Angiotensin II-stimulated secretion of arginine vasopressin is inhibited by atrial natriuretic peptide in humans

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Matsukawa T, Miyamoto T. Angiotensin II-stimulated secretion of arginine vasopressin is inhibited by atrial natriuretic peptide in humans. Am J Physiol Regul Integr Comp Physiol 300: R624–R629, 2011. First published December 1, 2010; doi:10.1152/ajpregu.00324.2010.—We investigated the effect of the intravenous infusion of atrial natriuretic peptide (ANP) on the response of plasma arginine vasopressin (AVP) levels to intravenous infusion of angiotensin II (ANG II) in healthy individuals. Intravenous infusion of ANP (10 ng·kg−1·min−1) slightly but significantly decreased plasma AVP levels, while intravenous infusion of ANG II (10 ng·kg−1·min−1) resulted in slightly increased plasma AVP levels. ANG II infused significant elevations in arterial blood pressure and central venous pressure (CVP). Because the elevation in blood pressure could have potentially inhibited AVP secretion via baroreceptor reflexes, the effect of ANG II on blood pressure was attenuated by the simultaneous infusion of nitroprusside. ANG II alone produced a remarkable increase in plasma AVP levels when infused with nitroprusside, whereas the simultaneous ANP intravenous infusion (10 ng·kg−1·min−1) abolished the increase in plasma AVP levels induced by ANG II when blood pressure elevation was attenuated by nitroprusside. Thus, ANG II increased AVP secretion and ANP inhibited not only basal AVP secretion but also ANG II-stimulated AVP secretion in humans. These findings support the hypothesis that circulating ANP modulates AVP secretion, in part, by antagonizing the action of circulating ANG II.

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Subjects and Methods

Subjects

We evaluated eight healthy male volunteers (42 ± 1 yr, mean age ± SE). All subjects were within 10% of their ideal body weight and were determined to be free from endocrine, metabolic, and cardiovascular disorders by preliminary examinations. The subjects received no medication for at least 2 wk before the study. Their plasma renin activity, measured in the supine position, ranged from 1.0 to 2.0 nmol·l−1·h−1 (1.3 ± 0.2 nmol·l−1·h−1, mean ± SE); i.e., all the subjects were normoreninemic subjects.

Written informed consent was obtained from each subject following a detailed explanation of the purpose of the study, the procedures, and the possible risks. This study was mainly performed in Research Institute of Environmental Medicine, Nagoya University, and the study protocols were approved by the Human Research Committee of Research Institute of Environmental Medicine, Nagoya University.

Procedures

All tests were carried out at 9:00 AM, after the subjects had fasted overnight, and were performed in the recumbent position. Both antecubital veins were cannulated, one for blood sampling and the other for infusion of saline, ANG II, nitroprusside, or ANP. Mean arterial pressure was measured every minute using an automatic sphygmmomanometer (model 1846SX; Critikon, Tampa, FL), and central venous pressure was monitored by means of a catheter in the superior vena cava.

ANG II and ANP

Synthetic ANG II (Delivert; Astellas, Tokyo, Japan) or synthetic ANP (α-human ANP; Daiichi-Sankyo, Tokyo, Japan) was diluted in saline and infused in a volume of 0.02 ml·kg−1·h−1 via the venous catheter by using a constant infusion pump (model STC-52103; Terumo, Tokyo, Japan).

Study Protocol

The study protocol is shown in Fig. 1. All eight subjects participated in three protocols: A, B, and C.

