Central Angiotensin-(1–7) Improves Vagal Function
Independent of Blood Pressure in Hypertensive
(mRen2)27 Rats

Manisha Nautiyal, Hossam A. Shaltout, Daniel C. de Lima, Kenia do Nascimento,
Mark C. Chappell, Debra I. Diz

Abstract—Hypertensive transgenic (mRen2)27 rats with overexpression of the mRen2 gene have impaired baroreflex sensitivity for heart rate control and high nicotinamide adenine dinucleotide phosphate oxidase and kinase-to-phosphatase signaling activity in medullary tissue compared with normotensive Hannover Sprague-Dawley control rats. They also exhibit insulin resistance at a young age. To determine whether blocking angiotensin II actions, supplementing angiotensin-(1–7), or scavenging reactive oxygen species in brain differentially alters mean arterial pressure, baroreflex sensitivity, or metabolic function, while altering medullary signaling pathways in these animals, we compared intracerebroventricular infusions of the angiotensin II type 1 receptor antagonist candesartan (4 μg/5 μL/h), angiotensin-(1–7) (0.1 μg/5 μL/h), a reactive oxygen species scavenger tempol (25 μg/5 μL/h), or artificial cerebrospinal fluid (5 μL/h) for 2 weeks. Mean arterial pressure was reduced in candesartan-treated rats without significantly improving the vagal components of baroreflex function or heart rate variability. In contrast, angiotensin-(1–7) treatment significantly improved the vagal components of baroreflex function and heart rate variability at a dose that did not significantly lower mean arterial pressure. Tempol significantly reduced nicotinamide adenine dinucleotide phosphate oxidase activity in brain dorsal medullary tissue but had no effect on mean arterial pressure or autonomic function. Candesartan tended to reduce fat mass, but none of the treatments significantly altered indices of metabolic function or mitogen-activated protein kinase signaling pathways in dorsal medulla. Although additional dose response studies are necessary to determine the potential maximal effectiveness of each treatment, the current findings demonstrate that blood pressure and baroreflex function can be essentially normalized independently of medullary nicotinamide adenine dinucleotide phosphate oxidase or mitogen-activated protein kinase in hypertensive (mRen2)27 rats. (Hypertension. 2012;60:1257-1265.)

Key Words: hypertension ■ baroreflex function ■ angiotensin peptides ■ oxidative stress ■ brain

Hypertensive (mRen2)27 transgenic rats overexpress the murine Ren2 gene, exhibit high levels of angiotensin (Ang) II in brain tissue, and develop hypertension at an early age.1 An elevated ratio of Ang II to Ang-(1–7) in brain medulla of hypertensive transgenic (mRen2)27 rats is accompanied by impaired baroreflex function3,4 and higher and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity5 in medullary tissue compared with normotensive Sprague-Dawley control rats. The (mRen2)27 rats also exhibit insulin resistance and excess body weight throughout life.5–8

Higher Ang II actions resulting from an imbalance between Ang II and Ang-(1–7) in brain have been implicated in several models of hypertension in addition to the (mRen2)27 hypertensive rats; however, the mechanisms for these effects are not well understood.4,9 At the level of the nucleus tractus solitarii (NTS) in normotensive Sprague-Dawley rats, Ang II is known to provide tonic inhibition of the sensitivity of the baroreceptor reflex (BRS) control of heart rate (HR), a vagally mediated component of the reflex control of arterial pressure.1,10 This effect of Ang II is counteracted within the NTS, in part, by Ang-(1–7), which provides tonic enhancement of the BRS in Sprague-Dawley rats.4,11,12 The acute actions of either peptide within the NTS on the BRS are not associated with changes in resting mean arterial pressure (MAP).9

Ang II–stimulated NADPH oxidase–derived reactive oxygen species (ROS) are major contributors of oxidative stress in animal models of renin-angiotensin system (RAS) overactivity and are implicated in both short-term and long-term pressor effects of Ang II through activation of mitogen-activated protein kinases (MAPKs) in brain13,14 and Ang II–mediated insulin resistance in hypertensive (mRen2)27 rats15,16 in the periphery. Thus, higher Ang II actions relative to Ang-(1–7) and increased ROS at this brain site may contribute to hypertension.
or impaired BRS or both in (mRen2)27 rats. Therefore, we compared intracerebroventricular (ICV) infusions of the Ang II type 1 (AT₁) receptor antagonist candesartan (CAN), Ang-(1–7), a ROS scavenger tempol, or artificial cerebrospinal fluid (aCSF) for 2 weeks to determine whether blocking Ang II actions, supplementing Ang-(1–7), or scavenging ROS has differential effects on blood pressure and baroreflex function. We further assessed whether central infusions of CAN, Ang-(1–7), or tempol could influence NADPH oxidase activity and MAPK signaling in brain dorsal medulla and peripheral indices of metabolism and renal function in hypertensive (mRen2)27 rats.

