Brain Microglial Cytokines in Neurogenic Hypertension

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Abstract—Accumulating evidence indicates a key role of inflammation in hypertension and cardiovascular disorders. However, the role of inflammatory processes in neurogenic hypertension remains to be determined. Thus, our objective in the present study was to test the hypothesis that activation of microglial cells and the generation of proinflammatory cytokines in the paraventricular nucleus (PVN) contribute to neurogenic hypertension. Intracerebroventricular infusion of minocycline, an anti-inflammatory antibiotic, caused a significant attenuation of mean arterial pressure, cardiac hypertrophy, and plasma norepinephrine induced by chronic angiotensin II infusion. This was associated with decreases in the numbers of activated microglia and mRNAs for interleukin (IL) 1β , IL-6, and tumor necrosis factor-α, and an increase in the mRNA for IL-10 in the PVN. Overexpression of IL-10 induced by recombinant adenoassociated virus-mediated gene transfer in the PVN mimicked the antihypertensive effects of minocycline. Furthermore, acute application of a proinflammatory cytokine, IL-1β, into the left ventricle or the PVN in normal rats resulted in a significant increase in mean arterial pressure. Collectively, this indicates that angiotensin II induced hypertension involves activation of microglia and increases in proinflammatory cytokines in the PVN. These data have significant implications on the development of innovative therapeutic strategies for the control of neurogenic hypertension. (*Hypertension.* 2010;56:297-303.)

Key Words: angiotensin II ■ hypertension ■ minocycline ■ interleukin 10 ■ microglia ■ paraventricular nucleus ■ cytokine

nflammation has been implicated in hypertension and Lacardiovascular diseases in both animal models and human diseases.^{1,2} Increases in levels of plasma proinflammatory cytokines (PICs) and other markers of inflammation are associated with the progression of hypertension, whereas immune suppression produces beneficial outcomes.^{3,4} Despite evidence for the participation of peripheral cytokines and inflammation in cardiovascular disease, little is known about their involvement in neurogenic hypertension. Studies from Francis and collaborators^{5,6} have indicated that angiotensin (Ang) II-induced hypertension involves activation of tumor necrosis factor- α (TNF- α) and nuclear factor κB and production of reactive oxygen species in the brain. These observations have led us to propose that Ang II-induced neurogenic hypertension involves activation of microglial cells and production of PICs within the brain. Our objective in the present study was to test this hypothesis.

We focused on the paraventricular nucleus (PVN) and a chronic Ang II infusion rat model of hypertension for this study, based on the following rationales. First, the PVN integrates signals/inputs from circumventricular organs and other cardiovascular-relevant brain areas and transmits them

to the rostroventrolateral medulla and other downstream areas to influence sympathetic nerve activity. Second, chronic Ang II infusion is an established animal model of hypertension with strong neurogenic components. Our studies demonstrate that Ang II—induced hypertension involves activation of microglia and increases in PIC within the PVN.

Materials and Methods

Animals

Adult male Sprague-Dawley (SD) rats (Charles River Laboratories) aged 5 weeks (n=118) were individually housed in a temperature-controlled room (22°C to 23°C) with a 14:10-hour light-dark cycle. Tap water and laboratory chow were available ad libitum. All of the experimental procedures were approved by the University of Florida Institute Animal Care and Use Committee.

Surgical Preparation

Implantation of Telemetry Transducers

Six-week-old male SD rats were anesthetized with a mixture of O_2 (1 L/min) and isoflurane (3% to 4%). Rat telemetry transducers (TA11PA-C40, DSI) were implanted into the abdominal aorta on day -10 (for minocycline experiments) or day -28 (for viral experiments), as described previously. A bolus injection of buprenorphine (0.05 mg/kg SC) was administered after each surgery.

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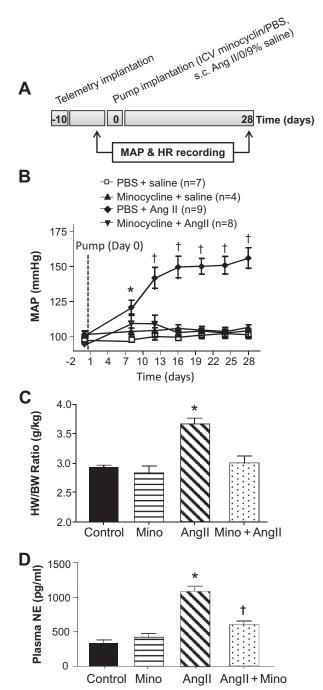