Baseline values. After the subject had rested supine for 30 min, the mean values of mean arterial pressure and central venous pressure, at the 5-min period immediately before each infusion, served as resting baseline values. Blood samples were taken immediately before the infusion of test agents via the venous catheter, and the baseline values of plasma concentrations of AVP and plasma osmolality values were determined.
Measurements of Plasma Concentrations of AVP, ANG II, and ANP

Venous blood samples were used to evaluate the plasma concentrations of AVP, ANG II, ANP, and osmolality. Blood samples for AVP determination were collected in chilled tubes containing EDTA-2Na. AVP was extracted from plasma using a reversed-phase C18 silica column and was measured by a highly sensitive RIA (Mitsubishi AVP RIA Kit), as described previously (17). All samples were run in triplicate with post hoc test (Scheffé’s test) for multiple comparisons (Table 1 and Fig. 2). F ratios and the related P values are shown in Table 1. P values for F ratios of < 0.05 were considered to be statistically significant, and the post hoc test (Scheffé’s test) was subsequently performed. Results are expressed as the means ± SE. Statistical significance was defined as P < 0.05.

RESULTS

Summarized results are shown in Table 1 and Fig. 2. Plasma concentrations of ANG II and ANP are shown in Table 1. The intravenous infusion of ANG II produced a significant increase in plasma ANG II concentrations, and ANP infusion induced a significant increase in plasma ANP concentrations.

Effect of Saline, Nitroprusside, or ANP on Mean Arterial Pressure, Central Venous Pressure, and Plasma AVP Concentration

