Microglia Participate in Neurogenic Regulation of Hypertension

Xiao Z. Shen, You Li, Liang Li, Kandarp H. Shah, Kenneth E. Bernstein, Patrick Lyden, Peng Shi

Abstract—Hypertension is associated with neuroinflammation and increased sympathetic tone. Interference with neuroinflammation by an anti-inflammatory reagent or overexpression of interleukin-10 in the brain was found to attenuate hypertension. However, the cellular mechanism of neuroinflammation, as well as its impact on neurogenic blood pressure modulation, is uncertain. Here, we found that hypertension, induced by either angiotensin II or L-N^3-nitro-l-arginine methyl ester, is accompanied by microglial activation as manifested by microgliosis and proinflammatory cytokine upregulation. Targeted depletion of microglia significantly attenuated neuroinflammation, glutamate receptor expression in the paraventricular nucleus, plasma vasopressin level, kidney norepinephrine concentration, and blood pressure. Furthermore, when microglia were preactivated and transferred into the brains of normotensive mice, there was a significantly prolonged pressor response to intracerebroventricular injection of angiotensin II, and inactivation of microglia eliminated these effects. These data demonstrate that microglia, the resident immune cells in the brain, are major factors in mediating neuroinflammation and modulating neuronal excitation, which contributes to the elevated blood pressure. 

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Online Data Supplement

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Hypertension, particularly resistant hypertension, is associated with enhanced sympathetic tone and can be substantially managed by renal nerve ablation and baroreceptor nerve stimulation. The sympathetic outflow is controlled by several important nuclei and their circuits in the central nervous system (CNS), especially the hypothalamic paraventricular nucleus (PVN) and the rostroventrolateral medulla and the nucleus tractus solitaries in hindbrain. Perturbations of these nuclei have been implicated in hypertension. Although neurons in these regions are the major cells in modulating sympathetic outflow, what factors modulate the elevation of neuronal activity in hypertension stay elusive. Emerging studies indicate that hypertension is accompanied with extensive neuroinflammation and that central anti-inflammatory treatment significantly alleviated hypertension. Thus, determining the cellular mechanism of neuroinflammation and neuronal modulation in hypertension is critical to fully understand central regulation of blood pressure.

The CNS has long been considered an immune privileged organ because of the blood–brain barrier, the brain’s lack of lymphatic drainage to lymph nodes, and suboptimal capacity to present antigen. Microglia are the primary immune cells in the CNS. They are derived from primitive macrophages emanating from the embryonic yolk sac. In the CNS, microglia proliferate and maintain homeostasis with limited contribution from peripheral blood-borne cells. Recent transcriptome analyses revealed that microglia have a distinct phenotype from macrophages in other tissues, suggesting unique characteristics of microglia. As surveillance cells, microglia are highly sensitive to pathological disturbance in the brain and play major roles in the progressive pathology of neurodegenerative diseases, such as Alzheimer’s disease. On stimulation, microglia promptly undergo a series of morphological and phenotypic changes, eventually releasing mediators that can directly modulate neuronal activities. Moreover, many lines of evidence indicate that microglia are highly involved in shaping neuronal behavior via sculpting dendritic spine formation and modulating neurotransmitter receptor presentation on the synaptic terminals in physiological conditions.

In the present study, we examined microglia in hypertension and found that microglia were activated in a different pattern from peripheral monocytes. When microglia were depleted by intracerebroventricular (ICV) administration of diphtheria toxin (DT) into the transgenic CD11b-diphtheria toxin receptor (DTR) mice, the neuroinflammation and blood pressure increase induced by either angiotensin (Ang) II or
L-NAME (L-N⁵-nitro-L-arginine methyl ester (L-NAME)) were significantly attenuated. In contrast, adoptive transfer of activated microglia prolonged pressor responses to central application of Ang II. Taken together, our findings indicate that microglia are the key players in the neurogenic regulation of hypertension.

Methods
All surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Cedars Sinai Medical Center. A detailed Methods section is available in the online-only Data Supplement.

