Atrial Natriuretic Factor Inhibits the Hypertension Induced by Chronic Infusion of Norepinephrine in Conscious Rats

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SUMMARY. To assess the physiological role of atrial natriuretic factors in the regulation of blood pressure and sodium-water excretion, we studied the chronic effects of continuous infusion of a synthetic atrial natriuretic factor of 25 amino acids for up to 3 days on systolic blood pressure, urine volume, and urinary excretion of sodium, prostaglandin E₂ and kallikrein in conscious rats, and also evaluated the antihypertensive effect of this substance in rats with hypertension caused by chronic infusion of norepinephrine. Continuous infusion of atrial natriuretic factor (150 μg/kg per day) into the jugular vein via osmotic minipumps did not induce any changes in systolic blood pressure, urine volume, and urinary excretion of sodium, prostaglandin E₂, and kallikrein for up to 3 days, compared with those in vehicle-infused rats. When the same dose of atrial natriuretic factor was administered simultaneously with 1.8 mg/kg per day of norepinephrine infused intraperitoneally by osmotic minipumps, the systolic blood pressure of conscious rats rose on day 1 to only 127.3 ± 6.3 mm Hg compared with the rise to 146.3 ± 1.6 mm Hg when norepinephrine alone was infused (P < 0.05). The antihypertensive effect of atrial natriuretic factor was sustained for 3 days in rats infused with norepinephrine. The administration of atrial natriuretic factor to rats made hypertensive by 3 days of infusion with norepinephrine alone returned the blood pressure to control levels, and the antihypertensive effect was sustained throughout the experimental period lasting for 3 days. These results indicate that a subdepressor dose of synthetic atrial natriuretic factor can modulate the vasopressor effect of norepinephrine, and may be involved in the regulation of blood pressure, independent of the renal effects of these substances. (Circ Res 57: 470-474, 1985)

IT is now well established that mammalian cardiac atria contain a group of biologically active peptides called atrial natriuretic factors (ANF), that are capable of producing natriuresis and diuresis (Sagnella and MacGregor, 1984). Moreover, extracts of cardiac atria have been found to cause relaxation of vascular smooth muscle during norepinephrine (NE)-induced contraction (Currie et al., 1983). It has been shown that natriuretic activity and smooth muscle relaxant activity reside inseparably in these peptides (Grammer et al., 1983; Kleinert et al., 1984). The physiological role of the peptides remains to be elucidated. The diuretic, natriuretic, and hypotensive effect induced by the administration of the extract of the cardiac atrium, or the purified or synthetic ANF, suggests that it could be involved in the regulation of blood volume and vascular resistance.

In the present study, we used an ANF of 25 amino acids [synthesized by Sugiyama et al. (1984)] which have been proven to be identical with those of the natural ANF extracted from rat atrium and sequenced by Misono et al. (1984). We studied the chronic effects of ANF on blood pressure and sodium-water excretion in conscious rats, and also evaluated the antihypertensive effect of this peptide in rats made hypertensive by the chronic infusion of norepinephrine (NE).

