Intranasal Angiotensin II in Humans Reduces Blood Pressure When Angiotensin II Type 1 Receptors Are Blocked

Inge Derad, Friedhelm Sayk, Hendrik Lehnert, Lisa Marshall, Jan Born, Martin Nitschke

Abstract—Intranasal administration of angiotensin II (ANGII) affects blood pressure in a mode different from intravenously administered ANGII via a direct access to the brain bypassing the blood–brain barrier. This clinical study investigated blood pressure regulation after intranasal ANGII administration in healthy humans, whereas systemic, blood-mediated effects of ANGII were specifically blocked. In a balanced crossover design, men (n=8) and women (n=8) were intranasally administered ANGII (400 μg) or placebo after ANGII type 1 receptors had been blocked by pretreatment with valsartan (80 mg; 12 and 6 hours before intranasal administration). Plasma levels of ANGII, aldosterone, renin, vasopressin, and norepinephrine were measured; blood pressure and heart rate were recorded continuously. Intranasal ANGII acutely decreased blood pressure without altering the heart rate. Plasma levels of vasopressin and norepinephrine remained unaffected. Plasma ANGII levels were increased throughout the recording period. Aldosterone levels increased despite the peripheral ANGII type 1 receptor blockade, indicating an aldosterone escape phenomenon. In conclusion, intranasal ANGII reduces blood pressure in the presence of selective ANGII type 1 receptor blockade. Intranasal ANGII administration represents a useful approach for unraveling the role of this peptide in blood pressure regulation in humans. (Hypertension. 2014;63:762-767.) ● Online Data Supplement

Key Words: administration, intranasal • aldosterone • angiotensin II • blood pressure • central nervous system

The peptide hormone angiotensin II (ANGII) displays a wide range of regulatory actions on blood pressure. Besides its systemic effects on the blood vessels and different organ systems, local ANGII is crucially involved in the central nervous regulation of blood pressure.1–6 ANGII receptors, mainly type 1 receptors (AT1) and also type 2 receptors (AT2), are expressed in several cerebral nuclei where they mediate effects of locally produced ANGII.7–10 Inhibition of central nervous ANGII synthesis reversed hypertension in spontaneously hypertensive rats.11

The differentiation of central nervous and peripheral actions of ANGII is important for understanding blood pressure homeostasis and the development of essential hypertension in humans. Peripheral ANGII increases blood pressure but cannot surpass the blood–brain barrier, except via the circumventricular organs (area postrema, organum vasculosum laminae terminalis).12,13 These structures express a magnitude of AT1 receptors and seem to serve as an interface gating effects of circulating ANGII to brain stem and hypothalamic nuclei (ie, nucleus tractus solitarius, supraoptic nucleus, and paraventricular nuclei). Such humoral ANGII input is known to increase tonic blood pressure levels by an upward resetting of the sympathetic baroreceptor reflex activity.14–17 and may also activate intrinsic hypothalamic neurohypophysial fiber pathways, inducing the release of vasopressin.3,18–20 When ANGII is directly injected into the nucleus tractus solitarius, the baroreceptor reflex is suppressed via activation of AT1 receptors. After intracerebral administration, however, pressor effects of ANGII were revealed to be variable and even contradictory depending on dose and location of the injection, therefore suggesting differential effects on blood pressure regulation.3,4,9,10,19,21–24

The intranasal administration of small peptide molecules represents an established approach to bypass the blood–brain barrier in humans and to achieve direct central nervous effects.25–29 These studies demonstrate that peptide molecules such as ANGII directly enter the cerebrospinal fluid after their intranasal administration without previous uptake to the blood but instead via diffusion along neuronal clefts in the nasal mucosa. Importantly, because of the relatively great amounts of substance administered to achieve central nervous effects of the peptide, it cannot be prevented that, rather than directly accessing the cerebrospinal fluid compartment, some portion of the substance spills over into the circulation. Peptide that enters the circulation might then mask direct central nervous effects after intranasal peptide administration. Thus, a viable approach to unravel direct brain effects of intranasal peptide administration is to combine this treatment with a peripherally acting receptor blocker of the peptide.
In a previous study, we found that the intranasal administration of ANGII in humans induced the release of vasoressin, whereas the prolonged neurogenic blood pressure increase was blocked. Those findings led us to suspect that within the brain, ANGII might act to decrease blood pressure, although such an effect was masked in that study because of ANGII that, after intranasal administration, spilled over into the bloodstream to activate angiotensin receptors in the circumventricular organs. With this knowledge, the present study aimed to investigate whether intranasal ANGII reduces blood pressure in healthy humans, whereas any afferent angiotensinergic effects via the blood were selectively blocked by the AT1 receptor blocker valsartan.