Results
Microglial Activation Pattern in Hypertension
To characterize activation states of microglia in established hypertension, C57BL/6 mice were treated with subcutaneous infusion of Ang II or by feeding L-NAME in drinking water for 4 weeks. Systolic blood pressure reached 130 mm Hg in the 1st week and sustained in the following 3 weeks (Figure S1A in the online-only Data Supplement). Four weeks after the induction of hypertension, mice were euthanized and microglia were analyzed. In both models, there was a significant increase of microglia in the PVN and motor cortex of hypertensive brains compared with the normotensive brains, as manifested by an increased area of Iba1 staining (Figure S1C–S1D). In contrast to the ramiﬁed appearance of naïve microglia, hypertensive microglia showed soma enlargement and process retraction. Thus, hypertension is associated with microgliosis, a characteristic of microglial activation.⁴⁷ To define the characteristics of microglia in hypertension, we dissociated microglia from the brains of normotensive mice or mice made hypertensive with Ang II or L-NAME. Because we previously found that there were increases of tumor necrosis factor α (TNFα) and interleukin (IL)-1β expression in the brain of hypertensive rats,⁵,⁶,¹⁶ we ﬁrst evaluated microglial expression of these proinflammatory cytokines using intracellular staining and ﬂow cytometry analysis. After dissociated from the mouse brains, microglia were cultured in vitro for 6 hours in the presence of brefeldin A, which blocks the secretion of protein from cells.¹⁸ There was elevation of TNFα- and IL-1β-expressing, as well as mild but signiﬁcantly increased IL-6-expressing, microglia in L-NAME-treated mice compared with those in normotensive animals (Figure 1A). Although the numbers of TNFα-, IL-1β-, or IL-6-expressing microglia from Ang II–treated mice were not conspicuously altered in the resting state (data not shown), there were remarkably more cells expressing these cytokines after lipopolysaccharide (LPS) treatment in Ang II hypertensive microglia than normotensive microglia, which indicates their preactivation (Figure 1B).

Current concepts of microglial activation arise, in part, from research into macrophage biology. In macrophages, M1 (proinflammatory classical activation) and M2 (alternative activation) represent extremes in a continuum of activation states.¹⁹ We thus investigated the M1-associated markers (MHC class II, CCR7, IFNγR, and iNOS) and the activation markers representing M2 state (CD36, mannose receptor, Tie2, CCR2, and IL-4Rα) in hypertension-associated microglia. After 4 weeks of Ang II or L-NAME treatment, microglia were dissociated and enriched by Percoll gradient centrifugation followed by ﬂow cytometry analysis. Intriguingly, all these molecules except iNOS were upregulated in the hypertensive microglia (Figure 2A and 2B). This activation proﬁle is speciﬁc to microglia because the monocytes from Ang II–induced hypertensive mice had only increased IL-4Rα and decreased MHC class II expression compared with their normotensive counterparts (Figure 2B).

Loss of Microglia-Alleviated Blood Pressure and Neuroendocrinological Factors Associated With Hypertension
To investigate the role of microglia in hypertension development, we ﬁrst used a microglial depletion strategy. Transgenic CD11b-DTR mice express the DTR under the control of the endogenous CD11b promoter.²⁰ In the CNS, only microglia but not neuronal or other glial cells express DTR in these mice. A single ICV injection of DT resulted in a dose-dependent reduction of microglia in both the PVN and motor cortex (Figure 3A). At a dose of 1000 pg/g, DT caused an over 90% loss of microglia, which was conﬁrmed by both immunohistochemistry (Figure 3A) and ﬂow cytometry analysis (the CD11b⁺CD45⁺ population in Figure 3B).