Mean arterial pressure, central venous pressure, and plasma AVP levels did not vary significantly before and during the infusion of saline (See Table 1). The infusion of nitroprusside produced a small increase in plasma AVP levels that was not statistically significant (P = 0.0961 < 0.1000) and produced a significant decrease in the mean arterial pressure and central venous pressure. ANP infusion did not result in significant changes in the mean arterial pressure and central venous pressure, but did result in a small but significant decrease in plasma AVP levels.
Table 1. Comparisons of changes in plasma concentrations of ANG II and ANP, mean arterial pressure, central venous pressure, and plasma concentrations of AVP among saline, nitroprusside (N) or ANP group, ANG II or ANG II + ANP group, and ANG II + N or ANG II + ANP + N group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Saline N</th>
<th>Saline</th>
<th>ANP</th>
<th>ANG II</th>
<th>ANG II + AMP</th>
<th>ANG II + N</th>
<th>ANG II + ANP + N</th>
<th>F value; P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma concentrations of ANG II, pg/ml</td>
<td>Baseline</td>
<td>19 ± 4</td>
<td>13 ± 4</td>
<td>17 ± 6</td>
<td>17 ± 5</td>
<td>19 ± 5</td>
<td>18 ± 3</td>
<td>F(6,49) = 3.58; P &lt; 0.0059</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>20 ± 5</td>
<td>22 ± 5</td>
<td>15 ± 6</td>
<td>106 ± 5†</td>
<td>106 ± 4†</td>
<td>107 ± 4†</td>
<td>108 ± 5†</td>
</tr>
<tr>
<td></td>
<td>Changes</td>
<td>1 ± 1</td>
<td>9 ± 2</td>
<td>−2 ± 1</td>
<td>89 ± 3 †</td>
<td>92 ± 4 †</td>
<td>88 ± 2 †</td>
<td>90 ± 5 †</td>
</tr>
<tr>
<td>Plasma concentrations of ANP, pg/ml</td>
<td>Baseline</td>
<td>30 ± 6</td>
<td>25 ± 8</td>
<td>28 ± 8</td>
<td>27 ± 7</td>
<td>28 ± 6</td>
<td>26 ± 7</td>
<td>27 ± 6</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>29 ± 6</td>
<td>22 ± 7</td>
<td>175 ± 21†‡§</td>
<td>27 ± 9</td>
<td>170 ± 20†‡§</td>
<td>27 ± 8</td>
<td>170 ± 20†‡§</td>
</tr>
<tr>
<td></td>
<td>Changes</td>
<td>−1 ± 1</td>
<td>−3 ± 2</td>
<td>147 ± 22†‡§</td>
<td>0 ± 2</td>
<td>142 ± 19†‡</td>
<td>1 ± 1</td>
<td>143 ± 20†‡§</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>Baseline</td>
<td>89 ± 4</td>
<td>85 ± 3</td>
<td>88 ± 3</td>
<td>87 ± 3</td>
<td>87 ± 2</td>
<td>89 ± 2</td>
<td>85 ± 2</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>88 ± 3</td>
<td>77 ± 3†§</td>
<td>87 ± 4§</td>
<td>113 ± 1†§</td>
<td>105 ± 2†</td>
<td>102 ± 1†</td>
<td>94 ± 2</td>
</tr>
<tr>
<td></td>
<td>Changes</td>
<td>0 ± 1</td>
<td>−8 ± 1†‡§</td>
<td>−1 ± 1§</td>
<td>26 ± 2†§</td>
<td>18 ± 1†‡</td>
<td>13 ± 2†‡</td>
<td>9 ± 2†‡</td>
</tr>
<tr>
<td>Central venous pressure, mmHg</td>
<td>Baseline</td>
<td>3.9 ± 0.4</td>
<td>4.2 ± 0.4</td>
<td>4.1 ± 0.5</td>
<td>4.2 ± 0.4</td>
<td>4.3 ± 0.4</td>
<td>4.4 ± 0.3</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>4.2 ± 0.5</td>
<td>2.0 ± 0.3†‡§</td>
<td>3.9 ± 0.5‡</td>
<td>6.3 ± 0.4‡§</td>
<td>6.2 ± 0.4‡§</td>
<td>4.4 ± 0.3‡§</td>
<td>4.2 ± 0.4‡</td>
</tr>
<tr>
<td></td>
<td>Changes</td>
<td>0.3 ± 0.2</td>
<td>−2.2 ± 0.1†‡§</td>
<td>−0.2 ± 0.1‡</td>
<td>2.1 ± 0.1‡</td>
<td>1.9 ± 0.2‡§</td>
<td>0.0 ± 0.0‡</td>
<td>0.0 ± 0.0‡</td>
</tr>
<tr>
<td>Plasma osmolality, mOsm/kg</td>
<td>Baseline</td>
<td>291 ± 3</td>
<td>288 ± 2</td>
<td>289 ± 2</td>
<td>288 ± 2</td>
<td>289 ± 2</td>
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<td>289 ± 2</td>
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<td></td>
<td>During</td>
<td>289 ± 2</td>
<td>289 ± 2</td>
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<td>288 ± 2</td>
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</tr>
<tr>
<td></td>
<td>Changes</td>
<td>1 ± 0</td>
<td>1 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>−1 ± 0</td>
</tr>
<tr>
<td>Plasma concentrations of AVP, pg/ml</td>
<td>Baseline</td>
<td>1.54 ± 0.13</td>
<td>1.68 ± 0.13</td>
<td>1.58 ± 0.08</td>
<td>1.58 ± 0.18</td>
<td>1.58 ± 0.11</td>
<td>1.60 ± 0.13</td>
<td>1.62 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>During</td>
<td>1.50 ± 0.14</td>
<td>1.90 ± 0.13§</td>
<td>1.26 ± 0.13§</td>
<td>2.21 ± 0.19§</td>
<td>1.57 ± 0.08§</td>
<td>3.60 ± 0.58‡§</td>
<td>1.58 ± 0.11§</td>
</tr>
<tr>
<td></td>
<td>Changes</td>
<td>−0.03 ± 0.03</td>
<td>0.22 ± 0.05†§</td>
<td>−0.32 ± 0.05†§</td>
<td>0.63 ± 0.03§</td>
<td>−0.02 ± 0.10‡</td>
<td>2.00 ± 0.46‡§</td>
<td>−0.04 ± 0.11§</td>
</tr>
<tr>
<td>% Recovery</td>
<td>110.4 ± 3.4</td>
<td>97.5 ± 7.2</td>
<td>101.2 ± 4.8</td>
<td>103.9 ± 3.4</td>
<td>103.9 ± 3.4</td>
<td>103.9 ± 3.4</td>
<td>103.9 ± 3.4</td>
<td>103.9 ± 3.4</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 8 each group. ANG, angiotensin; ANP, atrial natriuretic peptide; AVP, arginine vasopressin. †P < 0.05 vs. saline; ‡P < 0.05 vs. ANG II; §§ < 0.05 vs. ANG II + N.

