BRIEF COMMUNICATIONS

Failure of Atriopeptin II to Cause Arterial Vasodilation in the Conscious Rat

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SUMMARY. The cardiovascular actions of the synthetic natriuretic peptide, atriopeptin II, were examined in conscious unrestrained spontaneously hypertensive rats and normotensive Wistar-Kyoto rats. The animals were chronically instrumented with miniaturized pulsed Doppler flow probes to allow measurement of regional blood flow, or with an electromagnetic flow probe on the ascending aorta to facilitate the measurement of cardiac output in the conscious rat. Intravenous infusion of increasing doses of atriopeptin II (0.25–4 μg/kg per min) caused a dose-dependent fall in mean arterial pressure in the hypertensive and normotensive rats. Blood flow in the renal, mesenteric, and hindquarters vascular beds was markedly decreased during the infusion of atriopeptin II, and regional vascular resistance was significantly increased in both groups of rats. Heart rate was significantly elevated (47 ± 14 beats/min) in the spontaneously hypertensive rats during atriopeptin II infusion, but no change in heart rate was observed in the Wistar rats. In the hypertensive rats, atriopeptin II caused a marked dose-dependent decrease in cardiac output (maximal decrease = −39 ± 4%) and stroke volume (maximal decrease = −48 ± 4%). Central venous pressure and left atrial pressure were also significantly reduced during atriopeptin II infusion. Total peripheral resistance was increased over the infusion protocol by 26 ± 3%. These data suggest that atriopeptin II infusion markedly attenuated cardiac output in the conscious spontaneously hypertensive rat. Total and regional vascular resistances were increased, possibly through reflex compensatory mechanisms, to maintain arterial pressure in the face of decreased cardiac output. Also, similar changes in regional vascular resistance were observed in normotensive and hypertensive rats, suggesting that these responses were not confined to the spontaneously hypertensive rat. (Circ Res 56: 606–612, 1985)

IT has long been thought that an endogenous "natriuretic factor" was involved in the regulation of sodium excretion. Recently, atrial extracts have been demonstrated to contain several peptides [atrial natriuretic factor (ANF)] which possess powerful natriuretic and diuretic properties (deBold et al., 1981; Kleinert et al., 1984) and antagonize the vasoconstrictor actions of norepinephrine and angiotensin II in vitro (Kleinert et al., 1984; Camargo et al., 1984). With the subsequent purification, sequencing, and synthesis of ANF by several groups (Flynn et al., 1983; Currie et al., 1983; Thibaault et al., 1983; Atlas et al., 1984; Misono et al., 1984), more precise investigation of the vascular and renal actions of these peptides has been possible. Synthetic ANF was found to be a potent natriuretic agent and reduced arterial pressure (Currie et al., 1983; Atlas et al., 1984; Seymour et al., 1984), thus producing results similar to those reported when atrial extracts were used. The depressor actions of synthetic ANF were thought to be mediated through peripheral vasodilation. Synthetic ANF has been demonstrated to relax isolated vascular smooth muscle (Currie et al., 1983; Winquist et al., 1984), and injections of ANF caused a prolonged decrease in renal vascular resistance without affecting hindlimb blood flow or arterial pressure in anesthetized rats (Oshima et al., 1984). Similarly, bolus injections of ANF in conscious rats reduced arterial pressure and caused a sustained increase in renal blood flow, whereas blood flow in other vascular beds (heart, brain, skeletal muscle, gut) were not significantly altered (Koike et al., 1984). These data suggested ANF may cause selective vasodilation of the kidney. However, other investigators have reported conflicting results. Bolus intravenous administration of ANF failed to increase renal blood flow in conscious normotensive rats (M. J. Brody, unpublished data) and increased vascular resistance in isolated perfused rat kidneys (Atlas et al., 1984). In addition, continuous infusion of synthetic ANF failed to alter renal blood flow significantly in anesthetized dogs (Seymour et al., 1985). Thus, the vascular actions of ANF have become an area of controversy. Unfortunately, the actions of ANF were examined only after bolus...
administration of the peptide in all but one (Seymour et al., 1984) of the above studies. The hemodynamic effects of ANF were not studied during continuous infusion.

The present study was designed to examine the cardiovascular action of the synthetic ANF, atriopeptin II (APII), in conscious rats, as described by Currie et al. (1984). Spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats were chronically instrumented to allow continuous measurement of systemic blood pressure, heart rate, and regional blood flow. In addition, in a separate group of SHR, cardiac output, central venous pressure, and left atrial pressure were monitored in the conscious rats. Changes in the hemodynamic parameters were recorded during the steady state period of continuous infusions of APII.