**Methods**

**Subjects**

Sixteen normotensive student volunteers (8 men/8 women; age, 24±3 years; body mass index, 21.44±1.25 kg/m² [mean±SD]) participated in the study. All subjects were healthy nonsmokers and did not take any medication, as assessed before the experiment by a general medical examination. Pregnancy in women was excluded by urinary testing of β human chorionic gonadotropin. The participants were instructed to abstain from caffeine and alcohol ≥16 hours before experimental sessions and were examined in a postabsorptive state: a standardized continental breakfast had been served 6 hours before the test session (3 hours), the subjects remained in a supine position, and measurements were continued for 125 minutes. Throughout the test session (0–1 minute, arrow) of angiotensin II (ANGII; 400 μg) within 1 minute. The dosing of ANGII was adopted from a previous study of ours establishing dose–response curves for the effects of ANGII on blood pressure and for blood ANGII concentrations, which revealed that the intranasal administration of 400 μg ANGII and the intravenous infusion of 2.5 μg ANGII led to equivalent blood ANGII concentrations.

Before each test session, the AT1 receptor blocker valsartan (80 mg) was administered orally twice (12 and 6 hours before the intranasal substance), and blood pressure was controlled by oscillometric measurements at the upper arm to adjust and control continuous blood pressure recording via the 2-finger Portapress system (model 2 with a height correction system, TNO Institute of Applied Physics). Measurements switched automatically from one finger to the other after 30-minute periods. A cannula (19 gauge) was placed into the right cubital vein for blood sampling.

Continuous blood pressure registration started 15 minutes before intranasal substance administration. ANGII or placebo was administered, and measurements were continued for 125 minutes. Throughout the test session (3 hours), the subjects remained in a supine position, except for the 5-minute period during drug administration when they rose to a sitting position (90°). Blood was sampled every 10 minutes for determination of plasma concentrations of ANGII, aldosterone, renin, vasopressin, cortisol, and catecholamines (norepinephrine and epinephrine).

**Data Analysis**

Blood pressure and heart rate data were analyzed according to the beat-to-beat Modelflow Interpretation of the Portapress (BeatScope 1.0, TNO institute of Applied Physics Biomedical Instrumentation, Amsterdam, The Netherlands). Recordings (at a sampling rate of 1/s) were averaged off-line across subsequent 1-minute intervals. Plasma concentrations of ANGII, aldosterone, vasopressin, and renin were determined by commercially available radioimmunoassay (Bühlmann Laboratories, Switzerland; DPC, Germany; Sanofi Pasteur, France), with detection levels of 0.06 pg/mL (2s: 1–7.8 pg/mL) for vasopressin, 11 pg/mL (2s: 10–160 ng/L [recumbent]) for aldosterone, and 0.7 pg/mL (2s: 0–12.7 pg/mL) for ANGII. Norepinephrine and epinephrine plasma levels were assessed by high-performance liquid chromatography. All samples were assessed in duplicate.

For analysis, 6 time periods were defined as follows: the 15-minute blood pressure recording (or 2 blood samples) before drug administration served as baseline period; the following 5-minute period during drug administration (1 blood sample directly after drug administration), then data of succeeding 30-minute periods (or 3 blood samples) were averaged to epochs 1 to 4. Statistical analysis (performed with SPSS Statistics version 12.0) of systolic and diastolic blood pressure, heart rate, and hormone concentrations was based on multiple analyses of covariance with repeated measures (MANCOVA), including the factors treatment (ANGII or placebo), time, and order of treatment (first placebo or first ANGII). For the overall analysis, the baseline period served as covariate.