Methods

Male Sprague-Dawley rats weighing from 150–250 g were used. All rats were maintained in a humidity- and temperature-controlled room, each rat being housed in a metabolic cage during the study. The rats were fed a regular diet (Oriental CMF, 0.20 mEq of sodium/g, 0.27 mEq of potassium/g; Oriental Yeast Co.) and had free access to tap water. Studies were performed after a 7-day period of acclimatization to the housing, feeding, and drinking conditions. The rats were infused with 150 μg/
kg per day of a synthetic ANF or vehicle (0.9% physiological saline), delivered via osmotic minipumps (Alzet) into the jugular vein for up to 3 days. The vascular catheter (PE60) was tunneled subcutaneously, and the osmotic minipump was implanted in the interscapular region of the rat's back under pentobarbital sodium anesthesia (Abbott Laboratories Pty. Ltd.). An ANF of 25 amino acids synthesized by Sugiyama et al. (1984) was used in the present experiments and was dissolved in 0.9% physiological saline. The chemical characteristics and biological activities of this peptide have been proven to be identical with those of the natural ANF which was extracted from rat atrium and sequenced by Misono et al. (1984). Rats in the control group received the vehicle. To choose a subpressor dose of ANF, we assessed the effects of a continuous infusion of the synthetic ANF at doses of 0.1, 0.3, 1, and 5 μg/kg per min for up to 30 minutes in rats anesthetized with Inactin, and confirmed that, at 0.1 and 0.3 μg/kg per min, ANF alone induced significant diuresis and natriuresis but did not significantly affect blood pressure. Assuming that ANF did not degrade during the study, and that the pumps dispensed fluid at the specified rate of approximately 1 μl/hour, the infusion dose (150 μg/kg per day) was chosen to be sufficient to induce small but significant diuresis and natriuresis, but not to affect blood pressure in a rat bioassay system. The stability of ANF in the osmotic minipump was examined by comparing the diuretic and natriuretic activities remaining in the solution recovered from the minipump after 3 days of use in the rat to the activities in freshly dissolved ANF. No difference was observed in the activities between the fresh preparation and the solution recovered from the minipump.

The effect of combined administration of ANF with NE was assessed in rats on a regular diet. Following a 7-day control period, the rats were infused either with NE at a rate of 1.8 mg/kg per day dissolved in 5 ml glutathione containing ascorbic acid (50 μg/ml) or with NE in combination with 150 μg/kg per day of ANF for up to 3 days. The infusion of NE was delivered via the osmotic minipumps implanted intraperitoneally under light ether anesthesia. In subsequent experiments, ANF was administered for up to 3 days to rats made hypertensive by chronic infusion of NE. Intravenous infusion of ANF (150 μg/kg per day) or vehicle (0.9% physiological saline) was also initiated on day 3 after the infusion of NE alone had been started, and the combined administration of ANF or vehicle with NE was sustained for the following 3 days. In previous reports (Diz et al., 1981; Yasujima et al., 1984), it had been shown that chronic infusion of the same dose of NE induced a sustained increase in systolic blood pressure. Norepinephrine bitartrate was obtained from Sigma Chemical Company.

Systolic blood pressure in the rats was recorded daily by an indirect tail cuff method without anesthesia (Pfeffer et al., 1971). The daily fluid intake, urine volume, urinary sodium excretion, urinary prostaglandin E2 excretion, and urinary kallikrein excretion were determined. Urine was collected at 4°C and kept at -20°C until the assays. Urinary prostaglandin E2 was measured by the modified method described previously (Abe et al., 1978). Briefly, urine was acidified to pH 3 with hydrochloric acid and extracted with 5 volumes of ethyl acetate. The dried organic extract was then chromatographed on a silicic acid column for separation of prostaglandin E2, which was then eluted by a mixture of benzene and ethyl acetate (60:40) according to Jaffe's method (Jaffe et al., 1973). The prostaglandin E2 was dried and measured radioimmunologically using prostaglandin E2 antiserum (Pasteur Institute). The overall recovery of prostaglandin E2 was 60%. Urinary kallikrein was measured as kininogenase activity by the method of Abe et al. (1979). The enzyme kinetic assay was done using low molecular weight bovine serum kininogen as the substrate. With the present method, the extraction of kinin was not necessary, because bovine serum low molecular weight kininogen did not cross-react with the kinin antibody. The generated kinin was measured by Carretero's method (Carretero et al., 1976). Urinary sodium was measured with a flame photometer. All results were expressed as the mean ± SEM. The significance of differences between mean values was evaluated by Student's t-test.

**Results**

Body weight, systolic blood pressure, fluid intake, urine volume, urinary sodium excretion, urinary prostaglandin E2 excretion, and urinary kallikrein excretion were not significantly different between the groups prior to infusion of ANF or vehicle.

As shown in Table 1, continuous infusion of ANF (150 μg/kg per day) into the jugular vein via osmotic minipumps did not induce any significant changes in systolic blood pressure, urine volume, or in urinary excretion of sodium, prostaglandin E2, and kallikrein for up to 3 days, compared with those in vehicle-infused rats. On day 1, ANF induced a slight increase in urine volume, but the increase was not statistically significant compared with that in vehicle-infused rats.

