Antihypertensive Effect of Synthetic Atrial Natriuretic Factor in Vasopressin-Infused Rats

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To assess the pathophysiological role of atrial natriuretic factors in the regulation of blood pressure, we studied the effect of chronic infusion of a synthetic atrial natriuretic factor of 25 amino-acid residues on blood pressure and sodium-water excretion. Experimental subjects were rats with hypertension made by chronic infusion of vasopressin on regular intakes of sodium or on sodium loading with 1% NaCl as drinking water. When a subdepressor dose (150 μg/kg/day) of synthetic atrial natriuretic factor was delivered via an osmotic minipump into the jugular vein simultaneously with 7.2 U/kg/day of vasopressin infused intraperitoneally by another osmotic minipump, the expected elevation of systolic blood pressure was completely inhibited. This was not accompanied by any changes in urine volume and urinary sodium excretion. The antihypertensive effect was sustained throughout the experimental period lasting 3 days in rats on regular sodium intake (p < 0.01) or on sodium loading with 1% NaCl as drinking water (p < 0.01). These results indicate that a subdepressor dose of synthetic atrial natriuretic factor can modulate the vasopressor effect of vasopressin. Therefore it is suggested that an atrial natriuretic factor may be involved in the regulation of blood pressure via its antagonizing effect to vasopressin.

Potent natriuretic and diuretic substances present in the specific granules of mammalian atrial cardiocytes have been purified, sequenced and synthesized.¹⁻³ These atrial natriuretic factors (ANF) relax isolated vascular smooth muscle and also antagonize vascular smooth muscle contraction induced by angiotensin II and other vasoconstrictors.⁴,⁵ The diuretic, natriuretic and vasorelaxant activities of ANF suggest an important role for this family of peptides in the regulation of blood volume and vascular resistance clearly opposite to that of arginine-vasopressin (VP). Therefore it is reasonable to speculate that ANF antagonize the vascular and renal action of VP, although the functional interaction of ANF with VP in the regulation of blood pressure remains to be clarified in detail⁶,⁷

We have recently demonstrated that chronic infusion of synthetic ANF of 25 amino-acid residues synthesized by Sugiyama et al.⁸, which shares identical characteristics in the biological activities with a native form, inhibits hypertension induced by sustained infusion of norepinephrine in conscious rats.

Key Words:
- Rat atrial natriuretic polypeptide
- Blood pressure
- Antidiuretic hormone
- Sodium-water excretion
- Vascular smooth muscle

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TABLE 1 CONTROLS FOR EACH GROUP OF RATS

<table>
<thead>
<tr>
<th></th>
<th>Regular diets (n = 18)</th>
<th>Sodium loading (n = 18)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>VP (n = 6)</td>
<td>VP + ANF (n = 6)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>122.9 ± 2.6</td>
<td>122.5 ± 2.4</td>
</tr>
<tr>
<td>BW (g)</td>
<td>221.4 ± 5.2</td>
<td>226.8 ± 4.8</td>
</tr>
<tr>
<td>FI (ml/day)</td>
<td>22.0 ± 1.3</td>
<td>20.8 ± 1.0</td>
</tr>
<tr>
<td>UV (ml/day)</td>
<td>9.6 ± 1.1</td>
<td>9.2 ± 0.9</td>
</tr>
<tr>
<td>U_{Na}V (mEq/day)</td>
<td>0.74 ± 0.03</td>
<td>0.71 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>2.66 ± 0.48</td>
<td>3.18 ± 0.45</td>
</tr>
</tbody>
</table>

Results are mean ± SEM. Abbreviations: SBP = systolic blood pressure; BW = body weight; FI = fluid intake; UV = urine volume; U_{Na}V = urinary sodium excretion; VP = vasopressin; ANF = atrial natriuretic factor.

In the present study, we evaluated the effect of chronic infusion of synthetic ANF of 25 amino-acid residues in rats made hypertensive by chronic infusion of VP to determine if ANF inhibits the vascular action of VP.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing from 200 to 250g 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 metabolic cage was devised to prevent feces-urine contact (Model Metabolism cage ST type, Sugiyamagen Corp., Tokyo, Japan). The rats were fed a regular diet (Oriental CMF, 0.20 meq of sodium/g, 0.27 meq of potassium/g, Oriental Yeast, Tokyo, Japan) and allowed free access to tap water. Studies were performed after a 7 day period of acclimatization to the housing, feeding and drinking conditions.

