Pressor Hormones Regulate Atrial-Stretch-Induced Release of Atrial Natriuretic Peptide in the Pithed Rat

Heikki Ruskoaho, Olli Vakkuri, Olli Arjamaa, Olli Vuolteenaho, and Juhani Leppäläluoto

Atrial wall stretching is a known stimulus for atrial natriuretic peptide (ANP) secretion. The effects of the stimulation of autonomic nervous system, hemodynamic factors, and humoral factors (epinephrine, angiotensin, vasopressin, and brain extracts) on the release of ANP under basal conditions and during increased atrial pressure produced by acute volume loading in pithed rats were examined. In conscious rats, acute volume expansion by 0.9% of saline (4 ml) increased the plasma immunoreactive ANP (IR-ANP) concentrations by a factor of 4 (140±30 pg/ml vs. 521±140 pg/ml, p<0.001, n=8), whereas volume-induced ANP release was blocked in pithed rats (75±9 pg/ml vs. 99±13 pg/ml, NS, n=7). The ANP versus right atrial pressure curve shifted to the right, indicating that much smaller amounts of IR-ANP were released in pithed than in conscious rats for each given increase in right atrial pressure. Electrical vagal and sympathetic nerve stimulations or changes in heart rate had no effect on plasma IR-ANP concentrations and failed to restore the volume-load-induced release of ANP in pithed rats. When extracts of anterior pituitary lobe, brain cortex, or hypothalamus were infused, no effect on volume-expansion-induced plasma IR-ANP levels was seen. In contrast, acute volume expansion caused a fourfold increase in levels of circulating IR-ANP in pithed rats that received posterior pituitary extracts, and the ANP versus right atrial pressure curve shifted markedly to the left. Infusion of a V1 antagonist blocked the volume-expansion-induced ANP release produced by the posterior pituitary extract. When [Arg8]-vasopressin (0.025 or 0.05 µg/kg/min) was infused to pithed rats, mean arterial pressure increased but basal plasma IR-ANP did not change significantly. However, acute volume expansion in the presence of vasopressin infusion (0.05 µg/kg/min) increased the amount of circulating IR-ANP by a factor of 4 (113±14 pg/ml vs. 414±43 pg/ml, p<0.001, n=8). Thus, for a given increase in right atrial pressure, a similar amount of IR-ANP was released in the pithed rat during the vasopressin infusion as in the normal conscious animal. V1 antagonist blocked the increase in mean arterial pressure as well as the increase of plasma IR-ANP produced by [Arg8]-vasopressin. In addition, volume expansion during intravenous epinephrine (1.75 µg/kg/min) and angiotensin (1.0 µg/kg/min) doubled plasma IR-ANP levels. These results indicate that pressor hormones, especially vasopressin, restore the ability of volume expansion to induce ANP release in the pithed rat. The combined effect of right atrial pressure and pressor hormones in regulating ANP release is a new mechanism by which humoral stimulation modulates the direct, mechanical-stimulus-induced hormone secretion. (Circulation Research 1989;64:482-492)
studies correlated with the increase in atrial pressure or other hemodynamic variables or they were not measured, the role of pressor substances in the control of ANP release remains unclear.

In this study, the effect of the parasympathetic and sympathetic nervous systems, hemodynamic factors (mean arterial pressure, heart rate), and humoral factors (epinephrine, angiotensin, vasopressin, and brain extracts) on basal and atrial-stretch-mediated ANP release in the pithed rat was examined. Pithing, which removes both sympathetic and parasympathetic control of the heart and interrupts humoral influences of the brain and pituitary gland on the heart, blocks volume-induced release of ANP.

Materials and Methods

Male Wistar rats, weighing 240–320 g, from the colony of the Department of Pharmacology at the University of Oulu, Oulu, Finland, were used. The strain was originally obtained from the National Laboratory Animal Center, University of Kuopio, Kuopio, Finland. The rats were housed in plastic cages in a room with controlled humidity and a temperature of 22°C. A 6 PM to 6 AM dark environmental cycle was maintained. The experimental protocols were approved by the Committee of Animal Experimentation of the University of Oulu.

Synthetic rat ANP, angiotensin II acetate (human form), [Arg8]-vasopressin (AVP), and deamino-pentahexapeptide vasopressin (dVDAVP) were obtained from Sigma Chemical Co, St. Louis, Missouri. Adrenaline hydrochloride (epinephrine) was from Fluka AG (Busch SG, Switzerland), methoxamine hydrochloride was from The Wellcome Foundation Ltd (London, UK), d(CH2)4(Tyr(Me))AVP was from Peninsula Laboratories Inc (Belmont, California), and heparin was from Medica (Helsinki, Finland). Other chemicals were from Sigma.