**Materials and Methods**

**Animals**

Experiments were performed in 28- to 36-week-old male hypertensive (mRen2)27 transgenic rats obtained from the Hypertension and Vascular Research Center Colony at Wake Forest University School of Medicine. Animals were bred and housed in humidity- and temperature-controlled rooms (12-hour light/dark cycle) with free access to standard rat chow and water. They were housed overnight (4:00 pm to 8:00 am) in metabolic cages (Allentown, Inc., Allentown, NJ) for collection of urine on dry ice and assessment of food and water intake on day 13 of the ICV infusion. All experimental protocols were approved by the animal care and use committee of Wake Forest University Health Sciences.

**ICV Cannulation and Osmotic Minipump Implantation**

A 28-gauge stainless-steel cannula (brain infusion kit 2; Alzet, Palo Alto, CA) was implanted stereotaxically (David Kopf Instruments, Tujunga, CA) into the lateral cerebral ventricle (0.4 mm posterior, 0.21 mm lateral to bregma, and 3.5 mm with depth from skull surface) under 2.5% to 4% isoflurane anesthesia.17,18 Cannula placement was verified by microscopic examination of 30-μm thick frozen brain sections. The cannula was connected via poly-carbonate tube to an osmotic minipump (5.0 μL/h; model 2ML2; Alzet) placed under the skin in the lateral abdomen for infusion of AT₁ receptor antagonist CAN, CV11794 (Takeda, Osaka, Japan; 4 μg/5 μL/h); group 3 received Ang-(1–7) (Bachem, Torrance, CA; 0.1 μg/5 μL/h); and group 4 received 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (Calbiochem, Gibbstown, NJ; tempol, 25 μg/5 μL/h). The composition of aCSF (in mmol/L) was 150.0 sodium, 3.0 potassium, 1.4 calcium, 0.8 magnesium, 1.0 phosphate, and 155.0 chloride. All drugs were dissolved in aCSF and used at doses based on published literature.19-23

**Blood Pressure Recording and Spontaneous Baroreflex Study**

The femoral artery was catheterized on ICV day 10 for arterial pressure recordings. The animals were allowed to recover for ≥48 hours, and pulsatile arterial pressure was acquired (on ICV day 12, between 9:00 am to 11:00 am) via strain gauge transducer connected to an arterial catheter and data acquisition system (Acknowledgement software version 3.7.2, BIOPAC System Inc) in conscious rats. HR was determined from the arterial pressure wave. Spontaneous BRS was calculated by the frequency-domain analysis method24 using software designed for rats (Nevrokard SA-BRS, Medistar), as reported in our previous work.25-27 In brief, power spectral densities of systolic arterial pressure (SAP) and RR interval (RRI) were computed by 512-point fast Fourier transform and integrated over the specified frequency range for rats (ie, low frequency [LF], 0.25–0.75 Hz and high frequency [HF], 0.75–3.0 Hz). A Hanning window was applied, and the spectra of SAP and RRI series, as well as their squared coherence modulus, were computed if the coherence was >0.5, in accordance with reported criteria.24 The square roots of the ratio of RRIIs and SAP powers were computed to calculate LF and HF α indices, which reflect the BRS.24 The power of RRI spectra in the LF and HF range (LFREE and HREE) was calculated in normalized units, and the ratio of LF_{REE}/HF_{REE} was used as a measure of sympathovagal balance.26 The power of SAP spectra was calculated as low-frequency systolic arterial pressure as a measure of blood pressure variability (BPV).23 Heart rate variability (HRV) was also determined by sequence method analysis SD of a beat-to-beat interval. The SD of the MAP was used as a measure for BPV. Three time-domain parameters were used to measure hemodynamic variability, as in previous studies.22 BRS was calculated by sequence methods on the basis of quantification of sequences of ≥2 beats (n) in which SAP consecutively increases (up sequence) or decreases (down sequence), which are accompanied by changes in the same direction of the RRIIs of the subsequent beats (n+1).20,21

**Tissue Removal and Sample Collection**

All rats had free access to food and water until euthanized. Urine was collected on dry ice from rats housed in metabolism cages overnight on day 13 of the study. Rats were decapitated on ICV day 14 and the tissue and blood (serum) samples were collected at approximately the same time of day (8:00 am to 10:00 am) for all animals. Hearts (whole and left ventricle), brain (whole), and adipose tissue were weighed, and brain dorsal medulla was either used fresh (for NADPH oxidase assay) or frozen (for protein analysis) on dry ice for later use.