As shown in Figure 1a, the systolic blood pressure of the NE-alone group began to rise significantly on the 1st day of the infusion and remained high up to the 3rd day, whereas that of the vehicle-alone group did not change. When 150 μg/kg per day of ANF were administered simultaneously with 1.8 mg/kg per day of NE, the tail systolic blood pressure of conscious rats rose to only 127.3 ± 6.3 mm Hg, compared with 146.3 ± 1.6 mm Hg with NE alone on day 1 (P < 0.05). The antihypertensive effect of ANF was sustained for 3 days in rats infused with NE. This was not associated with significant changes in urine volume or urinary sodium excretion. Urine volume was 10.5 ± 1.0 ml/day (ANF and NE) and 8.9 ± 1.2 ml/day (NE alone) on day 1; on day 3 the values were 8.9 ± 1.2 ml/day (ANF and NE) and 11.9 ± 1.4 ml/day (NE alone). Urinary sodium excretion was 0.84 ± 0.18 mEq/day (ANF and NE) and 0.72 ± 0.28 mEq/day (NE alone) on day 1; on day 3, the values were 0.60 ± 0.16 mEq/day (ANF and NE) and 0.54 ± 0.06 mEq/day (NE alone). The blood pressure of the rats given NE with 150 μg/kg per day of ANF was not significantly different from that of the control rats given the vehicle.

Figure 1b shows the effect of ANF on the sustained hypertension caused by a 3-day infusion of NE alone. When ANF was infused along with NE on the 4th day, systolic pressure fell to 115.2 ± 1.8
TABLE 1
Effects of Continuous Infusion of Synthetic Atrial Natriuretic Factor in Conscious Rats

<table>
<thead>
<tr>
<th>SBP (mm Hg)</th>
<th>Day</th>
<th>ANF</th>
<th>Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>121.3 ± 4.8</td>
<td>117.0 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>120.3 ± 2.2</td>
<td>117.3 ± 0.9</td>
</tr>
<tr>
<td>U V (ml/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANF</td>
<td>1</td>
<td>10.4 ± 1.2</td>
<td>11.8 ± 2.4</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1</td>
<td>10.4 ± 0.4</td>
<td>9.0 ± 1.1</td>
</tr>
<tr>
<td>U N,V (mEq/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANF</td>
<td>1</td>
<td>0.87 ± 0.14</td>
<td>0.78 ± 0.17</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1</td>
<td>0.82 ± 0.10</td>
<td>0.64 ± 0.12</td>
</tr>
<tr>
<td>U PCEJ V (ng/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANF</td>
<td>1</td>
<td>3.16 ± 0.05</td>
<td>2.49 ± 0.04</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1</td>
<td>3.59 ± 0.04</td>
<td>3.34 ± 0.02</td>
</tr>
<tr>
<td>U ax V (µg/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANF</td>
<td>1</td>
<td>0.75 ± 0.07</td>
<td>0.75 ± 0.09</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1</td>
<td>0.83 ± 0.07</td>
<td>0.72 ± 0.08</td>
</tr>
</tbody>
</table>

Results are means ± SEM. Abbreviations: ANF, atrial natriuretic factor; SBP, systolic blood pressure; UV, urine volume; UN,V, urinary sodium excretion; UPCEJ V, urinary prostaglandin E2 excretion; U ax V, urinary kallikrein excretion.

mm Hg. This value was significantly lower ($P < 0.01$) than the value of systolic pressure obtained when vehicle was infused along with NE on the 4th day. The antihypertensive effect of ANF was sustained for the following 3 days, but it was not associated with significant changes in urine volume and urinary sodium excretion. The blood pressure of the rats given NE with 150 µg/kg per day of ANF was not significantly different from that of the control rats that did not receive any NE.