The effect of ANF on hypertension produced by chronic infusion of vasopressin was assessed in conscious rats on regular diet or under sodium loading with 1% NaCl as drinking water. Infusion of VP and ANF were simultaneously started, and changes in blood pressure were determined in comparison with the groups which received VP alone. Following a 7 day control period of acclimatization, rats were assigned to a regular diet or on sodium loading with 1% NaCl. After a subsequent 7 day period, the rats were infused VP at a rate of 7.2 U/kg/day dissolved in 0.01 N acetic acid, alone or in combination with 150 μg/kg/day of ANF dissolved in physiological saline for up to 3 days. After 3 days of combined administration of VP with ANF, the infusion of ANF was withdrawn by taking away the osmotic minipumps of ANF, whereas the infusion of VP was continued for an additional 3 days. Control rats received vehicle instead of VP.

A synthetic ANF (150 μg/kg/day) was delivered via an osmotic minipump (Alzet, Palo Alto, California, USA) into the jugular vein. The vascular catheter (PE 60) was tunneled through subcutaneous tissue, and the osmotic minipump was implanted in the interscapular region of the rat's back under anesthesia by pentobarbitone sodium (Abbott Laboratories Pty. Ltd., Japan). An ANF of 25 amino-acid residues synthesized by Sugiyama et al. was used in the present experiments and was dissolved in physiological saline. We chose the subpressor dose of ANF (150 μg/kg/day) as described previously and confirmed that continuous infusion of the dose employed in the present experiment into the jugular vein via an osmotic minipump did not induce any changes in systolic blood pressure, urine volume or urinary sodium excretion for up to 3 days compared to those in vehicle-infused rats. The stability of ANF in the osmotic minipump was examined by determining the diuretic and natriuretic activity remaining in the solution recovered from the minipump after 3 days of use in the rat in comparison with freshly dissolved ANF. No difference was observed in the activity between the fresh

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Fig. 1. Effect of atrial natriuretic factor (ANF) on the hypertension induced by chronic infusion of vasopressin (VP) in conscious rats on regular diets (panel a) and on sodium loading with 1% NaCl as a drinking water (panel b). Daily systolic blood pressure in rats infused with VP at a rate of 7.2 U/kg/day alone (●) or in combination with 150 µg/kg/day of ANF (●), and with vehicle alone (○). Results are means ± SEM. Analysis of variance for repeated measurements revealed a significant change in systolic blood pressure in rats given VP and ANF compared to that in rats given VP alone on a regular diet (p < 0.01) and on sodium loading (p < 0.01).

* p < 0.05 and † p < 0.01 compared to values in rats given vehicle alone.

preparation and solution recovered from the minipump.

Arginine-vasopressin (VP) (Protein Research Foundation, Osaka, Japan) dissolved in 0.01 N acetic acid was intraperitoneally delivered via another osmotic minipump placed in the abdominal cavity through a midline incision, which was then closed with autoclips. Assuming that VP did not degrade during the study, and the pumps dispensed fluid at the specified rate of approximately 1 µl/hour, the infusion dose (7.2 U/kg/day) was chosen to be sufficient to induce small but significant elevation of blood pressure in conscious rats. In the previous study, it had also been shown that chronic infusion of this range of VP induced a sustained increase in systolic blood pressure and a marked decrease in urine volume in conscious rats.

Systolic blood pressure in the rats was recorded daily by an indirect tail cuff method without anesthesia. Daily fluid intake, urine volume and urinary sodium excretion were determined.

All results were expressed as mean ± SEM. Statistical analysis of the data between groups was performed by two-way analysis of variance for repeated measurements. Statistically significant differences on each day were isolated by the unpaired t-test between the groups.

RESULTS

Initial parameters of each group of rats, systolic blood pressure, body weight, fluid intake, urine volume and urinary sodium excretion, before the infusion of VP alone or in combination with ANF, and of vehicle alone as a control on regular diet or under sodium loading with 1% NaCl were not significantly different among the groups (Table I).

As shown in Fig. 1, the systolic blood pressures of the VP alone group began to rise significantly on the 1st day of the infusion and remained high up to the 3rd day on a regular diet (p < 0.01) or sodium loading with 1% NaCl (p < 0.01), whereas those of vehicle did not change. When 150 µg/kg/day of ANF was administered simultaneously with 7.2 U/kg/day of VP, the elevation of systolic blood pressure was completely inhibited. The antihypertensive effect of this peptide was sustained throughout the experimental period of 3 days in rats on a regular diet.
Fig. 2. Effect of atrial natriuretic factor (ANF) on urine volume in the vasopressin (VP)-infused rats on regular diets (panel a) and on sodium loading with 1% NaCl as a drinking water (panel b). Daily urine volume in rats infused with VP at a rate of 7.2 U/kg/day alone (hatched column) or in combination with 150 μg/kg/day of ANF (close column) and with vehicle alone (open column). Analysis of variance for repeated measurements revealed no significant change in urine volume in rats given VP and AVF compared to that in rats given VP alone on both sodium conditions.

*p < 0.05 and †p < 0.01 compared to values in rats given vehicle alone.