Chronically Instrumented Rats

The surgical preparation and experimental setup have been described previously. Briefly, rats were anesthetized with chloral hydrate (30 mg/kg i.p.), the left femoral artery was cannulated for recording blood pressure and heart rate, and the left femoral vein was cannulated for volume expansion. A cannula was inserted into the right atrium via the jugular vein for measuring right atrial pressure. The day after the operation, the arterial and right atrial catheters were attached to pressure transducers (Micron Instruments Inc, model MP-15, Los Angeles, California) and a polygraph (Grass Instruments, model 7D, Quincy, Massachusetts) for mean arterial pressure (MAP), heart rate, and right atrial pressure recording. The venous catheter was connected to a syringe for saline infusion. After an equilibration period (30 minutes), hemodynamic variables were recorded in the conscious animals for 30 minutes. The rats were then given 4 ml of 0.9% saline intravenously over 1 minute. Arterial blood samples were taken 15 minutes before and 1 minute after the saline infusion. Each blood sample taken was replaced by an equal volume of saline. Preliminary experiments showed that there was no difference in plasma immunoreactive (IR)-ANP between the two samples.

Pithed Rats

To avoid any influence of anesthesia during the experiments on volume-expansion-induced ANP release, animals were anesthetized with diethyl ether for the pithing. The tracheae were cannulated, and the rats then pithed by passing a steel rod (2.1 mm in diameter) through the orbit and foramen magnum down into the spinal canal. Immediately after pithing, the trachea cannula was connected to a rodent respirator (model 681, Harvard Apparatus, Millis, Massachusetts), and the rats were artificially ventilated with moistened oxygen mixed with 5% CO2 at a frequency of 70 cycles/min, 10 ml/kg. To establish correlations between cardiovascular parameters and ANP release, MAP, heart rate, and right atrial pressure were measured continuously throughout the experiments as described above for conscious rats. The left femoral vein was cannulated for volume expansion and the intravenous administration of drugs. The preparation was allowed to stabilize for at least 30 minutes before the experiments.

The investigations in pithed rats consisted of three protocols (Figure 1). In Protocol 1, after an equilibration period, 4 or 7 ml of 0.9% saline intravenously were given over 1 minute. Arterial blood samples for plasma IR-ANP were taken 5 minutes before and 1 minute after the
before and 1 minute after volume expansion. One group of rats did not receive the volume load.

Protocol 2 consisted of nerve stimulations or drug administrations. To stimulate autonomic parasympathetic nerves, both vagus nerves were exposed in the neck and ligated. A platinum electrode was applied to the left vagus distal to the ligature. Impulses of 2 msec at 5 V and 5 Hz were delivered with a stimulator (model 345, Harvard Apparatus) for 22 minutes. To stimulate sympathetic nerves, the pithing rod was placed at the origin of the sympathetic preganglionic nerves and the stimulator was used to deliver a strain of 20 impulses (0.3 msec, 0.5 Hz, 50 V) through the uncovered tip, causing a tachycardia of 90–150 beats/min for 22 minutes. Drugs were administered intravenously as a continuous infusion via an infusion pump (B. Braun PerfusorED, Braun Melsungen AG, FRG) using a rate of 33 μl/min for 22 minutes. Finally, one group of rats received vehicle (0.9% saline) infusion. 

Preparation of Pituitary and Brain Extracts

Dawley rats (200-250 g) were removed. Anterior (100 mg) and posterior lobes of pituitaries were carefully prepared into two pools in an ice bath. Anterior (100 mg) and posterior pituitary (15 mg) pools in 2 ml of phosphate buffer saline (0.02 M phosphate, 0.15 M NaCl, pH 7.4) at +4°C were separated by double antibody in the presence of 0.5 ml 1.2 M (NH3)SO4, pH 7. The sensitivity of the assay was 1 pg/tube. The intra-assay and interassay coefficients were 5% and 14%, respectively. The ANP values were not corrected for recovery.

Hypophysectomized Rats

The rats were anesthetized with phentanyl fluanisone (0.2 ml/100 g i.m., Hypnorm, Janssen, Oxford, England) and hypophysectomized by a parapharyngeal approach. After the surgery, the animals were allowed to recover at the ambient temperature of 28°C for 2 weeks. Their drinking water contained 2.5 μg/ml dexamethasone for the first 2 days. The hypophysectomized rats were prepared for measurements of blood pressure, heart rate, and right atrial pressure and for volume expansion in conscious animals as described above.