Effect of ANG II and ANG II + ANP on Mean Arterial Pressure, Central Venous Pressure, and AVP Plasma Concentration

The infusion of ANG II induced a significant increase in the mean arterial pressure and central venous pressure, whereas plasma AVP levels showed a slight increase that was not statistically significant (Table 1) (P = 0.0721; <0.1000). The infusion of ANG II + ANP did not result in changes in plasma AVP levels in addition to the elevations in the mean arterial pressure and central venous pressure.

Effect of ANG II + Nitroprusside and ANG II + ANP + Nitroprusside on Mean Arterial Pressure, Central Venous Pressure, and AVP Plasma Concentration

In this protocol, nitroprusside was infused simultaneously with ANG II or ANG II + ANP to maintain the central venous pressure at the baseline level. The infused doses of nitroprusside were similar in the ANG II with nitroprusside group and in the ANG II + ANP with nitroprusside group (Table 1). The infusion of ANG II with the simultaneous infusion of nitroprusside produced a significant increase in plasma AVP levels and a significant elevation in mean arterial pressure. The infusion of ANG II + ANP with nitroprusside did not produce a significant change in plasma AVP levels but did produce a significant elevation in the mean arterial pressure.

The summarized results of important deviations of plasma AVP levels, and the values of mean arterial pressure and central venous pressure are shown in Fig. 2. Plasma AVP levels tended to increase during nitroprusside infusion and slightly, but significantly, decreased during the ANP infusion compared with the saline infusion. ANG II infusion produced a tendency for plasma AVP levels to increase, whereas the
infusion of ANG II + ANP did not produce a significant change in plasma AVP levels. The infusion of ANG II with nitroprusside produced a remarkable increase in plasma AVP levels, while maintaining the central venous pressure at the baseline level and the reducing arterial pressure, whereas additional infusion of ANP abolished the increase in plasma AVP levels produced by ANG II with nitroprusside (Fig. 2).

Plasma Osmolality

With the infusions of saline alone, nitroprusside alone, ANP alone, ANG II alone, ANG II + ANP, ANG II + nitroprusside, or ANG II with ANP + nitroprusside, plasma osmolality remained constant, and no significant changes in osmolality were observed from these infusions (Table 1).

DISCUSSION

In the present study, we investigated the effect of the intravenous ANP infusion on the response of the plasma AVP level to infused ANG II in normal individuals. This study had two key findings. First, the intravenous infusion of ANG II with simultaneous infusion of nitroprusside increased the secretion of AVP. Second, the intravenous infusion of ANP inhibited baseline AVP secretion and ANG II-induced AVP secretion.

Selection of ANG II and ANP Infusion Rates

We selected the infusion rate (10 ng·kg\(^{-1}\)·min\(^{-1}\)) of ANG II according to an earlier study (2). Previously, we examined the effects of ANG II infusion on muscle sympathetic nerve activity in healthy humans (14). We selected ANG II infusion doses of 5, 10, and 20 ng·kg\(^{-1}\)·min\(^{-1}\), which were sufficient to change hemodynamics and sympathetic activity. In our preliminary study, we examined the effects of the infusion of 5 ng·kg\(^{-1}\)·min\(^{-1}\) of ANG II on the plasma vasopressin level, but this dose of infused ANG II did not result in an adequate response of plasma AVP levels (data not shown). Therefore, we selected a dose of 10 ng·kg\(^{-1}\)·min\(^{-1}\) of ANG II in the present study.

We selected the infusion dose (10 ng·kg\(^{-1}\)·min\(^{-1}\)) of ANP according to our previous study (15). Infusions of this ANP dose blocked ANG II-evoked muscle sympathetic nerve activity in humans, so a dose of 10 ng·kg\(^{-1}\)·min\(^{-1}\) of ANP was used in the present study.