Figure 1. ICV minocycline attenuates Ang II-induced cardiovascular effects. A, Outline of experimental protocol. B, Minocycline abolishes Ang II-induced hypertension. *P<0.05, †P<0.01 vs the other 3 groups at the same time points. C, Minocycline infusion attenuates cardiac hypertrophy induced by Ang II. Bar graphs are mean ± SEM of heart weight vs body weight (HW/BW) ratio after each treatment (n=6 in each group). *P<0.05 vs other 3 groups. Mino indicates minocycline. D, Minocycline inhibits the increase in plasma NE induced by Ang II infusion. *P<0.05 vs control; †P<0.05 vs Ang II by 1-way ANOVA followed by Bonferroni. Control: n=5; n=6 in the other 3 groups.

Intracerebroventricular Minocycline Infusion

Ten days after implantation of telemetry transducers, one group of rats was implanted with intracerebroventricular (ICV) cannulae for infusion of minocycline on day 0 (Figure 1A), as described previously.^{9,10} In brief, rats were anesthetized with a 4% isoflurane/O₂ mixture, and the head was positioned in a Kopf stereotaxic apparatus.

An infusion cannula (ALZET, Durect Corp) was implanted into the left cerebroventricle (1.3 mm caudal to bregma, 1.5 mm lateral to the midline, and 3.5 mm ventral to the dura). A 4-week osmotic minipump (implanted SC) was connected to the infusion cannula via the catheter tube to deliver minocycline (Sigma, 5 μ g/h).

Implantation of SC Osmotic Minipump

On day 0, each group of rats was further randomly assigned to 2 subgroups to receive either Ang II (200 ng/kg per minute) or 0.9% saline delivered via an osmotic minipump (No. 2004, ALZET) implanted SC between the scapulae.

Injection of AAV Vector Into the PVN

For viral experiments, after 14 days of recovery from the transducer implantation surgery, rats were anesthetized with 4% isoflurane mixed with O2 and randomly assigned to 3 groups to receive AAV5-CBA-IL10 (1.2×10e12 genome copy), AAV5-CBA-GFP $(1.2 \times 10e12 \text{ genome copy})$, or 0.1 mol/L of PBS (vehicle control) injected into the PVN. A single injection of vector or PBS was made using a pneumatic picopump (WPI) through a glass micropipette (tip OD: 30 to 50 µm) bilaterally into the PVN (1.6 to 1.8 mm caudal to the Bregma, 0.2 to 0.3 mm lateral to the midline, and 7.6 to 7.8 mm ventral to the dura) by pressure microinjection (200 nL, 30 seconds).

AAV5-CBA-IL10 Vector

Rat interleukin (IL)-10 cDNA was amplified by PCR and cloned into a TA cloning vector, pCR-XL-TOPO (Invitrogen), generating construct pCR-XL-TOPO-IL10; DNA sequencing was performed to confirm that the nucleotide sequences of IL-10 cDNA on the TA cloning vector were correct. Then the rat IL-10 cDNA fragment was digested from pCR-XL-TPOP-IL10 plasmid, separated using 0.8% agarose gel, purified with QIAquick Gel extraction kit (Qiagen), and subsequently cloned into an AAV5-ITR-containing vector, PTR2-CB, under the control of hybrid cytomegalovirus enhancer/chicken-β actin (CBA) promoter, generating a new construct, AAV5-CBA-IL10.

Recombinant AAV Virus Production

The AAV virus production and titer determination were performed by the vector core of the University of Florida, Gene Therapy Center, as described previously.11 Briefly, rAAV production was performed by cotransfection of the rAAV5-ITR vector construct and a combined Ad/AAV helper construct. Transfections were performed by calcium phosphate coprecipitation in an Ad-E1a- and E1bexpressing permissive human cell line (HEK293). rAAV was purified from cells by iodixanol density gradient centrifugation.

RNA Isolation, Reverse Transcription, and **Real-Time PCR**

For analysis of cytokine mRNAs, the hypothalamic tissue including PVN was dissected, as described previously.¹² In brief, rat brains were isolated and cut into a coronal segment (-0.92 to -2.13 mm posterior to bregma, according to Paxinos and Watson¹³). From the coronal section we excised a block of the hypothalamus containing the PVN (≈2.0 mm wide and 1.5 mm high). Total RNA was isolated using RNeasy kits (Qiagen) according to the manufacturer's instructions, and 200 ng of purified RNA were reverse transcribed with a high-capacity cDNA reverse transcription kit (Bio-Rad Laboratories). The IL-1 β , IL-6, tumor necrosis factor- α , and IL-10 mRNA levels were analyzed by quantitative real-time PCR using specific primers and probes in a PRISM 7000 sequence detection system (Applied Biosystems).14 Data were normalized to 18s ribosomal RNA.