On the day of the experiment, 14 days after hypophysectomy, blood pressure, heart rate, and right atrial pressure were recorded for 30 minutes. Next, vehicle or [Arg9]-vasopressin was infused as a continuous infusion via the infusion pump (B. Braun PerfusorED) using a rate of 33 μl/min for 22 minutes. Volume expansion with 0.9% saline (4 ml) over 1 minute was done 20 minutes after the start of vehicle or vasopressin infusion. Arterial blood samples were taken 5 minutes before and 1 minute after the volume expansion.

Assay of Immunoreactive Atrial Natriuretic Peptide in Plasma

All blood samples (0.8 ml) were taken into precooled EDTA tubes on ice, immediately centrifuged at 2,000g for 10 minutes at +4°C, and the plasma was stored at −20°C until assayed by radioimmunoassay. ANP was extracted by Sep-pak C18 cartridges from plasma as described previously. The recovery of added rat ANP1-28 (50 pg) was 81.7 ± 3.3% (mean ± SEM, n = 6). The ANP values were not corrected for recovery.

The plasma extracts were diluted with the radioimmunoassay buffer and incubated in duplicates of 100 μl, with the same volume of the middle specific rabbit ANP antiserum at the final dilution of 1/25,000. Synthetic rat ANP1-28 (Sigma) ranging from 0 to 1,250 pg/tube were used as standards. The ANP tracer was rat [125I]ANP1-28 from Amersham, Buckinghamshire, England. After incubation for 20–44 hours at +4°C, the bound and free fractions were separated by double antibody in the presence of 0.5 ml 1.2 M (NH4)2SO4, pH 7. The sensitivity of the assay was 1 pg/tube. The intra-assay and interassay coefficients were 5% and 14%, respectively. The rat plasma extracts diluted serially displaced [125I]ANP parallel with the synthetic ANP1-28 standard and eluted (over 90%) in the reverse phase high performance liquid chromatography as rat ANP1-28.

Data Analysis

The results are expressed as mean ± SEM. For the comparison of statistical significance between two dependent groups, Student’s t test for paired data was used. For the multiple comparison, one-way ANOVA followed by the Bonferroni t test
Results

Protocol 1: Effect of Volume Expansion on Plasma ANP and Hemodynamics in Conscious and Pithed Rats

The plasma IR-ANP levels before volume expansion were lower in pithed rats (79±5 pg/ml, n=22) than in conscious rats (140±30 pg/ml, n=8, p<0.01) (Figure 2). Basal IR-ANP levels in these pithed rats were similar to those reported earlier. 313237 MAP was lower in pithed rats than in conscious rats, but their heart rates and right atrial pressures did not differ significantly (Table 1). In conscious animals with indwelling catheters, acute blood volume expansion with 0.9% saline increased the plasma concentration of IR-ANP by a factor of 4 (Figure 2). This volume-induced (4 or 7 ml saline) release of ANP was completely blocked by pithing (Figure 2). Volume expansion with 0.9% saline resulted in increases in right atrial pressure and MAP in both conscious and pithed rats while heart rates remained essentially unchanged (Table 1). The ANP versus right atrial pressure curve shifted to the right in the pithed rat; that is, smaller amounts of IR-ANP were released in pithed than in conscious rats for each given increase of right atrial pressure (Figure 2).

Protocol 2: Effect of Nerve Stimulation and Drug Infusions on Plasma ANP and Hemodynamics in Pithed Rats

During nerve stimulations and drug infusions in pithed rats, slight but insignificant changes (5–32 pg/ml) in plasma levels of IR-ANP were noted (Table 2). In these experimental procedures, plasma IR-ANP concentrations did not reach the values found in chronically instrumented intact rats (Figure 2). A 22-minute electrical vagal stimulation in pithed rats decreased heart rate significantly but had no effect on MAP, right atrial pressure, or plasma IR-ANP concentration (Table 2). Stimulation of preganglionic sympathetic outflow increased heart rate whereas right atrial pressure, MAP, and plasma IR-ANP remained unchanged (Table 2).

Intravenous infusions of epinephrine (1.5 μg/kg/min) in the pithed rats for 22 minutes produced significant increases in MAP, heart rate, and right atrial pressure, but plasma IR-ANP was unaffected (Table 2). Infusion of [Arg³]-vasopressin (0.05 μg/kg/min) alone for 22 minutes increased MAP but had no significant effect on right atrial pressure, heart rate, or plasma IR-ANP concentration. When the V₁ antagonist d(CH₂)₅(Tyr(Me)₂)AVP was infused (0.5 μg/kg/min), no effect was seen on hemodynamic variables or plasma IR-ANP levels (Table 2). Administering angiotensin II (0.5 μg/kg/min) alone for 22 minutes increased MAP but had no significant effect on right atrial pressure, heart rate, or plasma IR-ANP concentration (Table 2).