Intravenous Infusions of ANG II Stimulates Plasma AVP Levels

Intracerebroventricular administration of ANG II produced a pressor response accompanied by an increase in AVP secretion (22, 23), and circulating ANG II has been suggested to stimulate AVP secretion in animals (22, 23). In humans, however, it has been controversial whether or not circulating ANG II can...
stimulate AVP secretion. Several earlier studies found no evidence for a stimulatory effect of ANG II on plasma AVP levels (13, 20), while other studies reported a stimulatory action of ANG II on the release of AVP (27, 30). Recent reports revealed that infusions of high ANG II doses increased plasma AVP levels in humans (4, 5, 20). It is well known that the release of AVP is inhibited by stimulation of the baroreceptors (11, 27) and that the age-dependent reduction in baroreflex function is proposed to cause an enhanced AVP response to ANG II infusion (5). Thus, the pressor effect of ANG II could counteract its direct action of promoting AVP secretion.

Our previous studies in humans have shown a slight baroreflex-mediated decrease of muscle sympathetic nerve activity during the pressor response to infused ANG II (18, 19) with a contrasting increase in the sympathetic nerve activity during ANG II infusion when the pressor effect of the peptide was attenuated by nitroprusside (18, 19). Our present study demonstrated that ANG II infusion tended to increase AVP secretion and markedly increased AVP secretion when the baroreflex-mediated effects of ANG II were attenuated by nitroprusside. Thus, circulating ANG II stimulates AVP secretion in humans.

**Simultaneous Infusion of ANP Inhibits ANG II-induced Elevated Plasma AVP Levels**

Interactions between ANG II and ANP were suggested to occur in some organs and to involve the central nervous system (12, 26). Our observation that the intravenous ANP infusion inhibited ANG II-induced increase in plasma AVP levels was in agreement with previous studies demonstrating that the intraventricular administration of ANP inhibited the increase in blood pressure and AVP secretion induced by ANG II central administration (3, 12, 26, 32). However, it is not known whether or not circulating ANP inhibits the induction of AVP secretion by ANG II. Recent studies in humans and animals suggest that ANP inhibits basal (6, 8, 25, 26), osmotically-induced (1, 27), or hypovolemia-induced (24, 27, 31) AVP release. Accordingly, circulating ANP may inhibit not only the basal secretion of AVP, but also the stimulation of AVP secretion by circulating ANG II in humans.

**Physiological and Pathophysiological Relevance of Changes in Plasma Levels of AVP, ANG II, and ANP**

Previously, the effects of intravenous ANG II infusions of the same dose (21) or a similar dose (5) to our present study were examined for their effect on plasma AVP levels in humans. These studies measured plasma AVP levels at 30 and 45 min after starting ANG II infusions (21) or 60-min infusions of ANG II (5) and reported small but significant increase in plasma AVP levels. We determined the effect of ANG II infusions on plasma AVP levels 20 min after starting infusions and observed that plasma AVP levels tended to increase in plasma AVP ($P = 0.0721; < 0.1000$). Thus, if we were to determine the effects of ANG II infusions over a longer time interval, we might observe a significant increase in plasma AVP levels in humans.

Increased plasma AVP levels may be physiologically significant, and increased AVP could participate in regulating fluid balance and peripheral vascular resistance. Increased plasma ANG II levels may be a pathophysiological increase rather than a physiological one, because the increased ANG II could participate in fluid balance regulation and blood pressure regulation, such as in secondary aldosteronism. Moreover, this may also be true of increased plasma ANP levels, which may be a pathophysiological increase rather than physiological one, and the increase in ANP could also participate in fluid balance regulation and blood pressure regulation in secondary aldosteronism.