Methods

Regional Blood Flow

Spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY), approximately 18–21 weeks old, were chronically instrumented with miniaturized pulsed Doppler flow probes to allow measurement of renal, mesenteric, and hindquarters blood flow. The methods employed have been previously described (Haywood et al., 1982). In brief, rats were anesthetized with sodium pentobarbital (50 mg/kg, ip) and atropine (0.5 mg/kg). Through a midline incision the right renal artery, superior mesenteric artery and the left iliac artery were cleared of connective tissue. A small pulsed Doppler flow probe was placed around each artery and secured in place. The leads from the flow probes were exteriorized at the base of the skull, where they were soldered into a small head plug cemented to the skull.

Cannulae (P.E. 10’ fused to P.E. 50) were also inserted into the abdominal aorta and the inferior vena cava via the midline incision to facilitate the measurement of arterial pressure and allow administration of the natriuretic peptide. The cannulas were secured to the peritoneal wall, exteriorized in the scapular region, and sealed when not in use. Rats were allowed to recover for at least 4 days before experiments were begun.

On the day of the experiment, stable baseline hemodynamic parameters were recorded and increasing doses of APII (0.25, 0.5, 1.0, 2.0, and 4.0 μg/kg per min) or saline (0.625, 1.25, 2.5, 5, and 10 μl/min) were infused intravenously into WKY and SHR. The dose was increased at 15-minute intervals over the entire dose range. Hemodynamic measurements were made after a steady state condition was obtained with each infusion rate. Changes in mean arterial pressure, heart rate, and renal, mesenteric and hindquarters blood flow during ANF infusion in WKY and SHR were compared with responses in saline-infused rats using a one-way analysis of variance and a Student-Newman Kuels nonpaired t-test.

Cardiac Output

In additional separate experiments, the effects of APII on blood pressure and cardiac output were examined in conscious SHR. Rats were prepared as described by Smith and Hutchins (1978) and Smits et al. (1984). In brief, SHR were anesthetized with sodium pentobarbital (60 mg/kg, ip). The trachea was intubated and the rats were artificially ventilated (60 strokes/min 2.5 ml tidal volume). A thoracotomy was performed in the 3rd right intercostal space. The ascending aorta was carefully isolated and an electromagnetic flow probe (Skalar; 2.3–2.5 mm i.d.) was placed around the vessel. The cable was fixed to the ribs, tunneled subcutaneously to the neck where the connector was sutured to the skin. After the SHR had recovered for 4–6 days, cardiac output was monitored with a Skalar M 400 sinewave flowmeter. Cannulas were inserted into the femoral artery and vein and advanced into the abdominal aorta and inferior vena cava; respectively. The cannulas were exposed at the base of the skull.

In a separate group of SHR, changes in central venous pressure and left atrial pressure were monitored during APII administration. Central venous pressure was monitored through a cannula that was inserted into the femoral vein with the tip advanced 9 cm so that it lay in the thoracic section of the inferior vena cava (Smits et al., 1982). Left atrial pressure was measured directly through a catheter implanted in the left atrium. Prior to surgery, two small rims of Silastic adhesive were formed on a 10-cm piece of Silastic (602-135) tubing. The rats were anesthetized and artificially respirated as previously described. A thoracotomy was performed in the 4th left intercostal space, and a miniature hemostat was placed on the left atrial appendage. The catheter was inserted into the left atrium through a small incision and attached with a 5-0 silk ligature, tied between the Silastic rings. The catheter was exteriorized at the neck. The rats were allowed to recover for 2–3 days. On the day of the experiment, control levels for central venous pressure and left atrial pressure were taken to be 3 cm above the cage floor.

In these experiments, we employed the same experimental protocol described above. Increasing doses of APII were infused intravenously for 15-minute periods and changes in mean arterial pressure, heart rate, cardiac output, central venous pressure, and left atrial pressure were recorded during the steady state at each infusion level. Arterial blood samples were collected before and after the last infusion rate of APII for hematocrit determination. Responses were compared to preinfusion control values by Student’s t-test.

All data in this study are presented as the means ± sem. Statistical significance was accepted if P < 0.05. All synthetic APIII used in these studies was purchased from BaChem Inc. The peptide purity was found to be of greater than 97%, by high pressure liquid chromatography (HPLC).