Figure 1. Enhanced production of proinflammatory cytokines by microglia of hypertensive mice. A, The percentages of CD11b⁺CD45⁺ microglia expressing proinflammatory cytokines in normotensive mice and mice after 4 weeks L-N⁵-nitro-L-arginine methyl ester (L-NAME) treatment. B, The percentages of microglia expressing proinflammatory cytokines from normotensive mice and angiotensin (Ang) II–treated mice for 4 weeks are shown. Cells were stimulated with 10 ng/mL lipopolysaccharide (LPS). *P<0.05; †P<0.01 by unpaired t test. IL indicates interleukin; and TNF, tumor necrosis factor.
To be noted, ICV DT injection (1000 pg/g/d) did not change total blood monocytes (CD11b^Ly6G^F4/80^-) or inflammatory monocytes (CD11b^Ly6G^-Ly6Chigh; Figure 3B). At the dose we used, DT was not toxic to neurons or astrocytes in the CD11b-DTR mice because NeuN or GFAP staining of DT-treated PVN showed no change in the density or distribution of neurons or astrocytes, respectively, in comparison to the untreated CD11b-DTR mice (Figure 3C). To be noted, F4/80 and CD31 staining showed no difference in the density of perivascular macrophages between DT-treated and saline-treated CD11b-DTR mice (Figure 3C). Further, DT injection did not cause any change in body weight and general behavior of mice. Our results showed that ICV infusion of DT to CD11b-DTR mice is a valid model to investigate physiological changes in microglial loss without causing change in the brain or circulating monocytes. Because of the efficiency of microglial depletion with the dose of 1000 pg/g/d, we used this dose in all following studies.

CD11b-DTR mice were treated with Ang II or L-NAME. After 2 to 3 weeks, when hypertension was established and
neuroinflammation had developed, microglia were depleted by ICV infusion of DT. In both models, microglial depletion caused a gradual reduction in blood pressure and by 2 weeks, the mice had a 20 mm Hg lower blood pressure than the ICV saline-infused CD11b-DTR mice (Figure 4A). Notably, ICV DT did not alter resting blood pressure in naive CD11b-DTR or C57BL/6J mice, nor did it change the blood pressure responses when C57BL/6J mice were infused with Ang II (Figure S2). To examine the effects of microglial depletion on neuroinflammation induced by hypertension, brains were harvested and the PVN were dissected at the end of the protocol. The expression of TNFα and IL-1β were analyzed by ELISA. As shown in Figure 4B, hypertension resulted in significant increases of both cytokines. Remarkably, loss of microglia reduced these cytokines to normal levels. These data strongly support our hypothesis that microglia are central in neuroninflammation and blood pressure regulation.

Importantly, microglial depletion abolished such an increase, suggesting that loss of microglia may prevent hypertension-associated neuronal excitation. Vasopressin and norepinephrine are 2 CNS-regulated hormones, which are associated with blood pressure increase.3 To better understand the downstream events of neuronal excitation, we examined the levels of plasma vasopressin and kidney norepinephrine in hypertensive mice. Consistent with NMDA receptor expression, both hormones were significantly increased in Ang II–treated hypertensive mice (Figure 4D). Microglial depletion suppressed vasopressin and norepinephrine levels in Ang II–treated mice to the normal levels.

Transfer of Activated Microglia Changed Blood Pressure Response

To further confirm the central role of microglia on blood pressure regulation, we adoptively transferred N9 cells, a murine microglial cell line, to the cerebroventricle of naive C57BL/6J mice. N9 cells are homogeneous and they lack the contamination of astrocytes, which is a concern when using cultured microglia from newborn brain. Some cells were primed in vitro with either Ang II (100 nmol/L for 12 h) or LPS (10 ng/mL for 6 h). Twenty-four hours after their ICV transfer, the recipient mice were