(p < 0.01) or on sodium loading with 1% NaCl (p < 0.01) compared to the rise when VP alone was infused on both sodium conditions. The blood pressure of the rats given VP with 150 μg/kg/day of ANF was not significantly different from those of the control rats given the vehicle on both sodium conditions. The withdrawal of ANF after the simultaneous infusion of ANF and VP for 3 days caused the blood pressures to elevate to the levels in the VP alone-group on both sodium conditions.

The antihypertensive effect of ANF in VP-infused rats on a regular diet or under sodium loading with 1% NaCl was not associated with any significant changes in urine volume and urinary sodium excretion. As shown in Fig. 2, the infusion of VP induced a marked and significant decrease in urine volume on both sodium conditions when compared to that of the control rats given the vehicle alone. However, the infusion of VP in combination with ANF could not induce any further change in urine volume on both sodium conditions when compared to that of VP-infused rats.

DISCUSSION

We have previously shown that continuous infusion of a subdepressor dose of synthetic ANF of 25 amino-acid residues abolished completely the hypertensive effect of chronic infusion of norepinephrine and that the antihypertensive effect was independent of renal effects of this peptide. In the present study, we demonstrated

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that the administration of the same dose of synthetic ANF employed in our previous study also reduced blood pressure to control levels in the VP-infused rats on a regular intake of sodium or under sodium loading with 1% NaCl as drinking water. The antihypertensive effect of ANF in the VP-infused rats on both sodium conditions was not accompanied by any increase in natriuresis or diuresis, which suggest again, as in our previous report, that the marked reduction of blood pressure is not secondary to the renal effects of this peptide. In addition, it is interesting to note that chronically administered ANF did not induce any change in urine volume in the VP-infused rats, whereas the administration of VP caused a significant increase in body weight due to the volume expansion associated with the decrease in urine volume.10

Since the first report made by DeBold et al.,2 several studies have demonstrated the blood pressure reducing effect of ANF. However, most of the results that implicate a significant role of ANF in the regulation of blood pressure are obtained from short-term studies on anesthetized animals subjected to surgical stress or from those using pharmacological doses of ANF and may not be applicable to more physiological settings. To our knowledge, the antihypertensive effect of the subdepressor dose of ANF in rats made hypertensive by chronic infusion of VP has not been previously reported, although there have been a few reports to assess chronic effects of ANF in experimental models of hypertension.3-16 The suppression of VP-induced hypertension by ANF was not due to tachyphylaxis, since VP induced a sustained increase in systolic blood pressure in the absence of ANF.

The mechanism(s) by which ANF blocks the hypertension caused by chronic infusion of VP cannot be explained by the present set of experiment. Although the substance has natriuretic and diuretic action, we could not show any significant changes in urine volume and urinary sodium excretion in the VP-infused rats during the infusion of ANF on both sodium conditions. Therefore, it is unlikely that the antihypertensive effect of ANF is due to the loss of water and sodium. Recently, Koike et al.17 showed in conscious spontaneously hypertensive rats (SHR) 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 suggested that the hypotensive response to chronic infusion of ANF at the same dose range or less used in the present study may be, in part, due to vasodilation in experimental models of hypertension in conscious rats.3-15 Therefore, it is tempting to speculate that ANF antagonized the vascular effects of vasopressin, which may be consistent with previous studies in vitro.4,5 Manning et al.6 also reported that pharmacological levels of VP stimulated the cardiac endocrine system to release atriopeptin, a family of ANF, which may cause diuresis and vasodilation to antagonize the effects of VP. Given the antihypertensive effect of ANF in the VP-infused rats together with that in the NE-infused rats in the previous study,9 it is suggested that ANF interferes with intracellular calcium mobilization and/or some metabolic process causing vasoconstriction. It has also been reported that cyclic guanosine monophosphate (cGMP) is involved in the mechanisms of ANF action, because the in vivo injection of ANF increases urinary excretion and plasma levels of cGMP, and in vitro incubation of ANF with rat kidney homogenate induced an increase in tissue levels of cGMP and a decrease in cGMP phosphodiesterase.18 In support of this view, we also found that a synthetic ANF of 25 amino-acid residues increased the levels of cGMP in vascular smooth muscle cells from rat mesenteric arteries in culture, whereas it did not stimulate the biosynthesis of prostacyclin (unpublished observations). However, the exact mechanism of the effect of ANF on vascular smooth muscle has not been fully explained.

Another possible explanation for the antihypertensive effect of ANF on the hypertension induced by VP is a non-specific action of ANF to the vascular smooth muscle, induced by its unphysiological levels of ANF. However, its actual physiological levels are not yet known, and unfortunately we did not measure the circulating levels of ANF in the present study.

In conclusion, the present finding that continuous infusion of a subdepressor dose of synthetic ANF inhibits the hypertension caused by chronic infusion of VP, suggests that ANF is involved in the physiological regulation of blood pressure. However, its actual physiological relevance remains to be clarified.

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