**Results**

**Blood Pressure and Heart Rate**

After intranasal ANGII administration, compared with the placebo condition, systolic blood pressure significantly decreased (MANCOVA, $F(1,14)=5.12; P=0.03$; Figure 1). Compared with the baseline before intranasal treatment (mean systolic blood pressure, 107.6 mm Hg; n=16), blood pressure was reduced on average by 3.6 and 5.2 mm Hg during the epochs from 6 to 35 and 36 to 105 minutes after ANGII administration, respectively ($P=0.037$ and $P=0.028$ for subsequent paired t tests). The decrease occurred irrespective of the order of treatment condition (ie, ANGII first or second). Diastolic blood pressure decreased to a lower extent, paralleling systolic blood pressure. Figure S1 in the online-only Data Supplement shows the original systolic and diastolic blood pressure data sampled during shorter time intervals (5

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**Figure 1.** Systolic blood pressure (mm Hg) after intranasal administration (0–1 minute, arrow) of angiotensin II (ANGII; 400 μg, black bars) and placebo (empty bars). Continuous blockade of the ANGII type 1 receptors was provided by valsartan (80 mg, given 6 and 12 hours before each test session). Mean±SEM blood pressure for successive 1-minute postadministration epochs, that is, 0 to 5, 6 to 35, 36 to 65, 66 to 95, 96 to 125 minutes after intranasal ANGII or placebo. Data are based on ANCOVA, with the baseline values used as covariate. Baseline systolic blood pressure before intranasal administration was a mean of 103.0 mm Hg (placebo) and 107.6 mm Hg (ANGII) and did not differ significantly between the treatments. **P<0.05 for differences between the effects of the treatments.**
minutes). Heart rate remained unaffected from the intranasal treatments.

Of note, the order factor of the MANCOVA reached significance \((F[1,14]=5.86; \ P=0.02)\). Subgroup analyses revealed that baseline systolic blood pressure tended to be lowered by \(+7.4\ \text{mm Hg}\) (from \(107.4\pm3.6\) to \(100.2\pm3.8\ \text{mm Hg}\)) in the second compared with the first session in those subjects who had received ANGII first (ie, 2 weeks before; \(P=0.08\), pairwise \(t\) test). In contrast, baseline blood pressure did not change when placebo was administered in the first session (from \(107.9\pm2.8\) to \(106.0\pm3.9\ \text{mm Hg}\)).

**Plasma Levels of Vasoactive Hormones**

Plasma ANGII concentrations increased directly after intranasal administration, peaking at \(62\pm6\ \text{pg/mL}\) after 10 minutes and remained elevated for 95 minutes (Figure 2). Plasma aldosterone levels rose moderately but persistently, reaching a maximum of \(60\pm9\ \text{pg/mL}\) 30 minutes after ANGII administration, and then remained elevated throughout the session (Figure 2). As a consequence, the aldosterone–renin ratio increased. Vasopressin, renin, epinephrine, and norepinephrine plasma concentrations were not significantly affected by ANGII in the present setting (Tables 1 and 2).

**Discussion**

Blood pressure responses to intranasal administration of ANGII were examined in healthy subjects. ANGII is a small peptide hormone (1.2 kDa) that directly accesses the brain via the intranasal route. Previous studies have indicated that intranasal ANGII bypasses the blood–brain barrier, although there is a substantial spillover into the circulation.\(^{26,27,30}\) To distinguish the influences of intranasal versus blood-borne ANGII (via the circumventricular organs), the AT1 receptors of the body periphery were selectively blocked by valsartan, a nonlipophilic, nonpeptidic compound that does not cross the intact blood–brain barrier.\(^{31}\)

Because of its high (95%) plasma binding capacity, its penetration into tissue is limited. Due to its low oral bioavailability (25%), and because food intake may reduce area under the curve and \(t_{\text{max}}\) by 40% to 50%, food intake of our subjects was standardized, and this widely used antihypertensive drug (\(t_{\text{max}}\) 2–4 hours, \(t_{1/2}\) 6 hours) was administered twice (12 and 6 hours before ANGII administration). Valsartan successfully inhibited a direct increase in blood pressure after ANGII administration, which suggests that a substantial blockade of AT1 receptors was achieved, although it may have not been complete.

Consistent with a previous study of ours,\(^{30}\) the present placebo-controlled study confirmed an immediate decrease in blood pressure, although blood pressure levels in our healthy volunteers had already been at a rather low level. Of note, intranasal ANGII did not produce any vasopressin release, which concurs with findings in rodents showing that any vasopressin release after intracerebral ANGII administration can be inhibited by blocking AT1 receptors.\(^{32}\)

The decrease in blood pressure was also not counter-regulated via baroreflex-mediated sympathoactivation that develops in humans when ANGII levels rise because neither reflex tachycardia nor increased plasma norepinephrine levels were observed. Norepinephrine levels were on average even slightly lowered by ANGII, although this decrease was nonsignificant. Together, this pattern supports the view that intranasal ANGII produces a central nervous downregulation of the baroreflex set point.

