal blood flow tended to exhibit a biphasic response to bolus injection of the atrial peptides in several animals.
In approximately 50 to 60% of the SHR tested, renal blood flow fell below baseline levels after the initial increase. However, the reductions in flow were not dose-related and did not differ significantly from vehicle responses.

Atrial peptides also altered hindquarter and mesenteric vascular resistance (Figure 4). Changes in hindquarter vascular resistance were quite variable during the bolus administration of ANF and vehicle in conscious SHR. No significant, dose-related changes in hindquarter vascular resistance were observed after injection of the rat peptides as compared to vehicle effects; however, significant reductions in hindquarter vascular resistance were observed at high doses (3.6–18 nmol/kg) of Wy-47,663 and α-human atrial natriuretic polypeptide.

Modest, dose-dependent reductions in mesenteric vascular resistance were also observed in the SHR.

**FIGURE 1.** Effects of bolus administration of A) rat atriopeptin II (▲) and α-rat atrial natriuretic polypeptide (○) and B) human atrial natriuretic peptide (Wy-47,663; ●) and α-human atrial natriuretic polypeptide (○) on mean arterial pressure (MAP) and heart rate (HR) in conscious SHR. Asterisk indicates significant difference (p < 0.05) compared with vehicle responses (shown as x).

**FIGURE 2.** Typical cardiovascular responses to bolus administration of atriopeptin II (AP II) in conscious SHR.
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**Figure 3.** Maximal changes in renal blood flow and renal vascular resistance after bolus administration of A) α-rat atrial natriuretic polypeptide (○) and atriopeptin II (▲) and B) Wy-47,663 (●) and α-human atrial natriuretic polypeptide (○) in conscious SHR. Asterisk indicates significant difference (p<0.05) compared with vehicle responses (shown as x).

**Figure 4.** Maximal changes in hindquarter and mesenteric vascular resistances after bolus administration of α-rat atrial natriuretic polypeptide (○) and atriopeptin II (▲) and B) Wy-47,663 (●) and α-human atrial natriuretic polypeptide (○) in conscious SHR. Asterisk indicates significant difference (p<0.05) compared with vehicle responses (shown as x).
Again, the responses were more variable than those observed in the renal vascular bed, but significant falls in mesenteric vascular resistance were observed with all of the peptides.

Infusion of Wy-47,663 and α-rat atrial natriuretic polypeptide resulted in sustained, dose-dependent reductions in MAP that differed significantly from those in vehicle-treated SHR (Figure 5). A significant tachycardia was observed with Wy-47,663, while heart rate was unchanged in rats treated with α-rat atrial natriuretic polypeptide.

Continuous infusion of ANF in conscious SHR had marked effects on regional hemodynamics. In the renal vascular bed, dose-dependent reductions in blood flow were observed with α-rat atrial natriuretic polypeptide and Wy-47,663 infusion (Figure 6). These responses were sustained for the duration of the infusion period. Similarly, renal vascular resistance was significantly elevated during the infusion of both atrial peptides. Transient renal vasodilation occasionally was observed immediately after the initial infusion of the peptides; however, the renal vasodilation was quickly converted to renal vasoconstriction, and vasodilator responses were never observed throughout the remainder of the experimental protocol.

Vascular responses to the infusion of the atrial peptides in the hindquarter and mesenteric vascular beds (Figure 7) were similar to those in the renal vascular bed. Infusion of Wy-47,663 caused significant decreases in hindquarter vascular resistance. Hindquarter
vascular resistance also tended to increase in SHR treated with α-rat atrial natriuretic polypeptide. Marked dose-related increases in mesenteric vascular resistance were observed with the infusion of both atrial peptides. Again, these responses were sustained throughout the infusion period.