Figure 3. Verification of microglial depletion. A, Three days after intracerebroventricular (ICV) injection of diphtheria toxin (DT) in the indicated doses, the brain of CD11b–diphtheria toxin receptor (DTR) mice was perfused, and the coronal sections of paraventricular nucleus (PVN) and motor cortex were stained for Iba1. B, Flow cytometry (FCM) analysis of microglia (CD11b+CD45−) and blood total monocytes (CD11b+F4/80−) and inflammatory monocytes (CD11b+Ly6C+) in saline- and DT-treated CD11b-DTR mice. Representative dot plots from 10 mice of each treatment. C, The densities of neuron (NeuN+), astrocytes (GFAP+), and perivascular macrophages (blood vessels [CD31+] and macrophages [F4/80+]) in the PVN of saline- and DT-treated CD11b-DTR mice.
anesthetized, and their basal blood pressure and heart rate were recorded (Outline in Figure 5A). There was no difference in baseline blood pressure and heart rate across the groups (Table S1). When we induced a transient blood pressure increase by a single ICV injection of Ang II (50 ng), there was an acute pressor response with a 5 to 10 mm Hg rise (Figure 5B) in all groups. Interestingly, there was a significantly prolonged pressor response in mice receiving either Ang II- or LPS-primed microglia compared with the mice receiving naïve microglia or saline. This was specific for activated microglia because transferring Ang II–primed astrocytes did not change the duration of the pressor response. Minocycline has been widely used as an anti-inflammatory reagent affecting microglia. We incorporated another 2 cohorts of mice which were transferred with microglia primed with Ang II or LPS in the presence of minocycline. The prolonged pressor responses observed with activated microglia were completely abolished when microglia were coincubated with minocycline before transferring (Figure 5C).

To confirm these blood pressure changes were associated with changes in neuronal profiling, PVN tissues were dissected from the recipients 24 hours after transfer. There was a 2-fold increase in GluN2A level in LPS-primed group compared with the group receiving naïve microglia (Figure 5D). Minocycline coincubation with LPS fully abrogated this increase. These experiments suggest that activated microglia may potentiate neuronal responses to hypertensive stimulant by enhancing the expression of NMDA subunit GluN2A in the PVN.

**Discussion**

Hypertension is associated with neuroinflammation; however, the cellular mechanism of neuroinflammation is unknown, and it is unclear whether neuroinflammation contributes to the progression of hypertension. Here, we studied murine Ang II and l-NAME models and found that rampant microglial activation is a hallmark of hypertension-associated neuroinflammation. Because Ang II and l-NAME induce hypertension through different mechanisms, we
surmise that microglial activation is a characteristic of hypertension. To approve this hypothesis, future study of human samples will be critical. In both hypertensive models, microglial depletion significantly decreased blood pressure, neuroinflammation, and the levels of peripheral hypertensive hormones norepinephrine and vasopressin. In contrast, adoptive transfer of activated microglia predisposed recipients to hypertensive stimulant. In conclusion, this study shows that microglia are key modulators in the development of neurogenic hypertension.

The cause-and-effect relation between hypertension and neuroinflammation is under debate. In this present study, we focused on the role of microglia in established hypertension and in pressor responses to ICV Ang II stimulation. Our depletion data and adoptive transfer data clearly show that activated microglia contribute to blood pressure regulation. However, depletion of microglia did not correct blood pressure to normal level in established hypertension and transfer of microglia did not by itself give rise to blood pressure increase. Therefore, microglia activation is secondary to hypertensive insults but not an initiator of hypertension in our models. Microglia activation and hypertension forms a feed-forward loop.

Our study unveils a unique activation pattern of microglia associated with hypertension. Both M1 (classical activation) and M2 (alternative activation) markers are upregulated in hypertensive microglia but not in hypertensive monocytes. M1 and M2 represent extremes of a continuum in a universe of macrophage activation states. The upregulation of both M1 and M2 markers have not been reported in any other tissue macrophages in any other models. Such a distinctive phenotype of microglia echoes recent transcriptome analyses, which revealed that microglia, although considered the macrophages in brain, are distinguishable from peripheral myeloid cells. Whether this is caused by the unique brain environment or the hypertensive factors or both needs further investigation.