Cardiovascular Measurements

Measurements of mean arterial blood pressure (MAP) and heart rate (HR) were made via the telemetry transducers. The start of Ang II infusion via the osmotic pump was set as day 0. Raw data were analyzed using Dataquest IV software (DSI). MAP and HR were sampled between 9:00 AM and 5:00 PM every 3 to 4 days. The data from 10:00 AM to 12:00 PM were used for the analysis of MAP and HR. Plasma norepinephrine levels were measured using high-performance liquid chromatography, as described previously.¹⁵

Immunocytochemistry

Forebrains were cut into 35-μm coronal sections. For IL-10 immunostaining sections were incubated with a mixture of polyclonal rabbit anti–IL-10 antibody (1:200, Abbiotec Inc) and mouse monoclonal anti-NeuN antibody (1:100, Chemicon International) for 24 hours at 4°C. Secondary antibodies were Alex Fluor 594 antirabbit (1:500) for IL-10 and Alex Fluor 488 antimouse (1:1000) for NeuN (all from Molecular Probes). For microglial immunostaining, primary antibody was a mouse monoclonal antibody OX42 (BD Bioscience Inc, 1:250), which is a specific marker for microglia, 16 and the secondary antibody was a goat antimouse IgG (1:2000) conjugated with 3,3′-diaminobenzidine tetrahydrochloride. PVN sections were examined using an Olympus BX41 fluorescence microscope.

Microglia Quantification

OX42 antibody is a specific microglial marker and stains all microglia. When microglia are activated they undergo morphological changes. This is associated with retracted processes giving a thick and stubby appearance. 17,18 In addition, activated microglia have enlarged perikarya and concentrated OX-42 staining. We defined activated microglia as cells that exhibit strong OX-42 immunoreactivity, an enlarged soma, and fewer and shorter (less than the cell soma diameter: 4.0 to 4.5 μ m) processes. The dimensions of 30 activated microglia from 3 consecutive sections in each animal were measured, and average group (n=4) data were used to determine activated microglia in brain regions. The area of the PVN that was chosen for microglial measurements was the same anterior level (-1.8 to 1.9 mm from bregma) for all 3 of the groups. The number of microglia in a $0.2 \times 0.2 \text{ mm}^2$ area was counted from ≥ 3 different adjusted sections from each animal. Morphological analysis and quantification of microglia were performed within the PVN using a light microscope at ×400 magnification. Tissue sections from the cortex and lateral hypothalamus were used as control regions. There were no differences in the average length of microglial processes in these regions in both vehicle- and Ang II-treated animals, indicating a lack of microglial activation by Ang II. The analysis of activated microglia was performed in a double-blind fashion.

Cardiac Pathology

At the end of the experiment, rats were euthanized, and hearts were removed for cardiac morphology and histological analyses, as described previously. In brief, hearts were removed and weighed, the cross-sections of ventricles were fixed in 10% neutral-buffered formalin for 24 hours, embedded in paraffin, sectioned at 4 μm , and stained with hematoxylin-eosin for the myocyte diameter assay or stained with Picro-sirius red to determine interstitial fibrosis. Twenty images from different (nonoverlapping) regions of the left ventricle wall were examined using the Image J program from the National Institutes of Health.

Effect of Acute IL-1 β on Blood Pressure

Blood pressure was recorded via the indwelling transducer implanted 2 weeks ahead, as described earlier. To determine the effect of ICV IL-1 β on blood pressure, IL-1 β (10 ng/mL, 500 nL) was pressure injected to the left ventricle (-1.3 mm posterior to bregma; 1.2 mm lateral to midline; 3.4 mm ventral to brain surface) via a pneumatic picopump. In a different rat, the IL-1 receptor antagonist (IL-1RA; 100 ng/mL, 500 nL) was microinjected into the left ventricle, followed 20 minutes later by ICV delivery of IL-1 β . To further determine the site-specific effect of IL-1 β on blood pressure, this PIC was microinjected (10 ng/mL, 100 nL) into the left PVN (-1.8 mm posterior to bregma; 0.3 mm lateral to midline; 7.8 mm ventral to brain surface).

Statistics

Data were expressed as mean ± SE. Statistical significance was evaluated with the use of a 1-way or 2-way ANOVA followed by a layered Bonferroni post hoc test. Statistical tests were performed with Prism software (v4.0, GraphPad, Inc).