Results

Regional Blood Flow

Baseline measurements of mean arterial pressure (MAP) (159 ± 3 vs. 168 ± 8 mm Hg) and heart rate (328 ± 14 vs. 348 ± 16 beats/min) in the SHR were similar in APIII- and saline-treated groups, respectively. WKY had significantly lower MAP than the SHR, but heart rates were similar in the two groups of rats. There were no differences between the respective MAP (113 ± 2 vs. 118 ± 6 mm Hg) and heart rate (364 ± 11 vs. 326 ± 12 beats/min) values in the APIII- and saline-treated WKY rats.

The hemodynamic effects of APIII and saline were markedly different in the conscious SHR (Fig. 1).
Saline infusion failed to cause a significant alteration in MAP, heart rate, or renal, mesenteric, or hindquarters blood flow from baseline. However, APII infusion caused a dose-dependent fall in MAP and all three regional blood flows. With each increasing dose of APII, MAP fell rapidly to a steady state level within 5–7 minutes and remained stable for the duration of the infusion period. Heart rate was significantly elevated in the SHR during APII infusion (maximal Δ = 47 ± 14 beats/min), but these responses were not dose-related. Maximal depressor responses (—27 ± 3 mm Hg) were observed during the infusion of APII at 2 μg/kg per min. Similar to MAP, renal and mesenteric blood flow were also significantly reduced by APII infusion in the SHR. A transient increase in renal blood flow was initially observed immediately after the lowest infusion of APII was begun, but this response lasted only 30–45 seconds. Blood flow quickly returned to control levels, and no indication of increased renal blood flow was observed during the remainder of the experimental protocol. The maximal fall in regional blood flow was greater in the mesenteric vascular bed (—54 ± 3%) than in the renal vascular bed (—28 ± 5%). Interestingly, maximal reductions in renal blood flow were observed during APII infusions of 1 μg/kg per min. Increasing doses failed to reduce renal blood flow further. Hindquarter blood flow was also markedly reduced. However, due to variable responses in the saline-infused SHR, the effects of APII on hindquarters blood flow failed to achieve statistical significance.

In the normotensive WKY, saline infusion again failed to cause significant changes in baseline MAP or regional blood flow parameters (Fig. 2). However, APII produced significant dose-dependent reductions in MAP, as well as in renal, mesenteric, and hindquarter blood flow. The decreases in renal, mesenteric, and hindquarters blood flow were of a magnitude similar to those observed in SHR during the infusion of APII. However, unlike that in SHR, no significant change in heart rate was observed in the WKY during APII infusion.

Regional vascular resistance was increased significantly in the renal, mesenteric, and hindquarters vascular beds in both SHR and WKY during APII infusion (Fig. 3). Vasoconstrictor responses in the renal and mesenteric vascular beds were similar in SHR and WKY during APII infusion. However, significantly greater increases in hindquarter vascular resistance were observed in WKY, compared to SHR. Depressor responses were also consistently greater in SHR than WKY.

**Cardiac Output**

The APII infusion protocol was repeated in a separate group of conscious SHR chronically instrumented with an electromagnetic flowprobe on the
ascending aorta. APII again caused a dose-dependent fall in MAP, which was accompanied by a significant tachycardia (Table 1). Reductions in cardiac output (−13 ± 3%) and stroke volume (−18 ± 4%) were observed during the lowest infusion of APII (0.25 µg/kg per min) and continued to fall with increasing doses of APII. During the highest dose of APII (4 µg/kg per min), cardiac output and stroke volume were reduced by 39 ± 4% and 48 ± 4%, respectively. Total peripheral resistance (TPR), on the other hand, was increased during the infusion of APII. Dose-related increases in TPR of 10 ± 3% to 26 ± 3% were observed during the infusion protocol. In a separate group of SHR, central venous pressure and left atrial pressure fell significantly during the lowest infusion rate of APII and continued to fall in a dose-dependent fashion with increasing doses of APII (Table 1). Also, hematocrit was increased slightly from a preinfusion level of 46.4 ± 1.1 vol% by 2.3 ± 1 vol% (5 ± 2.5%) at the end of the infusion protocol.

**Discussion**

In the present study, the dose-dependent depressor actions of APII in the conscious SHR were mediated through suppression of cardiac output, rather than through reductions in peripheral vascular resistance. In fact, total and regional vascular resistances increased during APII infusion in SHR. Essentially similar hemodynamic changes were observed during APII infusion in conscious WKY rats.