Protocol 3: Effect of Volume Expansion on Plasma ANP and Hemodynamics During Various Experimental Manipulations in Pithed Rats

During vagus and preganglionic sympathetic nerve stimulations, changes in all hemodynamic variables were the same as seen in Protocol 2. A significant decrease in heart rate in response to vagus stimulation and an increase in response to sympathetic nerve stimulation were noted, and MAP and right atrial pressure were unaffected (Table 3). Volume infusion superimposed on vagus and sympathetic nerve stimulation at time=20, resulting in an elevation in right atrial pressure (Table 3), had no significant effect on plasma IR-ANP concentrations (Figure 3).

Intravenous administration of extracts of anterior pituitary, brain cortex, or hypothalamus had no hemodynamic effects in pithed rats, whereas the infusion of posterior pituitary extracts increased MAP significantly (Table 3). No significant effect on ANP release in response to acute volume expansion, resulting in an increase in right atrial pressure, was observed in the pithed rats infused with ante-
rior pituitary extract or with extracts of brain cortex and hypothalamus (Figure 3). In contrast, volume expansion interposed at time = 20 during the infusion of posterior pituitary extract resulted in a significant increase in plasma IR-ANP concentration (Figure 3). The relation between changes from control in plasma IR-ANP and right atrial pressure in response to volume expansion shifted to the left in the presence of posterior pituitary extract. When V₁ antagonist was infused with extracts of posterior pituitary lobe, the pressor effect and restoration of volume-induced ANP release by posterior pituitary extract were completely blocked, despite a comparable increase in right atrial pressure in response to volume expansion (Table 3, Figure 3).

The pressor agents angiotensin II, epinephrine, methoxamine, and [Arg⁸]-vasopressin all produced dose-dependent increases in MAP (Table 3). The maximum increment of MAP was similar for the three pressor hormones. Epinephrine infusion significantly increased heart rate and right atrial pressure, and infusion of [Arg⁸]-vasopressin (0.05 μg/kg/min) increased right atrial pressure in the pithed rats. During the administration of angiotensin II and [Arg⁸]-vasopressin, plasma IR-ANP levels tended to be elevated (Figure 3) as seen in Protocol 2 (Table 2) and reported previously by Haass et al.38 Acute saline infusion in the pithed rat during the intravenous administration of [Arg⁸]-vasopressin markedly increased plasma IR-ANP levels and the ANP versus right atrial pressure curve shifted dose-dependently to the right (Figure 3). When [Arg⁸]-vasopressin was infused together with V₁ antagonist, the pressor effect of [Arg⁸]-vasopressin and the ANP response to volume expansion were blocked (Figure 3). Infusion of V₂ agonist, dVDAVP, had no hemodynamic effect, and volume expansion in the presence of V₂ agonist infusion increased right atrial pressure but did not affect plasma IR-ANP (Figure 3).

To determine if volume expansion produces the release of ANP after discontinuing vasopressin treatment, [Arg⁸]-vasopressin (0.05 μg/kg/min) was infused for 22 minutes and the volume expansion was done 30 minutes after the end of infusion. In these experiments, during [Arg⁸]-infusion MAP increased from 57±2 to 141±4 mm Hg (p<0.001, n=8) and returned to the preinfusion levels within 30 minutes (Table 3). When volume expansion was superimposed at 30 minutes after the discontinuation of [Arg⁸]-vasopressin, the restoration of volume-

### Table 1. Mean Arterial Pressure (MAP), Heart Rate (HR), and Right Atrial Pressure (RAP) Before and After Volume Expansion in Conscious and Pithed Rats

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>RAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Conscious rats</td>
<td>4 ml saline</td>
<td>8</td>
<td>114±6</td>
<td>126±5</td>
</tr>
<tr>
<td></td>
<td>Pithed rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No load</td>
<td>8</td>
<td>59±3</td>
<td>59±3</td>
<td>371±11</td>
</tr>
<tr>
<td>4 ml saline</td>
<td>7</td>
<td>62±4</td>
<td>73±6*</td>
<td>325±10</td>
</tr>
<tr>
<td>7 ml saline</td>
<td>7</td>
<td>58±3</td>
<td>82±4*</td>
<td>334±16</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SEM; n refers to number of animals in each group. Hemodynamic measurements were taken immediately before and 1 minute after the intravenous infusion of 0.9% saline (see Figure 1, Protocol 1).