**Biochemical Measurements in Serum and Urine**

Glucose was measured in the serum of each animal using a Freestyle glucose monitor.7 Serum insulin and leptin were measured using radioimmunoassays specific for rat according to the manufacturer’s protocol (Linco, Inc.). Plasma angiotensin peptides (Ang I, Ang II, and Ang-(1–7)) were measured as reported.31,32 Because the decapitation procedure cuts through the tubing connecting the ICV cannula to the minipump, thus contaminating plasma collections, no values are reported for Ang-(1–7) in plasma. The oxidative stress marker 8-isoprostane F₂αt was determined in urine (normalized to urinary creatinine excretion [CR]) and plasma samples (Cayman Chemical, Ann Arbor, MI).33 Urinary creatinine, electrolytes, protein, and angiotensin peptides were normalized to urinary CR excretion, as described previously.31,32,34 Creatinine clearance (milliliters per minute per kilogram) was calculated by (creatinine excretion/serum creatinine) per kilogram of body weight.

**NADPH Oxidase Activity**

Freshly isolated brain dorsal medullary tissues were homogenized in cold lysis buffer (20 mmol/L of KH₂PO₄ [pH 7.0], 1 mmol/L of EGTA, containing Sigma protease inhibitor mixture), centrifuged at 1000g for 10 minutes at 4°C, and the pellet was resuspended in a lysis buffer containing protease inhibitors. NADPH oxidase activity was measured by a luminescence assay using 20 μL of homogenate (1 mg/mL of protein concentration) in a 50-mmol/L phosphate buffer (pH 7.0) containing 1 mmol/L of EGTA, 150 mmol/L of sucrose, 5 μmol/L of dark-adapted lucigenin (9,9’-bis[N-methylacridinium nitrate]; Sigma, St. Louis, MO) as the electron acceptor, and 100 μmol/L of NADPH (Sigma) as the substrate in a final volume of 180 μL. The relative luminescence units were obtained every 38 s for 15 minutes using a luminometer (Centro XS1/LB960 Microplate Luminometer, Berthold Technologies).35,36 Background-corrected values were normalized to the aCSF controls and expressed as percentage change. Superoxide anion production is expressed as relative luminescence unit per 20 μg of protein (% normalized to aCSF control).

**Western Blot Hybridization and Protein Analysis**

The dorsal medulla was homogenized in a lysis buffer containing protease and phosphatase inhibitors (250.0 mmol/L of sucrose, 0.5 mmol/L of EDTA, 50.0 mmol/L of NaF, and 10.0 mmol/L of Tris [pH...
7.4] containing 0.01 mmol/L of NaVO₄, 0.1 mmol/L of PMSF, and 0.6 μmol/L of leupeptin). SDS-PAGE and Western blot hybridization of proteins transferred to polyvinylidene fluoride membranes were carried out as described previously. Protein in the sample was determined by the Bio-Rad Bradford protein assay. Antibodies used were mitogen-activated protein kinase phosphatase-1 and phosphospecific extracellular-signal-regulated kinase 1 and 2, c-Jun N-terminal kinase-1 and p38 MAP kinases (Cell Signaling, Danvers, MA), and β-actin (Sigma). The optical density of the immunoreactive bands was normalized to the optical density of anti-β-actin immunoreactive bands to account for variation in the protein loading.

**Statistical Analyses**

Comparisons of blood pressure, HR, indices of spontaneous BRS, HRV, BPV, NADPH oxidase activity, body and tissue weights, biochemical measurements, and protein quantification by Western hybridization in the 4 groups were performed using 1-way ANOVA and Student-Newman-Keuls post hoc tests. The criterion for statistical significance was \( P < 0.05 \), and all tests were performed using Prism 4.0 and InStat 3 (GraphPad Software, San Diego, CA). Numerical values are presented as mean±SEM.

**Results**

**MAP and HR in Hypertensive (mRen2)27 Rats**

ICV infusion of CAN for 2 weeks significantly reduced MAP in 28- to 36-week-old (mRen2)27 rats compared with Ang-(1–7), tempol, or aCSF infusion (Figure 1). No significant differences in HR among the various groups were observed.

**BRS, HRV, and BPV**

Spontaneous BRS was determined using both spectral analysis and sequence methods (Figure 2). Ang-(1–7)-infused rats had an elevated high-frequency \( \alpha \) (HF-\( \alpha \)) index compared with aCSF-, CAN-, or tempol-infused rats, suggesting enhanced parasympathetic tone for BRS. No differences in low-frequency \( \alpha \) (LF-\( \alpha \)), an index of sympathetic tone for the BRS, were observed among the groups. Overall

![Figure 1. Central candesartan (CAN) infusion reduces mean arterial pressure (MAP) in transgenic (mRen2)27 rats. Heart rate was not influenced by artificial cerebrospinal fluid (aCSF), CAN, angiotensin (Ang)-(1–7), or tempol infusions for 2 wk. Mean±SEM (n=9 to 13 per group); \( ^* \) P<0.05 vs aCSF, Ang-(1–7), and tempol.](http://hyper.ahajournals.org/)