Results

ICV Minocycline Attenuates Ang II-Induced Hypertension

Chronic SC infusion of Ang II in SD rats caused an increase in MAP, which was sustained for 4 weeks (Figure 1B). Simultaneous ICV infusion of minocycline (5 μ g/h) resulted in attenuation of the Ang II–induced increase in MAP (Ang II: 156 ± 7.2 mm Hg [n=9]; Ang II+ minocycline: 104 ± 4.2 mm Hg [n=8]; P<0.05). A modest attenuation was observed as early as 8 days, an effect that became significant by day 12 and persisted thereafter throughout the experiment (Figure 1B). Minocycline treatment had no effects on HR. Furthermore, minocycline alone had no effect on MAP (control: 101 ± 2.7 mm Hg [n=7] versus minocycline: 106 ± 1.3 mm Hg [n=4]).

Minocycline Attenuates Ang II-Induced Hypertrophy

To determine the beneficial effects of minocycline on Ang II-induced cardiac hypertrophy, heart weight:body weight ratio and cardiac interstitial fibrosis were determined. As shown in Figure 1C, Ang II infusion leads to a significant increase in heart weight:body weight ratio. However, Ang II infusion in the presence of minocycline normalized the ratio to control values. Consistently, the fibrotic area was greater in Ang II-treated rats versus minocycline plus Ang II-treated rats (data not shown).

Minocycline Decreases Ang II-Induced Plasma Norepinephrine

Chronic Ang II infusion resulted in a 2.7-fold increases in plasma NE (Figure 1D), one index of increased sympathetic nerve activation. ICV infusion of minocycline at a dose that inhibits Ang II-induced hypertension abolished the increase in plasma NE produced by Ang II infusion (Figure 1D).

Effects of Minocycline on PVN Microglia and Cytokine mRNA Expression

Staining with OX-42 antibody showed a significant increase in the activated microglial cells in the PVN of Ang II–treated rats (Figure 2A). Chronic Ang II infusion resulted in a 23-fold increase in activated microglial cells (Figure 2B), and minocycline reduced these numbers by 80%. In addition, Ang II reduced the length of microglial cell processes, an effect that was attenuated by minocycline (Figure 2B). Ang II infusion increased the total number of activated microglia, and minocycline was able to attenuate this effect (Figure 2C). Figure 3 shows that Ang II infusion caused, respectively, 4.3, 2.8, and 3.0-fold increases in IL-1 β , IL-6, and tumor necrosis factor- α mRNA levels, whereas it decreased IL-10 mRNA by 38%. These effects were reversed in the PVN of Ang II–infused rats that were treated simultaneously with minocycline.

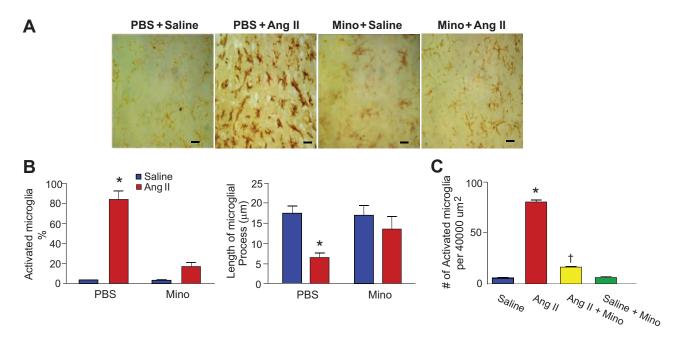


Figure 2. Effect of ICV minocycline on activation of microglia in the PVN. Microglia within the PVN under each treatment condition were identified via OX-42 immunostaining, and their activation was assessed as detailed in the Methods section. A, Representative micrographs showing the activation of PVN microglia under each treatment condition, 4 weeks after the start of Ang II infusions. The scale bar equals 10 μ m. B, Quantification of the length of processes of microglia in the PVN under each treatment condition. *P<0.001 vs other groups by 2-way ANOVA; n=4 in each group. C, Quantification of the numbers of activated microglial based on the morphological analysis. Mino indicates minocycline. *P<0.05 vs control by 1-way ANOVA followed by Bonferoni.