Discussion

The effects of ANF on regional vascular resistance in general and on renal vascular resistance specifically have been of great scientific interest. ANF has been observed to decrease renal vascular resistance in isolated, perfused kidneys, but in one instance, only after renal vasoconstrictor tone had been restored to the isolated organ. Initial findings in anesthetized rats also indicated that intrarenal injection of ANF reduced renal vascular resistance, while injection of the peptides into the iliac artery failed to alter hindquarter blood flow. Renal vasodilation was also observed after intrarenal bolus injection of the atrial peptides in anesthetized dogs. In conscious rats, Koike et al. using radioactive microspheres to assess regional blood flow, reported selective increases in renal blood flow 5 minutes after a single intravenous bolus injection of ANF. However, Garcia et al., using similar techniques, reported significant increases in renal, pulmonary, splenic, cardiac, and testes blood flow. Similar findings have also been reported in conscious dogs, in which ANF injection caused dose-dependent increases in renal blood flow but had no effect on mesenteric, iliac, or coronary blood flow. In the present study, renal vasodilator responses to ANF injection were observed in conscious SHR; however, mesenteric and, to a lesser degree, hindquarter vasodilation also occurred at higher doses of ANF. Even though these doses probably are excessively high, these data suggest that the renal-selective effects of ANF may be dose-related. At lower bolus doses, ANF may cause dilation only in the kidney, while at higher doses, the regional selectivity is lost and general vasodilator responses are initiated. Similar findings have been observed in anesthetized dogs after intra-arterial injections of ANF.

Unlike previous studies in conscious rats, the renal responses in the present study were observed immediately after injection of the atrial peptides and were very short-lived. Five minutes after injection of the peptide, when other investigators reported changes in renal blood flow, all hemodynamic parameters had returned to baseline in the present study. The differences between the studies may be related to the techniques employed. A pulsed Doppler flowmeter, which allowed continuous measurement of regional blood flow, was used in the present study, while other investigators relied on injection of radioactive microspheres into the left ventricle to measure regional blood flow. The latter technique allows the measurement of hemodynamic parameters at only a single time point. In addition, Garcia et al. examined normotensive Sprague-Dawley rats, while conscious SHR were employed in the study by Koike et al. Thus, species differences could have a bearing on the hemodynamic responses.

Infusion of the atrial peptides produced responses in the regional vascular beds that were markedly different from those produced by bolus injections of the peptides. Dose-dependent increases in renal, mesenteric,
and hindquarter vascular resistances were observed with the atrial peptides examined. These responses are similar to the previously reported hemodynamic actions of atriopeptin II infusion in conscious SHR and Wistar-Kyoto rats. The increase in regional vascular resistance appears to be mediated through increased sympathetic vasoconstrictor tone, rather than through direct vasoconstrictor actions of the peptide. In these studies, surgical denervation or chemical sympathectomy abolished the regional vasoconstrictor responses to ANF infusion in conscious SHR. Even in sympathectomized rats, however, sustained renal vasodilator responses were not observed during the infusion of ANF. In addition, intrarenal infusion of ANF in conscious rats failed to increase renal blood flow. Other investigators have also failed to observe significant increases in renal blood flow during the infusion of ANF in conscious and anesthetized rats. Thus, it does not appear that the transient renal vasodilator responses observed in rats after bolus administration of the atrial peptides can be sustained by a continuous infusion of ANF.

In dogs, the renal effects of infusions of ANF are not as clearly defined as in the rats. In general, most investigators have not observed sustained increases in renal blood flow during ANF infusion. However, transient increases in renal blood flow lasting for 2 to 3 minutes have been reported to occur immediately after the infusion of ANF was begun, though renal blood flow quickly returned to or below control levels for the remainder of the infusion period. In contrast, other investigators have reported dose-dependent but modest increases in renal blood flow during the infusion of ANF in anesthetized dogs. Further studies will be necessary to clarify the renal actions of ANF in dogs.

During infusion of the atrial peptides in the present study, steady state changes in the hemodynamic parameters were observed after 5 to 7 minutes of infusion and parameters remained unchanged throughout the remainder of the infusion period (15 minutes total). Although infusion of the peptide at each dose for longer periods might have produced somewhat greater responses, infusion of the atrial peptides for 30 minutes or longer (unpublished data) in conscious SHR caused quantitatively similar changes in the hemodynamic parameters as we observed during the 15-minute infusion period. Thus, even though the infusion periods for each dose of ANF were short (15 minutes), other studies from our laboratory indicate that the infusion periods are of ample duration to accurately illustrate the hemodynamic effects of ANF under steady state conditions.

The reasons for the different vascular responses to bolus injection and infusion of ANF have not been fully elucidated. It is doubtful that the lack of a vasodilator response during infusion can be attributed to the absence of functional ANF receptors in the renal vasculature since bolus administration caused marked dose-dependent reductions in renal vascular resistance. It is possible that neural or other vasoconstrictor mechanisms may be involved. After bolus administra-

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