In contrast, intravenous administration of ANGII elevates blood pressure via a resetting of the blood pressure set point at the level of central nervous baroreflex centers toward hypertensive levels.\(^{14,18}\) This well-known neurogenic increase in blood pressure was not found after intranasal administration of ANGII in a previous study in healthy men, although ANGII levels in the blood were elevated.\(^{30}\) Blood pressure reduction in the present study cannot arise from valsartan alone because this pretreatment was administered equally to the placebo group, and previous studies in healthy men had reported that
blood pressure remained unaffected by a subacute administration of the AT1 receptor blockers valsartan, eprosartan, or a single dose of captopril.13–15

In addition to peripheral synthesis, ANGII is locally produced by brain tissue itself. The intrinsic renin–angiotensin–aldosterone–ouabain system of the brain is characterized by a low renin production. With this limited renin availability, ANGII production is compartmentalized within specific nuclei.6 Intranasally administered ANGII, as well as brain ANGII, may act in a paracrine manner to influence neuronal activity of centers involved in blood pressure regulation.5,16 Hence, the function of ANGII and its degradation products seems to depend on the compartment of its action. Recent findings in rats underline this point of view: a chronic infusion of ANG 1–7 in the lateral ventricle of the brain attenuated the development of deoxycorticosterone acetate salt–induced hypertension.21 In contrast, microinjection of ANG 1–7 into the rostral ventrolateral medulla increased blood pressure in rats.37,38

Besides the distribution of ANGII within the brain, the distribution of its binding sites is also relevant. Recent data from spontaneously hypertensive rats support the finding of a reduction in blood pressure after increasing AT1 receptor RNA in the nucleus tractus solitarius, but, in contrast, deletion of AT1a receptors in the paraventricular nuclei reduced blood pressure in mice.39,40 Hence, ANGII-dependent central nervous function varies within the different brain locations and cannot be predicted easily in humans. The present intranasal approach might provide further insight into central nervous function varies within the different brain locations and cannot be predicted easily in humans. The present intranasal approach might provide further insight into central nervous function.

Mainly, AT1 receptors are expressed in human adults.9,10 Within the brain, however, AT2 receptors are expressed at substantial levels, and novel binding sites are still being identified.36,41,42 Their activation may protect from cerebral ischemia or hypertension.43–45 Our findings in healthy humans show that intranasal ANGII combined with systemic AT1 receptor blockade reduces blood pressure. The present combination of treatments might have functioned to specifically activate brain AT2 receptors. Brain AT2 activation might indeed be involved in the suppression of vasopressin release after central nervous ANGII administration32 and in the downregulation of the baroreflex set point mediated via reducing sympathetic outflow into resistance arteries of the body.19 However, whether brain AT2 receptors substantially contribute to the observed immediate decrease in blood pressure cannot be answered here. Considering its implications for the development of antihypertensive treatments by targeting the brain ANG system, this issue is clearly in need of further study.

Potentially, although more prominent in fetal tissue, peripheral vascular AT2 receptors may have been activated by the increased levels of circulating ANGII in the present study. This could in turn lower the blood pressure.46–48 With regard to kidney AT2 receptor activation, it has been shown to suppress renin biosynthesis. Because renin plasma levels were not significantly affected by the present protocol, it seems unlikely that peripheral renal AT2 receptors were activated in the present setting.46,49,50

The reduction in blood pressure after intranasal ANGII sustained during the first hour but faded as plasma levels of aldosterone increased. Aldosterone influences the salt–water homeostasis of the body via the kidneys and may elevate blood pressure with some delay.51 In our study, aldosterone concentrations started to rise during the first half hour after intranasal ANGII administration, although AT1 receptors were blocked. During chronic treatment with AT1 receptor blockade, an aldosterone escape phenomenon is known to occur when ANGII plasma levels are strongly elevated.52 In the present study, the fast aldosterone increase after intranasal ANGII might involve another more rapid pathway to stimulate adrenal aldosterone release.53

**Perspectives**

Using intranasal ANGII administration to bypass the blood–brain barrier in the healthy, normotensive volunteers of the present clinical study, a reduction in systolic blood pressure

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**Table 1. Plasma Vasopressin, Norepinephrine, and Renin (pg/mL; Mean±SEM) Before and After Intranasal Administration of ANGII or Placebo (N=16)**