![Figure 2. Central angiotensin (Ang)-(1–7) infusion improves indices of vagal but not sympathetic function, whereas candesartan (CAN) infusion reduced low-frequency systolic arterial pressure (LF-SAP)% , an index of blood pressure variability (BPV) and the vasomotor tone, consistent with lowering of blood pressure in transgenic (mRen2)27 rats. Mean arterial pressure (MAP) and heart rate recordings were analyzed for measurements of spontaneous baroreflex sensitivity (sBRS) (as LF-\( \alpha \), high-frequency (HF)-\( \alpha \), and sequence [Seq] ALL), heart rate variability (HRV) (as SD of beat-to-beat interval [SDRR]), and BPV (as power of the systolic arterial pressure [SAP] spectra in the LF range (LF-SAP%) and SD of MAP [SDMAP]). Mean±SEM (n=9–13 per group); \( ^* \) P<0.05 vs aCSF (artificial cerebrospinal fluid); \( \dagger \) P<0.05 vs CAN; \( \ddagger \) P<0.05 vs tempol.](http://hyper.ahajournals.org/)
sympathovagal balance to the heart, measured by LF_HRV/HF_HRV ratio, was not significantly different among groups (data not shown). BRS, measured by sequence method (Seq ALL), was also higher in Ang-(1–7)-infused rats compared with aCSF- or tempol-infused rats. HRV measured as SD of a beat-to-beat interval was significantly higher in Ang-(1–7)-infused rats compared with other groups. BPV was measured as SD of the MAP was not different among the groups; however, CAN treatment showed a significantly reduced low-frequency sympathetic nervous system (another index of BPV) compared with Ang-(1–7)- and aCSF-infused rats, consistent with lowering of pressure in these animals.

NADPH Oxidase Activity
NADPH oxidase activity in brain dorsal medulla was significantly reduced in (mRen2)27 rats with 2 weeks of tempol infusion compared with CAN or aCSF infusion (Figure 3). Pretreatment of the tissue extracts with diphenyleneiodonium (DPI, 10 µmol/L) was used for specificity of the reaction. Mean±SEM (n=6 to 7 per group); *P<0.05 vs artificial cerebrospinal fluid (aCSF) and candesartan (CAN).

MAPK Signaling
No significant differences were observed in phosphorylated MAPKs (extracellular-signal-regulated kinase 1/2, Jun N-terminal kinase-1, and p38), as well as the regulatory phosphatase, MAPK phosphatase-1 (negatively regulates MAPK pathway) levels in dorsal medulla among the various treatment groups (Table 1).

Metabolic and Biochemical Profiles
Metabolic and biochemical profiles of (mRen2)27 rats with aCSF, CAN, Ang-(1–7), or tempol infusion for 2 weeks are shown in Table 2. There were no significant differences in body weight, food, or water intake among groups. We did not detect differences in heart:body weight ratio; however, the CAN-infused rats had significantly lower left ventricle:body weight ratio (P<0.05) compared with aCSF-, Ang-(1–7), or tempol-infused rats. Brain:body weight ratio remained unchanged by treatments. There was a trend for lower retroperitoneal adipose tissue in CAN-infused rats compared with aCSF-infused rats (P=0.06); however, the adiposity index ([fat mass/lean mass]×100) was similar among groups. Although there was significantly higher serum glucose in CAN-infused rats (P=0.0489), there were no significant differences in serum insulin or leptin among the various treatment groups. Neither plasma concentration nor excretion of the ROS metabolite 8-isoprostane was different among groups. CAN-treated rats had significantly higher plasma Ang I compared with aCSF-, Ang-(1–7), or tempol-treated rats. Plasma Ang II was not different among groups. aCSF-, CAN-, and tempol-treated rats had similar plasma Ang-(1–7) concentrations. Excretions of Ang I and Ang II were significantly higher with a trend for higher Ang-(1–7) (P=0.06) in CAN-treated rats compared with aCSF-, Ang-(1–7),- or tempol-treated rats. Protein excretion was unchanged by treatments.

Discussion
The main finding of the present study is that the various treatments allowed us to demonstrate the distinct influence on blood pressure and vagal autonomic function apart from changes in NADPH oxidase or MAPK signaling within the dorsal medulla. Although central blockade of AT1 receptors normalized blood pressure, Ang-(1–7) enhanced vagal components of the BRS for control of HR and HRV independent of altering blood pressure in hypertensive (mRen2)27 rats. In addition, scavenging of ROS centrally had no effect on blood pressure or indices of the baroreceptor reflex function or HRV but significantly reduced NADPH oxidase activity in the brain dorsal medulla of hypertensive (mRen2)27 rats. None of the treatments altered MAPK signaling pathways in the dorsal medulla or substantially altered indices of metabolic function (circulating insulin, leptin, glucose, or fat mass). Although systemic treatment with RAS blockers improves oxidative stress in kidney and lowers blood pressure,20,21 there was no significant effect of the ICV treatment on markers of renal oxidative stress with any treatment, but the reduction in MAP in the CAN group was associated with increases in the urinary RAS peptides.