### Table 2. Effect of Various Experimental Manipulations on Mean Arterial Pressure (MAP), Heart Rate (HR), Right Atrial Pressure (RAP), and Plasma Immunoreactive Atrial Natriuretic Peptide (IR-ANP) in Pithed Rats

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>RAP (mm Hg)</th>
<th>IR-ANP (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Vehicle</td>
<td>7</td>
<td>53±3</td>
<td>49±1</td>
<td>307±8</td>
<td>305±8</td>
</tr>
<tr>
<td>Vagus stimulation</td>
<td>9</td>
<td>53±3</td>
<td>51±3</td>
<td>324±10</td>
<td>259±8*</td>
</tr>
<tr>
<td>Sympathetic stimulation</td>
<td>8</td>
<td>49±3</td>
<td>44±2</td>
<td>320±8</td>
<td>455±9*</td>
</tr>
<tr>
<td>Epi 1.5 μg/kg/min</td>
<td>7</td>
<td>52±2</td>
<td>109±2*</td>
<td>332±8</td>
<td>481±21*</td>
</tr>
<tr>
<td>AVP 0.05 μg/kg/min</td>
<td>8</td>
<td>61±2</td>
<td>144±6*</td>
<td>333±6</td>
<td>350±9</td>
</tr>
<tr>
<td>d(CH₂)₅(Tyr(Me))₂AVP</td>
<td>6</td>
<td>63±4</td>
<td>68±4</td>
<td>316±12</td>
<td>308±14</td>
</tr>
<tr>
<td>Ang 0.5 μg/kg/min</td>
<td>5</td>
<td>50±3</td>
<td>107±9*</td>
<td>340±12</td>
<td>348±13</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SEM; n refers to number of animals in each group. Epi, epinephrine; AVP, [Arg⁸]-vasopressin; Ang, angiotensin II; d(CH₂)₅(Tyr(Me))₂AVP, V₁ antagonist 0.5 μg/kg/min. Hemodynamic measurements were taken immediately before and 1 minute after the start of experiments. Blood samples were taken 5 minutes before and 22 minutes after the start of experiments (see Figure 1, Protocol 2).

*p<0.001 after vs. before (paired t test).

f <p<0.01.
induced ANP release induced by the infusion of vasopressin was markedly attenuated, despite comparable increase in right atrial pressure in response to volume expansion (Table 3, Figure 3). The relation between changes from control in plasma IR-ANP and right atrial pressure in response to volume expansion shifted markedly back to the left (Figure 3).

Volume expansion plus the infusion of higher doses of epinephrine and angiotensin increased right atrial pressure (Table 3) and doubled plasma IR-ANP concentration (165±24 pg/ml vs. 182±25 pg/ml, n=9, paired t test).

**Discussion**

In conscious animals, volume expansion with 0.9% saline resulted in an increase in right atrial pressure and plasma concentration of IR-ANP, whereas highly significant elevations in right atrial pressure in response to volume loading were incapable of eliciting the ANP response in the pithed rats. This observation is consistent with data presented by Zamir et al.31,32 in halothane- and pentobarbital-anesthetized pithed rats. Moreover, analysis of changes in plasma IR-ANP concentration against change in right atrial pressure demonstrate a dissociation between ANP release and atrial pressure elevations in the pithed rats in keeping with the hypothesis that atrial stretch is the primary stimulus for ANP secretion.39 The inability of increased atrial pressure to cause release of ANP in

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**Table 3. Effect of Volume Expansion on Mean Arterial Pressure (MAP), Heart Rate (HR), and Right Atrial Pressure (RAP) in Pithed Rats During Various Experimental Manipulations**