<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>Vasopressin</strong></td>
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<td></td>
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<tr>
<td>ANGII</td>
<td>4.10±2.40</td>
<td>4.57±2.96</td>
<td>4.17±2.39</td>
<td>4.15±2.34</td>
<td>4.42±2.59</td>
<td>4.47±2.52</td>
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<tr>
<td>Placebo</td>
<td>4.18±3.1</td>
<td>4.72±3.84</td>
<td>4.47±2.27</td>
<td>4.12±2.13</td>
<td>4.24±2.34</td>
<td>4.56±2.08</td>
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<tr>
<td><strong>Norepinephrine</strong></td>
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<tr>
<td>ANGII</td>
<td>240.5±24.7</td>
<td>257.7±24.2</td>
<td>208.8±20.9</td>
<td>204.1±22.7</td>
<td>208.4±23.0</td>
<td>226.9±27.5</td>
</tr>
<tr>
<td>Placebo</td>
<td>259.9±20.6</td>
<td>259.6±19.5</td>
<td>248.8±20.5</td>
<td>246.6±20.4</td>
<td>230.8±20.0</td>
<td>237.9±21.4</td>
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<tr>
<td><strong>Renin</strong></td>
<td></td>
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</tr>
<tr>
<td>ANGII</td>
<td>36.7±10.8</td>
<td>23.4±6.4</td>
<td>20.5±5.3</td>
<td>20.4±4.7</td>
<td>20.6±4.4</td>
<td>20.7±4.1</td>
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<tr>
<td>Placebo</td>
<td>44.7±8.0</td>
<td>26.5±4.9</td>
<td>25.4±4.7</td>
<td>22.7±3.5</td>
<td>20.9±3.1</td>
<td>20.3±3.3</td>
</tr>
</tbody>
</table>

ANGII indicates angiotensin II.

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**Table 2. Plasma Cortisol (pg/mL; Mean±SEM) After Intranasal Administration of ANGII or Placebo (N=16)**

<table>
<thead>
<tr>
<th>Cortisol</th>
<th>5 min</th>
<th>65 min</th>
<th>125 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANGII</td>
<td>7.46±0.79</td>
<td>6.04±0.80</td>
<td>8.74±1.19</td>
</tr>
<tr>
<td>Placebo</td>
<td>7.31±0.79</td>
<td>5.98±0.84</td>
<td>6.66±1.06</td>
</tr>
</tbody>
</table>

ANGII indicates angiotensin II.
has been found after central nervous ANGII administration. The pattern of effects speaks for the view that intranasal ANGII produces a central nervous downregulation of the baroreflex set point and is unmasked after selective blockade of the AT1 receptors by valsartan. Therefore, it may be speculated that central ANGII might act on binding sites other than AT1 receptors.

To further elucidate the underlying mechanism, investigations should include recordings of muscle sympathetic nerve activity, a direct measure of central sympathetic outflow in resistance arteries. Furthermore, imaging studies could help to detect the distribution of ANGII after intranasal administration and specify the location of action within the brain. The present intranasal approach might be of relevance to specify central nervous blood pressure regulation and neurohumoral dysfunctioning in patients.

In the absence of specific central nervous therapies such as selective AT2 agonists, our paradigm combining intranasal ANGII administration with selective AT1 receptor blockers might be of interest for the development of new antihypertensive strategies in humans.

Acknowledgments
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Disclosures
None.

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**Novelty and Significance**

**What Is New?**

- In this article, we approached the human brain via intranasal administration of the hormone angiotensin II (ANGII). This method seems to represent a promising approach to investigate blood pressure regulation in humans. However, it cannot be prevented that, rather than directly accessing the cerebrospinal fluid compartment, part of the substance spills over into the circulation. When this spillover of ANGII into the blood—activating ANGII type 1 receptors (AT1)—was prevented by a selective AT1 receptor blocker, the present study showed that intranasal ANGII decreased blood pressure in healthy subjects. Potential activation of AT2 receptors might play a role in this phenomenon.

**What Is Relevant?**

- Sixteen men and women volunteers developed a small but consistent reduction in blood pressure after intranasal ANGII administration in the present crossover study when the systemic AT1 receptors were blocked. These results coincide with recent studies in animals, manipulating the AT1 receptors of specific brain nuclei or infusing ANGII intracerebroventricularly.

**Summary**

Although the underlying mechanisms of the described phenomenon remain to be specified in humans, the present data shed new light on the putative therapeutic usage of intranasal ANGII conjoint with AT1 receptor blocker pretreatment.
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Intranasal angiotensin II in humans reduces blood pressure when AT1 receptors are blocked

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