In this study, we devised a microglial depletion strategy by infusing DT ICV to CD11b-DTR mice. A previous study also investigated microglia biology through a DT depletion strategy. In that study, the authors subcutaneously injected DT to CD11b-DTR mice at the age of P3 when their blood–brain barrier was incomplete. However, peripheral monocytes and macrophages were also ablated by that approach. Our approach with ICV DT infusion efficiently depleted microglia but leaving other CNS cells and circulating monocytes...
intact, suggesting the exclusive depletion of microglia. Thus, our study may provide a unique model to investigate microglia biology to a variety of pathophysiological settings in the future.

Microglia are the major sources to produce inflammatory molecules and neurotrophic factors. Proinflammatory cytokines, such as IL-1p and TNFp, can directly or indirectly modulate neuronal activities.23,24 One mechanism is that they can enhance neuronal excitation by increasing NMDA receptor expression in the postsynapses.25,26 A recent study shows that brain-derived neurotrophic factor produced by microglial are of importance in the regulation of neuronal activity by increasing the expression of NMDA receptors on the postsynaptic terminals.16 Given the essential role of NMDA receptors in neuronal excitation and plasticity,21 signals triggering NMDA receptor presentation are the key factors on neuronal activity. NMDA receptor is a tetrameric glutamate-gated ion channel, assembled by an essential subunit, NR1, and metric glutamate-gated ion channel, assembled by an essential

Perspective
Our work provides direct evidence that hypertension elicits a unique activation pattern of microglia. Using depletion and adoptive transfer strategies, we evidenced that microglia are the major cellular modulators of neurogenic hypertension. Hypertension is generally accompanied with elevated sympathetic tone. To understand the central regulation of hypertension is critical to develop efficient treatment to essential hypertension.

Sources of Funding
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Disclosures
None.

References


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**Novelty and Significance**

**What Is New?**

- Hypertension elicits a unique activation pattern of microglia with the upregulation of markers associated with both classical and alternative activation.
- Using depletion and adoptive transfer strategies, we evidenced that microglia are the major cellular modulators of neurogenic hypertension.

**What Is Relevant?**

- Hypertension is generally accompanied with elevated sympathetic tone. To understand the central regulation of hypertension is critical to develop efficient treatment to essential hypertension. Our study unveils that microglia are central to neuroinflammation and neuronal regulation of hypertension, which provides a mechanistic insight to this disease.

**Summary**

Our study shows that rampart activation of microglia is a characteristic of hypertension. We provide direct evidence that microglia are the major modulator of neuroinflammation and central control of blood pressure.
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Supplemental Material

Microglia participate in neurogenic regulation of hypertension

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\textbf{Short title}: Microglia and neurogenic hypertension

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Methods

Mice and hypertension models. C57BL/6 and CD11b-DTR mice were purchased from Jackson Laboratories. All mice were maintained in micro isolator cages, and all experimental protocols were approved by the Institutional Animal Care and Use Committee at Cedars Sinai Medical Center. We used 8-10 weeks old male C57BL/6 mice for studying microglial activation (Figures 1, 2, S1), microglial adoptive transfer (Figure 5) and DT effect (Figure S2B). For microglial depletion studies, both male and female CD11b-DTR mice (8-10 weeks old) were identically mixed in each group. Hypertension was induced by s.c. infusion of Ang II (1000 ng/kg/min) (Pheonix Pharmaceuticals) via an osmotic minipump (Alzet) or by L-NAME treatment (1.5 mg/ml in the drinking water) (Bachem). Blood pressure (BP) was monitored in conscious mice using a computerized non-invasive tailcuff system (Visitech Systems, BP-2000 series II, Apex), and invasively using radiotelemetry as described\(^1\). For the former, mice were trained for 5 continuous days before data acquisition. BP was determined by averaging 20 measurements, with tracings manually reviewed to verify proper BP determination. For the latter, mice were anesthetized with isoflurane, and a catheter connected to a radiotelemetry device (HD-X11; Data Sciences International) was inserted in the left carotid artery. After a 10-14-day recovery phase, baseline BP and HR were recorded before osmotic pump implantation, followed by sampling every 2-3 days to the end of the protocol. Data were collected, stored, and analyzed using Dataquest A.R.T. 4.0 software (Data Sciences International). For acute BP recording, mice were anesthetized (2% isoflurane mixed with oxygen, 1 l/min). Blood pressure was recorded through a cannulated PE-50 catheter into the right common carotid artery. The head was positioned onto the stereotaxic frame. After baseline blood pressure and heart rate recording, a single dose of Ang II (50 ng in 1 µl) was injected through a 5 µl Hamilton syringe driven by a digital stereotaxic injector (Stoelting). BP and heart rates were recorded via a Statham Transducer (P23XL), continuously monitored and digitally recorded at 100 Hz using PowerLab software (ML870, AD Instrument). Body temperature was maintained at 37°C with a water-circulating pad throughout the procedure.