The fall in cardiac output during APII apparently was due to marked reductions in venous filling pressure in both right and left atria, rather than to a direct cardiac depressant action of APII. The fall in central venous pressure and, particularly, left atrial pressure could account entirely for the observed changes in stroke volume and cardiac output in the present study. APII could reduce venous return via reductions in plasma volume caused by the diuretic actions of APII or by direct venodilator effects similar to those of sodium nitroprusside (Winquist et al., 1984). However, only a small increase in hematocrit (5 ± 2.5%), an index of plasma volume, was observed over the entire infusion period. In addition, cardiac output began to fall immediately after each dose of APII, reaching a steady state level within 5–8 minutes. Thus, the rapid onset of the response and the relatively small change in hematocrit would suggest that reduction of plasma volume could not solely account for the precipitous fall in cardiac output observed after APII. Rather, it would appear that APII caused venodilation in the conscious rats. Synthetic ANF has been demonstrated to relax rabbit facial veins but was weakly active in relaxing the phasic contractions of rat portal veins in vitro (Winquist et al., 1984). Interestingly, the rabbit facial vein
FIGURE 3. Comparisons of the effects of APII on mean arterial pressure (MAP) and renal (RVR), mesenteric (MVR) and hindquarters (HQVR) vascular resistance in conscious SHR (●) and WKY (▲). *P < 0.05, comparison to responses in WKY; %Δ = percent change from preinfusion baseline.

appeared to resemble arterioles more closely than other venous tissues, in its smooth muscle composition and intrinsic tone (Winquist et al., 1984), and may not accurately represent venous responses to ANF. The fall in central venous pressure in the present study would suggest that peripheral venous tone was reduced in the intact rat by APII. In addition, since left atrial pressure fell more than central venous pressure, it was possible that APII may also reduce pulmonary vascular resistance.

Interestingly, other investigators (Koike et al., 1984) did not observe reductions in cardiac output after ANF in conscious rats. However, ANF was administered by intravenous bolus, and cardiac output was not measured continuously with an electromagnetic flow probe. Rather, microsphere techniques were employed by Koike et al. (1984). It is possible that the mode of administration could effect the cardiovascular actions of ANF. Owing to an apparent short plasma half-life, the diuretic effects

<p>| Table 1: Hemodynamic Effects of APII in Conscious SHR (n = 7)* |
|---------------------------------|------|-----|-----|-----|------|-----|</p>
<table>
<thead>
<tr>
<th>Infusion (µg/kg/min)</th>
<th>MAP (mmHg)</th>
<th>HR (b/min)</th>
<th>CO (ml/min)</th>
<th>SV (µl)</th>
<th>TPR (mmHg/ml)</th>
<th>CVP† (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>148 ± 4</td>
<td>356 ± 9</td>
<td>78.4 ± 5</td>
<td>220 ± 14</td>
<td>1.92 ± 0.1</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>0.25</td>
<td>–9 ± 5</td>
<td>+40 ± 8*</td>
<td>–9.8 ± 2.2*</td>
<td>–46 ± 3*</td>
<td>0.18 ± 0.06*</td>
<td>–0.5 ± 0.2*</td>
</tr>
<tr>
<td>0.5</td>
<td>–19 ± 5*</td>
<td>+30 ± 17*</td>
<td>–16.5 ± 1.9*</td>
<td>–66 ± 9*</td>
<td>0.19 ± 0.05*</td>
<td>–0.8 ± 0.1*</td>
</tr>
<tr>
<td>1.0</td>
<td>–28 ± 6*</td>
<td>+61 ± 13*</td>
<td>–23.2 ± 2.2*</td>
<td>–87 ± 8*</td>
<td>0.28 ± 0.06*</td>
<td>–1.2 ± 0.1*</td>
</tr>
<tr>
<td>2.0</td>
<td>–29 ± 4*</td>
<td>+66 ± 10*</td>
<td>–25.5 ± 2.0*</td>
<td>–95 ± 7*</td>
<td>0.39 ± 0.06*</td>
<td>–1.4 ± 0.1*</td>
</tr>
<tr>
<td>4.0</td>
<td>–33 ± 6*</td>
<td>+65 ± 11*</td>
<td>–30.3 ± 2.5*</td>
<td>–105 ± 7*</td>
<td>0.49 ± 0.05*</td>
<td>–1.5 ± 0.3*</td>
</tr>
</tbody>
</table>

MAP = mean arterial pressure; HR = heart rate; CO = cardiac output; SV = stroke volume; TPR = total peripheral resistance; CVP = central venous pressure; LAP = left atrial pressure.