Discussion

In the present study, we demonstrated that continuous infusion of a subdepressor dose of a synthetic ANF of 25 amino acids for up to 3 days failed to induce any significant changes in urine volume, urinary sodium excretion, urinary prostaglandin E2 excretion, and urinary kallikrein excretion in conscious rats. Moreover, it is interesting to note that a continuous infusion of a subdepressor dose of ANF exerted an antihypertensive effect in rats made hypertensive by chronic infusion of NE alone.

Since the first report made by De Bold et al. (1981), several studies have demonstrated the blood pressure-lowering effect of atrial extracts. The hypotension was attributed to loss of body fluid because the hematocrit increased after intravenous injection of the extract in their experiment. Recently, Koike et al. (1984) showed in conscious sponta-
neously hypertensive rats that the acute hypotensive effect of synthetic ANF, which is identical with the peptide used in the present study, appeared to result from vasodilation, especially in the kidney. In addition, it has been shown that extract of cardiac atrium (Currie et al., 1984; Kleinert et al., 1984; Misono et al., 1984), or synthetic ANF (Sugiyama et al., 1984) counteracts the vasoconstrictive action of NE, angiotensin II or potassium chloride in vitro. However, the precise mechanism of the contribution of ANF to the regulation of blood pressure has been controversial. To our knowledge, the antihypertensive effect of ANF in rats made hypertensive by chronic infusion of NE alone has not been previously reported, whereas it has been well documented that, in vitro, ANF attenuates the acute vasopressor effects of exogenous NE. The suppression of NE-induced hypertension by ANF was not due to tachyphylaxis per se, since NE alone induced a sustained increase in systolic blood pressure in the absence of ANF.

Although, in preliminary experiments, continuous infusion of ANF at this dose range induced slight but significant increases in urine volume and urinary sodium excretion in rats anesthetized with Inactin, sustained infusion of ANF for 3 days failed to cause such changes. We have no definite explanations for the discrepancy, since the stability and bioavailability of ANF during the infusion has been confirmed in the preliminary experiments. In addition, we could not find a significant difference in body weight between the ANF and vehicle groups, and did not evaluate any additional parameters related to the state of hydration.

The mechanism(s) by which ANF blocks the hypertension caused by chronic infusion of NE cannot be elucidated by the present experiments. Although the substance has natriuretic and diuretic action (Sagnella and McGregor, 1984), we could not show any significant changes in urine volume and urinary sodium excretion in rats infused with ANF alone or in combination with NE. In addition, ANF alone did not induce significant changes in urinary prostaglandin E₂ excretion or urinary kallikrein excretion. Therefore, it is unlikely that the antihypertensive effect of ANF is due to loss of water and sodium, or to the renal kallikrein-kinin-prostaglandin E₂ system. Since chronic hypertension induced by the administration of NE is not volume dependent, but is characterized by an increase in total peripheral resistance (Kleinjans et al., 1984), it cannot be excluded that ANF interferes with intracellular calcium mobilization and/or some metabolic process causing vasoconstriction. It has been reported that an inhibition of Na⁺,K⁺-ATPase is not involved in the effect of ANF on vascular smooth muscle (Pollock et al., 1983). It has been suggested that cyclic guanosine monophosphate (cGMP) is involved, since the in vivo injection of ANF increases urinary excretion and plasma levels of cGMP, and in vitro incubation of ANF with rat kidney homogenate induces an increase in tissue levels of cGMP and a decrease in cGMP phosphodiesterase (Hamet et al., 1984). In addition, ANF has been shown to mimic the effects of nitroprusside, which is associated with changes in cGMP (Garcia et al., 1984). However, the exact mechanism of the effect of ANF on vascular smooth muscle is still unknown.

In conclusion, the result of the present study that continuous infusion of a subdepressor dose of a synthetic ANF blocks the hypertension caused by chronic infusion of NE, suggests that ANF may be involved in the physiological regulation of blood pressure. However, its actual physiological relevance remains to be elucidated, since the release of ANF into the circulation has not yet been shown.

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INDEX TERMS: Atrial natriuretic factor • Blood pressure • Sodium-water excretion • Norepinephrine • Vascular smooth muscle
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