IL-10 Overexpression in the PVN Attenuates Ang II-Induced Hypertension

In view of our data that Ang II-induced hypertension involves microglial activation, increases in PIC and decreases in IL-10 levels in the PVN, we hypothesized that overexpression of IL-10 would overcome and attenuate this hypertensive effect. AAV5-IL-10 or AAV-GFP was injected bilaterally into the PVN 14 days before chronic Ang II infusion (Figure 4A). In the AAV5-IL-10-injected rats there was a significant decrease in the Ang II-induced hypertension compared with rats injected with AAV-GFP or PBS at the same site (Figure 4B). This inhibition became statistically significant at 17 days after the start of Ang II infusion (Ang II+AAV-GFP: 149±6.0 mm Hg [n=6] versus Ang II+AAV-IL-10:

 109 ± 5 mm Hg [n=6]; P<0.001). IL-10 overexpression had no effect on basal MAP in control SD rats, and none of the treatments produced significant changes in HR (data not shown). Overexpression of IL-10 in the PVN also attenuated the cardiac hypertrophy produced by Ang II infusion (Figure 4C) similar to the effect of minocycline (Figure 1C). Heart weight:body weight ratio was reduced by 22% in the Ang II+AAV-IL-10 group compared with the Ang II+AAV-GFP group (3.0 ± 0.12 versus 3.8 ± 0.14 g/kg; P<0.05). Similarly, enhanced IL-10 expression attenuated the increase in myocyte diameter and interstitial fibrosis produced by Ang II infusion (Figure 4C).

The locations of the AAV microinjections and IL-10 overexpression were confirmed by real-time RT-PCR and

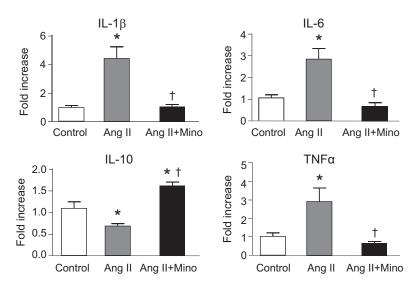


Figure 3. Effect of ICV minocycline on PVN cytokine mRNA expression. After euthanization of the rats used in the experiment in Figure 1, brains were removed, and mRNA levels of cytokines in the PVN were assessed by real-time RT-PCR. $^*P < 0.05$ vs corresponding control; $^*P < 0.05$ vs Ang II by 1-way ANOVA (n=6 in each group). Mino indicates minocycline.

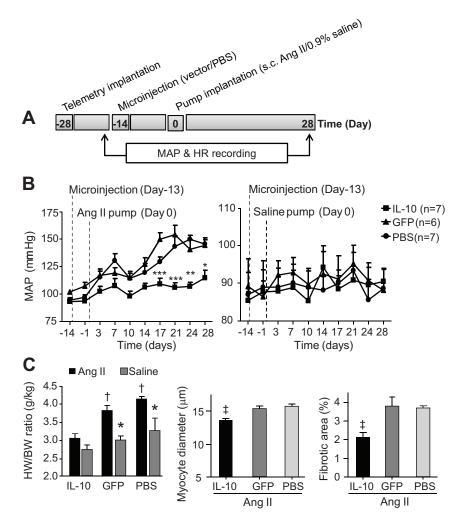


Figure 4. Increased expression of IL-10 in the PVN blunts Ang II-induced hypertension. SD rats were microinjected with AAV5-IL-10, AAV5-GFP, or PBS into the PVN as detailed in the Methods section. A, Outline of experimental protocol. B, Timedependent changes in MAP in each treatment group. Dashed lines indicated the treatment time points. *P<0.05, **P<0.01, ***P<0.001 vs green fluorescent protein (GFP) or PBS groups at the same time points. C, Effect of IL-10 transduction on Ang II-induced cardiac hypertrophy. *P<0.05 vs IL-10+saline; †P<0.05 IL-10+Ang II; ‡P<0.05 vs GFP or PBS plus Ang II infusion (n=4 in each group).

immunocytochemistry (Figure 5). The data indicate that microinjection of AAV5-IL-10 bilaterally into the PVN produced increases of IL-10 mRNA levels in rats that had received either Ang II or 0.9% saline infusion (Figure 5A). Furthermore, AAV5-mediated transduction was highly efficient, robust, and restricted to the PVN region. To further determine the cellular distribution, NeuN, a neuronal marker, was costained with IL-10 in the same coronal section. The majority of IL-10 immunoreactivity was colocalized with NeuN (Figure 5B).