* Data are presented as the maximal changes in the respective parameters from baseline measurements.

† Measurements taken in separate group of conscious SHR.

‡ P < 0.05, comparison to baseline values.
of injected ANF are of relatively short duration (debold, 1981). Similarly, only minor or transient reductions in cardiac output may occur in conscious rats after bolus administration of ANF. Without continuous measurement of cardiac output, such changes could go undetected. However, Ackermann et al. (1984), using thermodilution techniques, were able to demonstrate a small but significant reduction in cardiac output in anesthetized rats after bolus administration of atrial extracts. In the present experiment, sustained reductions in cardiac output were observed at each infusion rate of APII, indicating that with continuous infusion, the suppression of cardiac output and stroke volume could be maintained.

APII failed to reduce total or regional vascular resistance in the conscious SHR and WKY. To the contrary, increases in renal, mesenteric, and hindquarters vascular resistance were consistently recorded, with little variability observed in the dose-dependent reductions in the regional blood flow caused by APII. Similar responses to APII infusion have also been observed in normotensive Sprague-Dawley rats (unpublished observation). These findings suggest that the vasoconstrictor effects of APII infusion are not confined to a particular strain of rat.

It could be argued that other natriuretic peptides may elicit somewhat different responses, and that the present study is applicable only to APII, a 23-amino acid peptide. However, limited studies to date with equimolar infusions of rat atrial natriuretic peptide (28 amino acids, Ba Chem, Inc.) also demonstrate increased regional vascular resistance in the conscious SHR (data not shown). Thus it would appear that atrial natriuretic peptides in general possess similar cardiovascular actions in the conscious rat. Likewise, it is doubtful that impurities in the synthetic APII could be responsible for the cardiovascular responses of the peptide. All studies were performed with APII purchased from Ba Chem, Inc., and the samples were found to be 97% pure by HPLC analysis.

It is doubtful that regional vascular resistance was increased through direct vasoconstrictor actions of APII. Several studies have demonstrated that ANF possesses potent vasorelaxant properties in isolated vascular smooth muscle (Currie et al., 1983; Winquist et al., 1984; Kleinert et al., 1984). Similarly, intrarenal administration of APII reduced renal vascular resistance in anesthetized rats (Oshima et al., 1984; Seymour et al., 1985). ANF did, however, increase renal vascular resistance in isolated perfused kidneys, but only if all vasoconstrictor agents were removed from the perfusate. (Camargo et al., 1984; Atlas et al., 1984).

APII may, indeed, cause measurable vasodilator responses in the rat at much lower concentrations. However, at the doses examined in the present study, the vasodilatory actions of ANF may have been obscured by reflex neural vasoconstrictor responses. With the progressive fall in cardiac output and arterial pressure stimulating baroreflex mechanisms, increased sympathetic vasoconstrictor tone could mask any moderate ANF-induced vasodilation. Humoral mechanisms could also be involved. A fall in renal perfusion pressure may stimulate renin release in the conscious rats, resulting in increased vascular resistance. However, ANF has been reported to inhibit renin release directly (Atlas et al., 1984), suggesting that the renin-angiotensin system may not be involved in increasing vascular resistance during APII infusion. Similarly, ANF has been reported to inhibit the release of vasopressin (Januszewicz, unpublished data), suggesting that increased plasma levels of this vasoconstrictor peptide may not contribute to the cardiovascular actions of APII. The precise vasoconstrictor mechanism is, as yet, unknown, and further studies are necessary to define possible reflex mechanisms involved in the cardiovascular responses to APII infusion.

The doses of APII used in the present study were chosen to cover a drug range which elicited minimal to marked depressor effects on arterial pressure during acute infusion in the conscious rat. Also, the diuretic and natriuretic threshold for the synthetic APII in our laboratories was found to occur between 0.12 and 0.24 μg/kg per min in anesthetized assay rats (unpublished observation). Thus, lower infusion rates were not examined in the conscious instrumented rats. It would be of interest, however, to determine whether chronic infusion of very low doses of ANF would eventually elicit cardiovascular responses similar to those observed in the present study. Regardless, the present study clearly indicates that even at moderate infusion levels, APII decreased cardiac output and increased peripheral resistance while lowering arterial pressure in the conscious rat.

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