Microglial depletion. CD11b-DTR Mice were anesthetized and positioned in the stereotaxic frame. A small piece of skull was removed according to the following coordinates (0.3-0.5 mm post Bregma, 1.5-2 mm lateral to the midline). A 2-wk osmotic mini-pump infusing DT (Bio Academia) was connected to a brain infusion kit (Alzet), and the tip of the cannula was positioned in the left cerebroventricle with a depth of 3 mm, and then stabilized on the skull surface using bio-adhesive glue. The osmotic mini-pump was placed along the neck pocket.

Microglial dissociation. Following transcardiac perfusion with sterile heparinized PBS (100 ml, 4°C), mice were decapitated and the brains were removed. The procedures have been documented previously with slight modification\(^3\). In brief, whole brains were chopped to small pieces followed by enzymatic digestion with 1.6 mg/ml collagenase IV (Worthington) and 15 units of DNase I (Sigma) at 37°C for 1 h. Digested tissues were passed through 70 µm strainer (BD) and centrifuged in 37% and 70% Percoll (GE Healthcare) gradients. Microglia were enriched from the interface between 37% and 70% Percoll.
**Antibodies and Flow Cytometry.** The following antibody clones were used: M1/70 (anti-CD11b), 30-F11 (anti-CD45), AF6-120.1 (anti-I-A^b_), mIL4R-M1 (anti-IL-4Rα), 4B12 (anti-CCR7), 2E2 (anti-IFNγR), MR5D3 (anti-mannose receptor), TEK4 (anti-Tie2), 72-1 (anti-CD36), MP6-XT22 (anti-TNFα), NJTEN3 (anti-IL-1β) and MP5-20F3 (anti-IL-6). All the antibodies above were purchased from either eBioscience, BioLegend or Pharmingen. For staining intracellular proteins, fixation and permeabilization buffers were purchased from eBioscience. PE-labeled anti-iNOS was from Santa Cruz Biotechnology. Microglia was defined as CD11b^+CD45^{low}. To detect microglia cytokine expression, cells were cultured for 6 h in dish in the presence of brefeldin A (eBioscience). Some cells were stimulated with LPS (Sigma) in the 6-h culture. The stained samples were analyzed on a Beckman Coulter CyAn ADP and data were analyzed by FlowJo software.

**Immunohistochemistry.** After being deeply anesthetized with 5% isoflurane mixed with oxygen, mice were perfused transcardially with heparinized saline followed by 4% paraformaldehyde. Brains were cut into 30 µm coronal sections. Brain sections were incubated with monoclonal mouse anti-NeuN (Millipore, MAB377) antibody followed by Alexa 488-labeled goat anti-mouse IgG for detecting neurons; sections were incubated with polyclonal rabbit anti-Iba1 antibody (Wako; 019-19741) followed by Alexa 594-labeled goat anti-rabbit IgG for detecting microglia; sections were incubated with chicken anti-GFAP (Millipore, AB5541) antibody followed by Alexa 594-tagged goat anti-chicken IgG for detecting astrocyte; and sections were incubated with cocktail of goat anti-CD31 (R&D Systems) and rat anti-F4/80 (AbD Serotec) antibodies followed by cocktail of donkey anti-goat Alexa 594 and donkey anti-rat Alexa 488 IgG for detecting endothelial cells and brain macrophages, respectively. Sections were imaged using a laser scanning confocal microscope (Olympus, FV10i).