The physiological roles of AVP, ANG II, and ANP observed in the present study are described below. Because one of its principal physiologic effects is the retention of water by the kidney, AVP is often called the antidiuretic hormone. The processes induced by ANG II produce the permeability of the kidney collecting ducts so that water enters the hypertonic interstitium of the renal pyramids. The urine becomes concentrated, and its volume decreases. The overall effect is, therefore, retention of water in excess of solute, and, consequently, the effective osmotic pressure of body fluids is decreased (9, 10). Moreover, the increase in AVP induced by ANG II has a potent vasoconstrictor effect via vascular smooth muscle stimulation (10). ANG II produces arteriolar constriction and increases blood pressure. ANG II also acts directly on the adrenal cortex to increase the secretion of aldosterone, and the renin-angiotensin system is a major regulator of aldosterone secretion. ANG II additionally infuses contraction of mesangial cells with a resultant decrease in glomerular filtration, which directly causes increased Na reabsorption by the renal tubules (10). Circulating ANP acts on the kidney to increase Na excretion by dilating afferent arterioles and relaxing mesangial cells. Both of these actions increase glomerular filtration. Finally, ANP inhibits Na reabsorption by the renal tubules and relaxes vascular smooth muscle in arterioles and venules, which leads to a decline in blood pressure (10).

The pathophysiological roles of AVP, ANG II, and ANP observed in the present study are described below. Plasma ANG II levels were suppressed in patients with Conns syndrome before treatment, but were increased in patients with malignant hypertension, with Addison’s disease before treatment, and in patients with liver cirrhosis (7). Moreover, an extensive myocardial infarction results in significant disturbances of hemodynamics that cause increased activation of vasoconstriction mechanisms aimed at maintaining an appropriate perfusion pressure within the cardiovascular system. Among these mechanisms are increased release of ANG II, aldosterone, and AVP, and activation of the sympathetic system. Activation of these mechanisms may cause retention of body fluids, and in the later stage, the postinfarct cardiac failure is associated with the compensatory increased release of ANP (28).

**Mechanisms or Sites of Interaction between ANG II and ANP that Affect Plasma AVP Levels**

The mechanisms or sites of interaction between ANG II and ANP that affect the secretion of AVP could not be deduced from the present study, but evidence from previous studies suggests the involvement of the central nervous system. It has been postulated that the central effect of circulating ANG II on AVP secretion is mediated via circumventricular organs that have no blood-brain barrier such as the subfornical organs, the organum vasculosum laminae terminalis, or the posterior pitu...
ity (22, 23). Binding sites for ANP (26) and ANG II (22) have been demonstrated in the circumventricular organs. Therefore, it is possible that circulating ANP inhibits AVP secretion induced by circulating ANG II at the level of the central nervous system.

Intravenous infusion of ANP was recently demonstrated to cause sympathetic inhibition by activating cardiac vagal afferents (16, 29), and cardiac baroreceptor reflexes are known to affect AVP secretion (11, 27). It is therefore possible that this action of ANP participates in its inhibitory effect on the stimulation of AVP secretion by ANG II.

In conclusion, circulating ANG II stimulates AVP secretion and the intravenous infusion of ANP appears to inhibit this stimulatory action of ANG II in humans. These findings support the hypothesis that circulating ANP modulates the secretion of AVP in part by antagonizing the action of circulating ANG II. This suggests that ANP plays a role in the regulation of body fluid homeostasis and arterial pressure.

**Perspectives and Significance**

The results of this study suggest that circulating ANP has an inhibitory action on ANG II-activated AVP secretion, and our previous study showed that ANG II-induced sympathetic activation is inhibited by ANP in humans (19). Thus, the actions of ANG II on AVP secretion and the sympathetic nervous system may play a role in the regulation of fluid homeostasis and arterial pressure. ANG II also stimulates ACTH secretion (23), and the peptide-induced steroid hormones are known to be important regulators of the fluid balance and arterial pressure. ANP can inhibit ANG II-induced activation of ACTH secretion. In future studies, we shall determine the effect of ANP infusion on ANG II-produced activation of ACTH in plasma in humans.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**