<table>
<thead>
<tr>
<th></th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>RAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Basal</td>
<td>Before</td>
</tr>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>56±2</td>
<td>56±2</td>
</tr>
<tr>
<td>Ang 0.5 µg/kg/min</td>
<td>6</td>
<td>54±2</td>
<td>100±2</td>
</tr>
<tr>
<td>Ang 1.0 µg/kg/min</td>
<td>6</td>
<td>59±1</td>
<td>127±4</td>
</tr>
<tr>
<td>Epi 1.0 µg/kg/min</td>
<td>6</td>
<td>56±3</td>
<td>108±1</td>
</tr>
<tr>
<td>Epi 1.75 µg/kg/min</td>
<td>6</td>
<td>60±1</td>
<td>134±3†</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>8</td>
<td>57±3</td>
<td>119±8†</td>
</tr>
<tr>
<td>Vagus stimulation</td>
<td>9</td>
<td>62±2</td>
<td>57±2</td>
</tr>
<tr>
<td>Sympathetic stimulation</td>
<td>9</td>
<td>61±3</td>
<td>73±5</td>
</tr>
<tr>
<td>Posterior lobe</td>
<td>9</td>
<td>62±3</td>
<td>135±6†</td>
</tr>
<tr>
<td>Posterior lobe + d(CH2)5(Tyr(Me))AVP</td>
<td>6</td>
<td>59±2</td>
<td>66±2†</td>
</tr>
<tr>
<td>Anterior lobe</td>
<td>6</td>
<td>55±1</td>
<td>57±1</td>
</tr>
<tr>
<td>Cortex</td>
<td>6</td>
<td>57±2</td>
<td>60±2</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>6</td>
<td>56±2</td>
<td>52±2</td>
</tr>
<tr>
<td>AVP 0.025 µg/kg/min</td>
<td>7</td>
<td>56±2</td>
<td>105±5</td>
</tr>
<tr>
<td>AVP 0.05 µg/kg/min</td>
<td>8</td>
<td>56±1</td>
<td>136±6†</td>
</tr>
<tr>
<td>AVP 0.05 µg/kg/min + d(CH2)5(Tyr(Me))AVP</td>
<td>7</td>
<td>57±2</td>
<td>61±2</td>
</tr>
<tr>
<td>AVP 0.05 µg disc.</td>
<td>8</td>
<td>57±2</td>
<td>55±3</td>
</tr>
<tr>
<td>dVDAVP 0.5 µg/kg/min</td>
<td>7</td>
<td>65±2</td>
<td>65±3</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SEM; n is number of animals in each group. Epi, epinephrine; AVP, [Arg²]-vasopressin; Ang, angiotensin II; d(CH2)5(Tyr(Me))AVP, V¹ antagonist 0.5 µg/kg/min; methoxamine 30 µg/kg/min; AVP 0.05 µg disc., infusion of 0.05 µg/kg/min of AVP discontinued 30 minutes before volume expansion; dVDAVP=V¹ agonist. Hemodynamic measurements were taken immediately before the start of experiments (=basal) and 5 minutes before and 1 minute after the intravenous infusion of 0.9% saline (see Figure 1, Protocol 3).

*tp<0.001 before vs. basal or after vs. before (paired t test).
†tp<0.01.
‡tp<0.05.

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Results from previous in vitro experiments suggest that the autonomic nervous system may influence ANP secretion. Alpha- and/or β-adrenergic agonists stimulate ANP secretion from isolated perfused rat heart,17,18 paced left atria,20 or isolated atrial cells.19 The muscarinic cholinergic agonists have been reported to increase15-17 or inhibit20 ANP secretion in vitro. To investigate the role of the nervous system on atrial-stretch-induced ANP release, we studied the effects of sympathetic and parasympathetic nerve stimulation on the capacity of the heart to release ANP in response to volume expansion in pithed rats. As clearly demonstrated in each experiment, the nerve stimulations produced expected and reproducible hemodynamic alterations that in no case resulted in an ANP response. These experiments suggest that the autonomic nervous system does not seem to modulate the ANP secretory response to acute volume expansion in vivo. Our results are consistent with those of Rankin and colleagues28 showing that parasympathetic and sympathetic nerve stimulation has no significant effect on ANP secretion in the anesthetized dog.
Further, sympathetic or parasympathetic nervous system blockade, by surgical or pharmacological means, did not affect the acute volume-expansion-induced ANP release. Because the in vivo results of the influence of the autonomic nervous system on ANP secretion do not support the in vitro findings, the potential role of the autonomic nervous system in modulating ANP secretion remains to be determined.

Recent results suggest that ventricular ANP in addition to atrial ANP responds to volume and pressure overload and may contribute to the plasma ANP levels produced by volume expansion. The decreased MAP in the pithed rat, through reduced ventricular stretch, could contribute to the blockade of stimulated ANP release in these animals. This is unlikely, however, since methoxamine increased MAP to the level found in conscious rats but had no effect on volume-expansion–induced ANP release. Our results show that neither alterations in heart rate induced by nerve stimulation nor increases in MAP produced by methoxamine infusion were associated with any change in plasma IR-ANP levels in response to volume expansion.

The inability of the autonomic nervous system or hemodynamic factors to restore the volume-expansion–induced release of ANP led us to seek a humoral mechanism regulating atrial-stretch–induced ANP release. Recent observations that plasma concentrations of IR-ANP are decreased after hypophysectomy together with our finding that acute volume loading, despite an increase in right atrial pressure, had no effect on plasma IR-ANP levels in hypophysectomized animals suggest that the pituitary gland has a regulatory role in ANP release.