If overexpression of IL-10, an anti-inflammatory cytokine, blocks Ang II-induced hypertension, then one would anticipate that microinjection of PICs (eg, IL-1 β) in the PVN would increase MAP. Figure 6A is a representative experiment demonstrating that microinjection of IL-1 β (10 ng/mL, 500 nL) into the left ventricle resulted in a 25-mm Hg increase in MAP in normal SD rats. This effect was attenuated by IL-1 receptor antagonist (100 ng/mL, 500 nL). Furthermore, microinjection of IL-1 β (100 ng/mL, 100 nL) unilaterally into left PVN induced an 8-mm Hg increase in

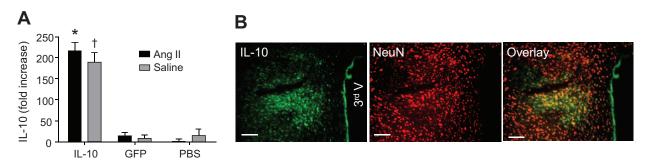


Figure 5. AAV5-IL-10 induced neuronal expression of IL-10 in the PVN. A, IL-10 mRNA levels in the PVN at 28 days after microinjections of AAV5-IL-10, AAV5-GFP, or PBS. *P<0.05 vs GFP or PBS group. †P<0.05 vs GFP or PBS groups. B, Representative fluorescence micrographs showing IL-10 and NeuN immunoreactivity in the PVN following AAV5-CBA-IL10 microinjection. The right panel shows the merged images of IL10 and NeuN. Bar=50 μ m.

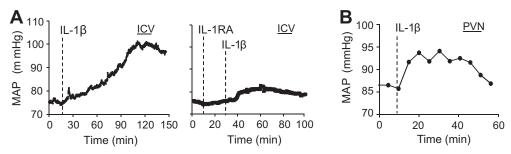


Figure 6. IL-1 β increases MAP in anesthetized rats. A, Representative tracings showing the effects of ICV injection of IL-1 β (10 ng/mL, 500 nL) on MAP in the absence or presence of the IL-1 receptor antagonist (IL-1RA: 100 ng/mL, 500 nL). B, Representative tracing showing effect of PVN injection of IL-1 β (10 ng/mL, 100 nL) on MAP.

MAP in normal SD rats, as shown in Figure 6B. This is consistent with observations from the literature.¹⁹

Discussion

The present study provides evidence that microglial cells in the PVN and cytokines produced within the brain play a central role in neurogenic hypertension. Inhibition of microglial activation or overexpression of IL-10 in the PVN attenuates Ang II-induced hypertension and its associated cardiac pathophysiology. The evidence for these conclusions are as follows: (1) ICV infusion of minocycline, an antiinflammatory antibiotic with demonstrated neuroprotective effects,20 attenuates Ang II-induced hypertension and raised plasma NE; (2) minocycline also inhibits Ang II-stimulated microglial activation in the PVN, consistent with its demonstrated actions in the brain,21 and also the Ang II-induced increases in PIC levels at this nucleus; and (3) IL-10 overexpression in the PVN and not in the rostral ventrolateral medulla (data not shown) attenuates hypertension, whereas IL-1 β increases MAP.

Evidence has emerged from the last several years that implicate the involvement of cytokines in cardiovascular diseases, such as hypertension and heart failure.2,5 For example, levels of PVN PICs are increased in animal models of hypertension, and hypothalamic infusion of IL-1 β increases blood pressure and sympathetic outflow.²² In addition, central gene transfer of IL-10 reduces hypothalamic inflammation in rats with heart failure after myocardial infarction.²³ Our present observations complement these findings. Furthermore, they provide evidence that the production of cytokines within the brain during hypertension involves microglial cells. This includes the observations that ICV infusion of minocycline and overexpression of IL-10 attenuate Ang II-induced hypertension. Thus, it is tempting to propose a novel mechanism of dysregulation in neurogenic hypertension. We propose that circulating Ang II activates cardiovascular-responsive areas in the circumventricular organs, resulting in the generation and transmission of hypertensive signals to the PVN. Microglia in the PVN are activated and release PICs, which directly or indirectly increase the activity of PVN sympathetic neurons. These and other pathways converge into the rostral ventrolateral medulla leading to increases in sympathetic outflow.7 So what is (are) the hypertensive signal(s) from the circumventricular organs that activates PVN microglial? Available evidence suggests that Ang II is a likely candidate. First, levels of PVN Ang II and Ang II type 1 (AT₁) receptors are increased in many animal models of hypertension.²⁴ Second, our preliminary data show that microglial cells express AT₁ receptors and that chronic silencing of the PVN AT₁ receptor by AAV-mediated expression of an AT₁ receptor–specific short hairpin RNA causes a significant decreases in high blood pressure in the spontaneously hypertensive rat (data not shown). However, the role of other signaling molecules downstream of the AT₁ receptor, such as reactive oxygen species,^{25,26} cannot be ruled out at the present time. In addition, the role of other cell types (ie, astroglial cells and neurons) in the PVN in cytokine actions remains to be explored.²