Although the ICV dose for effective BP lowering by CAN is well established in our transgenic (mRen2)27 rats,22 doses for Ang-(1–7) and tempol were based on published studies.23–25 Because we did not use additional doses of CAN, Ang-(1–7),

Table 1. MAPK Signaling Protein Expression in Brain Dorsal Medulla

<table>
<thead>
<tr>
<th>MAPK Pathway</th>
<th>aCSF</th>
<th>CAN</th>
<th>Ang-(1–7)</th>
<th>Tempol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylated ERK1/2</td>
<td>0.45±0.10</td>
<td>0.34±0.12</td>
<td>0.40±0.14</td>
<td>0.58±0.11</td>
</tr>
<tr>
<td>Phosphorylated JNK-1</td>
<td>0.27±0.04</td>
<td>0.54±0.25</td>
<td>0.20±0.06</td>
<td>0.64±0.40</td>
</tr>
<tr>
<td>Phosphorylated p38</td>
<td>0.38±0.12</td>
<td>0.51±0.12</td>
<td>0.43±0.17</td>
<td>0.40±0.20</td>
</tr>
<tr>
<td>MKP-1</td>
<td>0.68±0.22</td>
<td>0.51±0.13</td>
<td>0.60±0.21</td>
<td>0.53±0.21</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=3–7 per group). MAPK indicates mitogen-activated protein kinase; aCSF, artificial cerebrospinal fluid; CAN, candesartan; Ang, angiotensin; ERK1/2, extracellular-signal-regulated kinase 1 and 2; JNK-1, c-Jun N-terminal kinase-1; MKP-1, mitogen-activated protein kinase phosphatase-1.
or tempol, or different durations of treatments, we do not know whether improvements in all end points studied might eventually be achieved with higher doses or longer treatment times. Nonetheless, the pattern of changes in MAP and autonomic function were clearly dissociated from dorsal medullary changes in NADPH oxidase and MAPK and circulating indices of metabolism. In addition, the different chemical makeup (metabolism/clearance) and off target effects of ICV infusion may limit the interpretation of results in terms of specific sites of action of the treatments within the central nervous system. It is entirely possible that changes in NADPH oxidases or MAPK in other brain areas (eg, paraventricular nucleus of the hypothalamus or ventral medulla) would accompany the reduction in MAP or improvement in BRS or HRV. We predict that ICV CAN would primarily block central receptors. However, circulating Ang II acts at central sites, such as paraventricular nucleus and subformicle organs, to produce increase in blood pressure. Therefore, we cannot rule out possible blockade of some actions of circulating Ang II. In fact, blockade of AT1 receptors in the paraventricular nucleus by CAN may account for the blood pressure-lowering effects of treatment, because this would block pressor actions of central or circulating Ang II.40 It is well established that both circulating41 and central Ang II have sites of action within the blood-brain barrier.18 We do not know whether ICV CAN blocked peripheral actions of Ang II in addition to central blockade in our longer-term studies. However, our focus on the brain dorsomedial medullary region for MAPK and NADPH oxidase reflects previous studies demonstrating the role of Ang II and Ang-(1–7) to influence MAP and BRS at this site.3,4 Moreover, (mRen2)27 rats exhibit increased NADPH oxidase activity when compared with normotensive Sprague-Dawley rats at this brain site.5

AT1 receptor blockade by ICV CAN normalized blood pressure as expected but did not correct the sympathovagal