We therefore studied the effect of the infusion of anterior and posterior lobe extracts on basal and volume-expansion–induced plasma IR-ANP levels in the pithed rat and compared these with those observed after the infusion of extracts of brain cortex and hypothalamus. Infusion of posterior pituitary extracts increased MAP while infusion of anterior pituitary, brain cortex, and hypothalamic extracts had no hemodynamic effects. In subsequent experiments, we infused the amount of posterior pituitary extract that had previously raised the MAP in pithed rats to that found in conscious rats. Acute volume expansion caused a fourfold increase in levels of circulating IR-ANP in rats that received posterior pituitary extracts, and the ANP versus right atrial pressure curve shifted markedly to the left. Thus, for a given increase in right atrial pressure, a similar amount of IR-ANP was released in the pithed rat during the posterior pituitary extract infusion as was released in the normal conscious animal. No significant effect on ANP release in response to acute volume expansion was observed in the pithed rats infused with extracts of anterior pituitary, brain cortex, or hypothalamus. Our results show that extracts of posterior pituitary gland contain one or more factors that restore, in the pithed rat, the heart's ability to release ANP in response to acute volume expansion.

Recently, Zamir et al reported that basal and stimulated ANP release in hypophysectomized rats were restored (10 days posthypophysectomy) when the resected anterior pituitary was reimplanted under the renal capsule. Our results do not support these findings, however, perhaps because of differences in the experimental models used and the types of experiments performed (i.e., acute versus long-term studies).

The posterior pituitary secretes two established hormones, oxytocin and vasopressin. Arginine vasopressin plays a central role in the regulation of water balance through its action on the renal tubular V2 receptors. The vasoconstrictor effects of this hormone and its other actions, such as effects on glycogenolysis and platelet aggregation, are mediated via vascular V1 receptors. The fact that the posterior pituitary gland extract, which had a pressor effect, increased ANP release suggests that this effect may be due to arginine vasopressin. We investigated this possibility by infusing a selective V1-receptor antagonist d(CH2)5(Tyr(Me)²)AVP together with posterior pituitary extract to assess whether ANP release was blocked. When the V1 antagonist was infused alone at the concentrations that blocked the pressor effect of arginine vasopressin, no effect was seen on plasma IR-ANP concentrations, MAP, heart rate, or right atrial pressure. However, infusion of the V1-receptor antagonist blocked both the pressure increase and restoration of volume-expansion–induced ANP release produced by the infusion of posterior pituitary gland extract in pithed rats. Thus, arginine vasopressin appears to be the factor in posterior pituitary extracts that restores the atrial-stretch–induced release of ANP in pithed rats. Further, no evidence for brain ANP-releasing or inhibiting factor(s) other than arginine vasopressin was found in our present experiments.

To investigate whether synthetic vasopressin has an effect similar to that of the posterior pituitary gland extract, we examined the effect of intravenous [Arg⁸]-vasopressin on basal and volume-expansion–induced plasma IR-ANP levels in pithed rats. Infusion of [Arg⁸]-vasopressin alone increased MAP but had no significant effect on right atrial pressure, heart rate, or plasma IR-ANP levels. However, acute volume expansion during [Arg⁸]-vasopressin infusion resulted in a dose-dependent increase in the amount of circulating IR-ANP; and as observed during the infusion of posterior pituitary extract, the ANP versus right atrial pressure curve shifted markedly to the left. When [Arg⁸]-vasopressin infusion was discontinued, the restoration of volume-expansion–induced ANP release was markedly attenuated. Thus, atrial stretching, which is a potent stimulus for ANP release, seems to require vasopressin for the induction of ANP secretion in the pithed rat.
We have further investigated the type of vasopressin receptors involved in the regulation of stretch-mediated ANP release by studying the effects of the selective V₁-receptor antagonist d(CH₂)₅Tyr(Me)₂ AVP and a selective V₂-receptor agonist dVDAVP on volume-load–stimulated IR-ANP release in pithed rats. V₁ antagonist blocked the increase in MAP produced by [Arg⁸]-vasopressin as well as the increase of plasma IR-ANP levels in response to volume load observed during AVP infusion. The right atrial pressure increased normally and the ANP versus right atrial pressure curve shifted back to the right. In contrast, the administration of V₂-receptor agonist had no effect on MAP, heart rate, right atrial pressure, or plasma IR-ANP concentration in response to volume expansion. These experiments suggest that vasopressin regulates atrial-stretch–mediated ANP release in the pithed rat by stimulating V₁ receptors.