Finally, this study raises important questions. First, are antihypertensive effects of minocycline truly central, or does the ICV-infused antibiotic cross the blood-brain barrier and exert a peripheral effect? The latter seems to be an unlikely possibility, because the dose of minocycline that normalizes Ang II-induced increases in hypertension after ICV administration is only ≈30% as effective when administered subcutaneously (data not shown). Because minocycline is able to freely pass through the blood-brain barrier, would it be possible to administer it in a high enough dose peripherally that it could influence the PVN and, thus, be beneficial for the control of neurogenic hypertension?²⁷ Experiments are under way to support/refute this view. The mechanism of minocycline actions in the PVN needs to be elucidated. Minocycline is a broad-spectrum antibiotic of the tetracycline family and has been implicated to be neuroprotective in several diseases, for example, sclerosis, neurodegenerative disorder, stroke, and Parkinson disease.²⁸ It has been shown to inhibit protein kinase C-α phosphorylation and nuclear translocation of protein kinase $C-\alpha/\beta II$ and interferon- γ regulatory factor, which further inhibit class II activator, another transcription factor, to promote the gene expression for microglial activation.29 In addition to anti-inflammation, minocycline and other tetracycline derivatives are proposed to attenuate apoptosis and inhibit production of reactive oxygen species via an action on mitochondria.²⁸ Thus, the antihypertensive effect of minocycline may involve ≥1 of these signaling pathways leading to inhibition of microglial activation.

Perspectives

The present study described that central application of minocycline, an anti-inflammatory agent and inhibitor of micro-

glial activation, increased expression of the antiinflammatory cytokine IL-10 in the PVN, elicited an antihypertensive effect, and alleviated cardiac damage in an Ang II–infused rat model. Thus, microglia may serve as a source of inflammatory cytokines in the central nervous system cardiovascular control centers and have a key role in the development of neurogenic hypertension. Thus, these results may indicate novel targets for the treatment of hypertension.

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Disclosures

None.

References

- Ferrario CM, Strawn WB. Role of the renin-angiotensin-aldosterone system and proinflammatory mediators in cardiovascular disease. Am J Cardiol. 2006;98:121–128.
- Shi P, Raizada MK, Sumners C. Brain cytokines as neuromodulators in cardiovascular control. Clin Exp Pharmacol Physiol. 2010;37:e52–e57.
- Schillaci G, Pirro M, Gemelli F, Pasqualini L, Vaudo G, Marchesi S, Siepi D, Bagaglia F, Mannarino E. Increased C-reactive protein concentrations in never-treated hypertension: the role of systolic and pulse pressures. *J Hypertens*. 2003;21:1841–1846.
- Stumpf C, John S, Jukic J, Yilmaz A, Raaz D, Schmieder RE, Daniel WG, Garlichs CD. Enhanced levels of platelet P-selectin and circulating cytokines in young patients with mild arterial hypertension. *J Hypertens*. 2005;23:995–1000.
- Kang YM, Ma Y, Zheng JP, Elks C, Sriramula S, Yang ZM, Francis J. Brain nuclear factor-kappa B activation contributes to neurohumoral excitation in angiotensin II-induced hypertension. *Cardiovasc Res.* 2009; 82:503–512.
- Sriramula S, Haque M, Majid DS, Francis J. Involvement of tumor necrosis factor-α in angiotensin II-mediated effects on salt appetite, hypertension, and cardiac hypertrophy. *Hypertension*. 2008;51: 1345–1351.
- Guyenet PG. The sympathetic control of blood pressure. Nat Rev Neurosci. 2006;7:335–346.
- King AJ, Fink GD. Chronic low-dose angiotensin II infusion increases venomotor tone by neurogenic mechanisms. *Hypertension*. 2006;48: 027, 032
- Li H, Gao Y, Qi Y, Katovich MJ, Jiang N, Braseth LN, Scheuer DA, Shi P, Sumners C. Macrophage migration inhibitory factor in hypothalamic paraventricular nucleus neurons decreases blood pressure in spontaneously hypertensive rats. FASEB J. 2008;22:3175–3185.
- Mecca AP, O'Connor TE, Katovich MJ, Sumners C. Candesartan pretreatment is cerebroprotective in a rat model of endothelin-1-induced middle cerebral artery occlusion. *Exp Physiol*. 2009;94:937–946.
- Mu W, Ouyang X, Agarwal A, Zhang L, Long DA, Cruz PE, Roncal CA, Glushakova OY, Chiodo VA, Atkinson MA, Hauswirth WW, Flotte TR, Rodriguez-Iturbe B, Johnson RJ. IL-10 suppresses chemokines, inflam-