| Table 2. Tissue Weights and Biochemical Profile of 28- to 36-wk-Old Male (mRen2)27 Rats With 2 wk of ICV Treatment |
|-------------------------------------------------|-------------|-------------|-------------|-------------|
| Variables aCSF CAN Ang-(1–7) Tempol |
| BW, g | 501±13 | 487±16 | 494±6 | 503±19 |
| Food intake, g | 13±3 | 12±4 | 12±3 | 14±2 |
| Water intake, mL | 34±4 | 22±6 | 30±3 | 29±5 |
| Heart:BW ratio, % | 0.400±0.06 | 0.300±0.02 | 0.312±0.02 | 0.320±0.02 |
| Left ventricle:BW ratio, % | 0.250±0.01 | 0.211±0.01* †‡ | 0.245±0.01 | 0.253±0.01 |
| Brain:BW ratio, % | 0.423±0.02 | 0.450±0.03 | 0.371±0.01 | 0.440±0.03 |
| Retro fat:BW ratio, % | 1.28±0.12 | 0.74±0.13* †‡ | 1.00±0.08 | 1.02±0.20 |
| Epi fat:BW ratio, % | 1.14±0.25 | 0.73±0.21 | 0.86±0.23 | 0.88±0.23 |
| Ing fat:BW ratio, % | 0.80±0.20 | 0.60±0.15 | 0.60±0.20 | 0.60±0.20 |
| IBAT fat:BW ratio, % | 0.08±0.01 | 0.08±0.01 | 0.07±0.01 | 0.10±0.02 |
| Adiposity Index, % | 3.5±0.5 | 2.2±0.5 | 2.6±0.4 | 2.7±0.5 |
| Serum glucose, mmol/L | 117±4 | 137±8* | 117±4 | 117±5 |
| Serum insulin, ng/mL | 1.0±0.22 | 1.3±0.34 | 1.0±0.14 | 1.0±0.12 |
| Serum leptin, ng/mL | 5.1±1.2 | 4.2±0.7 | 4.8±1.1 | 3.7±1.2 |
| Plasma 8-isoprostanates, pg/mL | 245±23 | 209±27 | 200±8 | 209±20 |
| Urine 8-isoprostanates, ng/mg of CR | 1.5±0.2 | 1.3±0.3 | 1.5±0.1 | 1.1±0.1 |
| Plasma Ang I, pg/mL | 477±221 | 930±211†‡ | 204±41 | 296±59 |
| Plasma Ang II, pg/mL | 38±18 | 48±8 | 26±6 | 52±23 |
| Plasma Ang-(1–7), pg/mL | 53±7 | 65±6 | N.D. | 47±7 |
| Urine Ang I, pmol/mg of CR | 0.1±0.07 | 0.26±0.08†| 0.02±0.00 | 0.04±0.01 |
| Urine Ang II, pmol/mg of CR | 0.12±0.04 | 0.26±0.04* | 0.09±0.01 | 0.12±0.02 |
| Urine Ang-(1–7), pmol/mg of CR | 0.16±0.05 | 0.36±0.08* †‡ | 0.13±0.02 | 0.11±0.01 |
| Urine sodium, mmol/d per kg | 2.8±0.6 | 1.0±0.3 | 1.9±0.6 | 2.0±0.3 |
| Urine potassium, mmol/d per kg | 2.8±0.6 | 2.5±0.3 | 3.0±0.8 | 3.1±0.5 |
| CR excretion, mg/d per kg | 26±3 | 23±4 | 21±3 | 23±4 |
| CR clearance, mL/min per kg | 7.0±1.3 | 5.3±1.5 | 6.1±0.8 | 5.5±1.4 |
| Protein excretion, mg/d per kg | 10.4±2.8 | 12.0±4.9 | 8.8±1.9 | 22±7.5 |

Retro indicates retroperitoneal fat; Epi, epididymal fat; Ing, inguinal fat; IBAT, interscapular brown adipose tissue; Adiposity index, (fat mass/lean mass)×100; CR, creatinine; aCSF, artificial cerebrospinal fluid; CAN, candesartan; ICV, intracerebroventricular; ND, not determined. Values are mean±SEM (n=5–10 per group). *P<0.05 vs aCSF. †P<0.05 vs Ang-(1–7). ‡P<0.05 vs tempol.
imbalance (BRS or HRV) or improve metabolic function. The lowering of blood pressure in hypertensive (mRen2)27 rats was accompanied by reduced left ventricle:body weight ratio and showed a trend toward reduced retroperitoneal adipose tissue but not changes in metabolic hormones, glucose, or body weight. There was evidence of reduced blood pressure variability as measured by low-frequency systolic arterial pressure, suggesting reduced sympathetic tone, a factor that may contribute to the above target organ and adipose tissue changes as well. The lack of response to acute Ang II receptor blockade in the NTS to improve BRS in (mRen2)27 rats has been attributed to a deficit in medullary Ang-(1–7) in these animals that can be restored by angiotensin-converting enzyme inhibitor treatment within the NTS of (mRen2)27 rats.4,42,43 This effect was independent of changes in resting blood pressure and reversed by blockade of Ang-(1–7) receptors,43 suggesting that the Ang-converting enzyme inhibition restored the Ang-(1–7) facilitatory tone in the (mRen2)27 rats. Although the present study markedly extends the time frame for lack of effects of AT1 receptor blockade on BRS (from 2 to 3 hours in the acute study to 2 weeks), systemic AT1 receptor blockade carried out for 12 months in normotensive rats showed evidence of upregulation of components of the dorsal medullary RAS that would favor Ang-(1–7) formation.44 However, neither BRS nor Ang-(1–7) concentration in brain tissue was measured after treatment. Therefore, it is currently unclear whether longer term AT1 receptor blockade would eventually correct the medullary peptide imbalance in the hypertensive animals and improve BRS.

Although central Ang-(1–7) acutely and often transiently lowers blood pressure in animal models of hypertension,12,19,21 the lack of blood pressure–lowering effects in our study could be attributed to differences in animal models or dose-/duration-dependent effects of Ang-(1–7). The independent improvement in vagal indices of autonomic function apart from lowering MAP is supported by a recent study in heart failure,45 suggesting that this particular finding is not specific to the (mRen2)27 model of hypertension. In addition, Britto et al46 demonstrate that the effects of long-term oral angiotensin-converting enzyme inhibition on BRS but not MAP were reversed by ICV blockade of Ang-(1–7) receptors. However, there are studies suggesting that Ang-(1–7) may have depressive effects when administered in the brain stem4,49 but not in the lateral ventricles.20 In addition, both Ang II and Ang-(1–7) exhibit similar pressor responses in rostral, or caudal, ventrolateral medulla, suggesting differential effects of these peptides in different areas of the brain.20,48,49 Thus, it is difficult to predict overall effects of the peptide on MAP during ICV infusion. Regardless of the sites of action for the improvement in vagal function, the ICV effects of Ang-(1–7) were not associated with changes in NADPH oxidase or MAPK in the medulla or circulating indices of metabolism or body weight.