The role of vasopressin on ANP release was further studied in hypophysectomized, conscious rats, 14 days after surgery, by the infusion of AVP to assess its effect on basal and stimulated circulating IR-ANP levels. We have previously shown that acute volume expansion has no significant effect on plasma IR-ANP levels in hypophysectomized rats, and the ANP versus right atrial pressure curve was markedly shifted to the right in these animals. In the present study, we report that [Arg⁸]-vasopressin infusion removed the blockade of stimulated release of ANP in hypophysectomized rats. A 20-minute infusion of [Arg⁸]-vasopressin in conscious hypophysectomized rats with indwelling catheters increased MAP, and acute volume expansion in the presence of vasopressin increased the amount of circulating IR-ANP. These results support our findings that AVP regulates atrial-stretch–mediated ANP release.

To examine whether other pressor hormones have effects similar to those of vasopressin, we studied the effect of infusions of epinephrine and angiotensin on basal and volume-expansion–induced plasma levels. When epinephrine or angiotensin alone were infused, no significant effect was seen on plasma IR-ANP concentrations. In subsequent experiments, volume expansion in the presence of both epinephrine and angiotensin induced significant increases in plasma IR-ANP concentrations, although the ANP responses were smaller than in the presence of vasopressin, in spite of the fact that equipressor doses of all hormones were used. Thus, epinephrine, and angiotensin mimic the stimulatory action of vasopressin on atrial-stretch–induced ANP release in the pithed rat. Our results suggest that the release of ANP from the cardiac atria in response to stretching appears to be hormonally mediated.

Taken together, volume expansion or pressor hormones, when used alone, did not markedly influence plasma IR-ANP levels in pithed rats, but volume expansion in the presence of humoral stimulation caused marked increases in ANP release. The mechanism of this evoked release of ANP by various pressor hormones is still not clear. The observations of Mancini et al. during cardiac tamponade in open-chest dogs underscore the importance of atrial stretch on the relation between atrial pressure and ANP release. They found a dissociation between atrial pressure elevation and ANP release in cardiac tamponade and suggested that atrial distension, rather than change in intra-atrial pressure, is the primary stimulus of ANP release. Our observations in a relatively uncomplicated experimental preparation clearly demonstrate the requirement of pressor hormones for ANP secretion in response to volume expansion in the pithed rat. In view of present results and previous studies, it seems likely that pressor hormones elevate plasma ANP levels by two different mechanisms: First, they directly increase atrial stretch as changes in plasma ANP concentration with the hormones correlate with changes in atrial pressure, and second, pressor hormones may enhance the stretch-mediated ANP release.

The intracellular mechanism underlying the coupling of humoral stimulation and atrial stretch to the regulation of ANP release remains to be studied. It could be that pressor hormones act directly on atrial myocytes to facilitate the stretch-mediated release of ANP. In other systems, occupancy of V₁ receptors, angiotensin II, or α-receptors activate the breakdown of phosphoinositides with the formation of the two intracellular signals diacylglycerol and inositoltriphosphate. Diacylglycerol is thought to activate protein kinase C, which is implicated in the release of many hormones, including ANP.

Recently, protein kinase C activation has been found to augment stretch-induced vascular smooth muscle tone. Thus, it is quite feasible that humoral stimulation and the concomitant activation of protein kinase C could explain the stimulatory effect of these pressor hormones on the stretch-activated release of ANP.

The physiological significance of the stimulatory effect of pressor hormones on ANP secretion observed in this study is not obvious. First, release of ANP has been observed in response to atrial stretch in vitro, in isolated perfused hearts, and in isolated atria, where possible neural and humoral effects on ANP release do not occur. Second, in volume loading, when ANP is increased, increase of plasma volume will suppress vasopressin release. In contrast, in volume depletion when plasma ANP tends to decrease, vasopressin, renin, angiotensin, and catecholamines are stimulated. Nevertheless, the stimulatory action of vasopressin on atrial-stretch–induced release of ANP may be important during rapid rehydration, such as occurs in the correction of hypovolemia or when dehydrated animals drink, that has been associated with rapid changes in plasma IR-ANP and vasopressin levels. The high concentrations of pressor hormones that contribute to the vasoconstriction in congestive heart failure may be related to the
increased plasma ANP levels observed in these patients—by facilitating atrial-stretch-induced ANP secretion from the heart. Finally, the fact that in vitro atrial stretch alone produces ANP release does not necessarily exclude the regulatory action of humoral stimulation on ANP release.

In conclusion, we have shown that pressor hormones, especially vasopressin, regulate the atrial-stretch–induced release of endogenous IR-ANP in pithed and hypophysectomized rats. The combined effect of atrial distension and pressor hormones in regulating ANP secretion is a new mechanism by which humoral stimulation modulates direct, mechanical-stimulus–induced hormone secretion. However, further studies are required to elucidate the physiological role of vasopressin and other pressor hormones on atrial-stretch–mediated ANP secretion.

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