- mation, and fibrosis in a model of chronic renal disease. *J Am Soc Nephrol.* 2005;16:3651–3660.
- Jiang N, Shi P, Li H, Lu S, Braseth L, Cuadra AE, Raizada MK, Sumners C. Phosphate-activated glutaminase-containing neurons in the rat paraventricular nucleus express angiotensin type 1 receptors. *Hypertension*. 2009;54:845–851.
- Paxinos G, Watson C. The Rat Brain in Sterotaxic Coordinates. 4th ed. San Diego, CA: Academic Press; 1998.
- Ferreira AJ, Shenoy V, Yamazato Y, Sriramula S, Francis J, Yuan L, Castellano RK, Ostrov DA, Oh SP, Katovich MJ, Raizada MK. Evidence for angiotensin-converting enzyme 2 as a therapeutic target for the prevention of pulmonary hypertension. Am J Respir Crit Care Med. 2009; 179:1048–1054.
- Guggilam A, Haque M, Kerut EK, McIlwain E, Lucchesi P, Seghal I, Francis J. TNF-α blockade decreases oxidative stress in the paraventricular nucleus and attenuates sympathoexcitation in heart failure rats. Am J Physiol Heart Circ Physiol. 2007;293:H599–H609.
- Robinson AP, White TM, Mason DW. Macrophage heterogeneity in the rat as delineated by two monoclonal antibodies MRC OX-41 and MRC OX-42, the latter recognizing complement receptor type 3. *Immunology*. 1986;57:239–247.
- Marco P, Sola RG, Ramon y Cajal S, DeFelipe J. Loss of inhibitory synapses on the soma and axon initial segment of pyramidal cells in human epileptic peritumoural neocortex: implications for epilepsy. *Brain Res Bull.* 1997;44:47–66.
- Nakajima K, Kohsaka S. Microglia: activation and their significance in the central nervous system. J Biochem. 2001;130:169–175.
- Lu Y, Chen J, Yin X, Zhao H. Angiotensin II receptor 1 involved in the central pressor response induced by interleukin-1 βin the paraventricular nucleus. Neurol Res. 2008;31:420–424.
- Graber JJ, Dhib-Jalbut S. Protective autoimmunity in the nervous system. *Pharmacol Ther*. 2009;121:147–159.
- Miyaoka T. Clinical potential of minocycline for schizophrenia. CNS Neurol Disord Drug Targets. 2008;7:376–381.
- Kannan H, Tanaka Y, Kunitake T, Ueta Y, Hayashida Y, Yamashita H. Activation of sympathetic outflow by recombinant human interleukin-1 βin conscious rats. Am J Physiol. 1996;270:R479–R485.
- 23. Yu Y, Zhang ZH, Wei SG, Chu Y, Weiss RM, Heistad DD, Felder RB. Central gene transfer of interleukin-10 reduces hypothalamic inflammation and evidence of heart failure in rats after myocardial infarction. Circ Res. 2007;101:304–312.
- Veerasingham SJ, Raizada MK. Brain renin-angiotensin system dysfunction in hypertension: recent advances and perspectives. Br J Pharmacol. 2003;139:191–202.
- Itani H, Liu X, Sarsour EH, Goswami PC, Born E, Keen HL, Sigmund CD. Regulation of renin gene expression by oxidative stress. *Hypertension*. 2009; 53:1070–1076.
- Zimmerman MC, Lazartigues E, Sharma RV, Davisson RL. Hypertension caused by angiotensin II infusion involves increased superoxide production in the central nervous system. Circ Res. 2004;95:210–216.
- Brogden RN, Speight TM, Avery GS. Minocycline: a review of its antibacterial and pharmacokinetic properties and therapeutic use. *Drugs*. 1975;9:251–291.
- Orsucci D, Calsolaro V, Mancuso M, Siciliano G. Neuroprotective effects of tetracyclines: molecular targets, animal models and human disease. CNS Neurol Disord Drug Targets. 2009;8:222–231.
- Nikodemova M, Watters JJ, Jackson SJ, Yang SK, Duncan ID. Minocycline down-regulates MHC II expression in microglia and macrophages through inhibition of IRF-1 and protein kinase C (PKC)α/βII. J Biol Chem. 2007;282:15208–15216.