Ang I and Ang II excretion increased in CAN-treated rats with a similar trend for Ang-(1–7). There was no change in urinary peptides in the other treatment groups, suggesting this may be related to activation of the intrarenal or circulating or both systems accompanying the reduced pressure in the CAN-treated rats. Plasma concentrations of Ang peptides remained unchanged in CAN-treated rats with the exception of Ang I, which was increased. The increase in Ang I in concert with the large reduction in pressure suggests upregulation of renin release to counteract the reduction in MAP in these animals, rather than direct brain-to-kidney regulation. However, the peptides were measured at the end of the study, after the initial fall in MAP with CAN treatment. Therefore, set point resetting for MAP would be expected by this time. Thus, it is surprising that activation of the renal RAS would persist via this mechanism alone. The ICV treatments did not alter urinary or plasma isoprostanes or urinary electrolytes and protein.

In the central nervous system, NADPH oxidase–derived ROS are implicated in Ang II–mediated pressor effects in several models of hypertension.50–53 Ang-(1–7) reduces NADPH oxidase in the periphery of diabetic hypertensive rats,45 but its effects in the dorsal medulla have not been evaluated. Surprisingly, we failed to see a lowering of NADPH oxidase activity in the brain dorsal medulla with central blockade of AT1 receptors at a dose that completely normalized MAP or with Ang-(1–7) ICV infusion at a dose that improved BRS and HRV in the (mRen2)27 rats. In addition, NADPH oxidase–activated MAPK pathways are implicated in both short- and long-term pressor effects of Ang II in brain.13,14 However, we observed no changes in activity (phosphorylated MAPKs) or expression of regulatory phosphatase (MAPK phosphatase-1) in rats after AT1 receptor blockade or Ang-(1–7) augmentation. The failure of the 2-week AT1 receptor blockade or Ang-(1–7) infusion to alter these components suggests that these signaling pathways in the dorsal medulla may be influenced by inputs other than changes in resting MAP, activation of AT1, or Ang-(1–7) receptors, or reduced NADPH oxidase via tempol. Collectively, these results suggest that the antihypertensive effect of CAN and improvement in vagal function with Ang-(1–7) are independent of either MAPK or NADPH-derived ROS in the brain dorsal medulla of male hypertensive (mRen2)27 rats.

The above discussion highlights the fact that the normalization of MAP or improvement in BRS and HRV occur independent of changes in dorsal medullary NADPH oxidase activity. Nevertheless, we cannot rule out that a higher dose of tempol or direct inhibitors of NADPH oxidase would have resulted in changes in hemodynamic or metabolic end points. Increased ROS are implicated in pathogenesis of neurogenic hypertension in several animal models, including Ang II–dependent increases in blood pressure.50–53,55 The contribution of central ROS in transgenic (mRen2)27 rats, a model of chronically overactive brain-RAS and higher Ang II actions relative to Ang-(1–7) in brain medullary tissues,2–4 is not known. Hypertension in (mRen2)27 rats is suggested to be independent of oxidative stress in peripheral tissues, because 3 weeks of treatment with tempol in the drinking water did not lower MAP; however, the current study is the first to assess changes in central ROS on blood pressure and baroreflex regulation chronically in this strain.56,57 Central infusion of the ROS scavenger tempol did not lower pressure or influence indices of baroreflex function in (mRen2)27 rats. The failure to influence baroreflex function by this treatment is consistent with the absence of an effect on the MAPK systems as well. One possibility for lack of hypertensive effects of tempol could be the insufficient dose of tempol in this animal model. Tempol treatment reduced NADPH oxidase activity in brain dorsal medulla in (mRen2)27 rats.
however, the magnitude of the reduction was only 22%, and whether a larger reduction would be accompanied by correction of MAP or BRS remains to be determined. In spontaneously hypertensive rats, Ang II may increase oxidative stress through lowering antioxidant enzyme activities in brain. It is possible that (mRen2)27 rats have increased ROS through NADPH oxidase or other enzymatic sources, and the antioxidant enzyme activities are either unaltered or upregulated so that ROS does not play a significant role in the pathogenesis of neurogenic hypertension in this strain.

Although acute treatment (10–30 minutes) with tempol has been reported to prevent Ang II/NADPH oxidase/ROS-stimulated activation of MAPKs in brain, longer-term effects of central tempol infusion on NADPH oxidase–derived ROS and effects on MAPK activation in brain have not been reported. Because tempol reduced NADPH oxidase activity in brain dorsal medulla by only 22% compared with aCSF controls, the reduction in NADPH oxidase activity may not be sufficient to see differences in phosphorylated MAPKs. Again, the duration or level of MAPK phosphorylation may be transient and influenced by various other inputs. Several studies have postulated that the phosphoinositol 3-kinase signaling pathway is recruited in hypertensive animals and contributes to hypertension and impaired BRS in (mRen2)27 rats. In this regard, future studies will assess whether CAN or Ang-(1–7) treatments would lower activated phosphoinositol 3-kinase pathway components as part of their mechanism of action for blood pressure or autonomic effects in these animals.

In summary, we show distinct actions for antihypertensive, NADPH oxidase lowering, and autonomic improvement by the 3 treatments used in the present study. AT1 receptor blockade by ICV essentially normalized blood pressure independent of a dorsal medullary NADPH oxidase–ROS–MAPK pathway and without correcting the vagal component of the BRS, HRV, or indices of metabolic function. Augmentation of central Ang-(1–7) improved baroreflex control of HR at a dose that did not alter blood pressure and circulating metabolic hormones, NADPH oxidase, or MAPK signaling pathways in this model of hypertension. Tempol infusion by ICV modestly reduces NADPH oxidase without an accompanying change in MAPK signaling pathways, autonomic, metabolic, or blood pressure actions. In conclusion, in the (mRen2)27 rat model of hypertension, we demonstrate that manipulation of blood pressure and BRS/HRV can be achieved independently of changes in dorsal medullary NADPH oxidase or MAPK activity and indices of metabolic function by brain treatments that chronically inhibit or activate AT1 and Ang-(1–7) receptors, respectively. Thus, to achieve the most beneficial therapeutic profile, targeting oxidative stress, AT1 receptors, as well as replacing Ang-(1–7) centrally, may be required.

Perspectives

There are 2 main concepts to be derived from the present study. First, brain selective modulation of Ang II/AT1, or Ang-(1–7) systems in a model of genetic hypertension associated with insulin resistance differentially influenced MAP, BRS/HRV, and metabolic function. The caveats and limitations of the study were noted above with respect to site specificity of the actions or whether higher doses or longer-term treatments of some of the agents might produce additional effects. However, that the normalization of MAP or BRS can occur independent of changes in metabolic and renal function during brain-selective interventions argues against involvement of a common pathway for regulation of cardiometabolic function. In addition, that the effects on MAP and BRS/HRV occurred in the absence of changes in medullary NADPH and MAPK pathways further dissociates a requisite involvement of these pathways at this brain site in the regulation of pressure and autonomic function. This does not rule out the importance of ROS and other signaling pathways at other brain sites, but the data provide new insights with respect to the dorsal medulla.

The second concept is that determining the key factors influencing the vagal component of baroreflex function may facilitate the development of new therapeutic mechanisms for reducing cardiometabolic pathologies. Decreased vagal function (impaired BRS for control of HR and reduced HRV) is associated with increased overall mortality, independent of blood pressure. Therefore, targeting improved vagal function in addition to lower MAP by elevating brain Ang-(1–7) may provide better end-organ protection. The present data further suggest brain Ang-(1–7) as an important therapeutic target to improve cardiovagal baroreflex function in forms of hypertension where a deficiency is observed, although the peptide may be devoid of additional blood pressure–lowering actions in the chronic setting. However, there were no improvements in renal function or indices of renal injury (proteinuria, increased RAS peptides) during any of the ICV treatments, in contrast to well-established improvements seen with systemic Ang-(1–7) treatments independent of blood pressure reductions. Thus, the greatest benefits of Ang-(1–7) supplementation may be achieved with systemic treatments likely to have local vascular, cardiac, and renal effects in addition to modulation of central autonomic function.

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Disclosures

None.

References


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What Is New?

• We evaluated the effects of long-term intracerebroventricular infusion of angiotensin-(1–7) versus AT1 receptor blockade on mean arterial pressure, baroreflex sensitivity, and peripheral indices of metabolic function in transgenic (mRen2)27 rats along with dorsal medullary NADPH oxidase and mitogen-activated protein kinase pathways.

What Is Relevant?

• We demonstrate that manipulation of blood pressure and baroreflex sensitivity can be achieved independently of changes in dorsal medullary NADPH oxidase or mitogen-activated protein kinase activity and indices of metabolic function by brain treatments that chronically inhibit or activate AT1 and angiotensin-(1–7) receptors, respectively, or provide reactive oxygen species scavenging.

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Summary

In a model of hypertension with chronic overactivity of the brain renin-angiotensin system, treatments that restore angiotensin-(1–7) and reduce both angiotensin II and oxidative stress may be required for maximal cardiometabolic improvements.
Central Angiotensin-(1–7) Improves Vagal Function Independent of Blood Pressure in Hypertensive (mRen2)27 Rats

Manisha Nautiyal, Hossam A. Shaltout, Daniel C. de Lima, Kenia do Nascimento, Mark C. Chappell and Debra I. Diz

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