**Fractional area analysis.** The morphological changes of microglia, identified by Iba1 immunoreactivity, were analyzed by measuring the fractional area as described previously. The fractional areas were defined as the fraction of area immunoreactive for Iba1 antigen. In brief, the sections that covered the entire PVN (Bregma -0.82 to -1.06 mm) were analyzed based on the ratio of the calculated area of Iba1-positive staining to the entire images (0.2x0.2 mm^2 area), and 10 fields from 3 different sections of each animals were analyzed. Motor cortex was selected on the dorsal part of the same brain section.

**Western blot.** PVN tissues were homogenized in RIPA buffer (Thermo) mixed with protease inhibitor cocktail (Thermo), loaded on 4-12% gradient Bis-tris gels (Invitrogen) followed by electrophoretic transfer to polyvinylidene difluoride (PVDF) membranes in a conventional method as described previously. The membrane was blocked (Odyssey) and incubated with rabbit anti-GluN2A (Cell signaling) and mouse anti-α-tubulin (Developmental Studies Hybridoma Bank) 4°C overnight, and then reacted with the fluorescence-conjugated goat anti-rabbit or goat anti-mouse antibodies (Li-Cor). The fluorescent intensities were visualized by Odyssey Clix imaging system (Li-Cor). Fluorescent signals were captured and stored digitally; and quantified using Odyssey software (Li-Cor, v3.0).

**ELISA.** The ELISA kits used in this research are listed below: vasopressin (Enzo; ADI-900-017), norepinephrine (Abnova; KA1891), IL-1β (eBioscience; 88-7013-88) and
TNFα (eBioscience; BMS607/2INST). ELISA was performed according to the manufacturers’ instructions.

**Primary astrocyte culture.** Astrocytes were dissociated from one-day-old C57BL/6J mouse pups as previously stated

In brief, brains were removed, mechanically and enzymatically dissociated, and plated in 75-cm² flask. When the cells reached 100% confluency, cells were suspended and re-seeded to a new flask for culture for another 7 days. The attached cells, of which 99% are astrocytes, were suspended, washed and re-seeded to 35-mm dishes one day before Ang II treatment (100 nM, 12 hr).

**Adoptive transferring of primed microglia or primary astrocytes.** After being stimulated with Ang II (100 nM) or LPS (10 ng/ml, Sigma) with or without minocycline (100 µM, Sigma; M9511), N9 cells

or primary astrocytes were harvested, washed once with DMEM medium and re-suspended in PBS. Each recipient mouse received 5x10⁵ cells via a pump-driven Hamilton microsyringe based on the following coordinates: 0.3-0.35 mm posterior to Bregma; 1.5-2 mm lateral to midline; 2 mm below the dura). In this process, cells were injected in a volume of 1 µl at 0.1 µl/min.

**Statistics.** Data are summarized as mean ± S.E.M.. Statistical analysis was performed with GraphPad Prism version 6. Differences between groups were compared by unpaired t-test, one- or two-way ANOVA analysis by Newman-Keuls Multiple comparison test, accordingly.
References


Table S1. Baseline mean arterial pressure (MAP) and heart rate (HR) in the mice receiving i.c.v. transfer of saline (Sham) or pre-treated microglia or astrocytes.

<table>
<thead>
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<th>Treatment</th>
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<td></td>
<td>sham</td>
<td>Naive</td>
<td>Ang II</td>
</tr>
<tr>
<td>n</td>
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<td>7</td>
<td>10</td>
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<td>MAP (mmHg)</td>
<td>81±1</td>
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<td>85±2</td>
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<tr>
<td>HR (beat/min)</td>
<td>327±10</td>
<td>327±20</td>
<td>303±10</td>
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</table>
S1. Characterization of microglia associated with hypertension. (A) Systolic blood pressure of naïve mice or mice treated with Ang II (1000 ng/kg/min) or L-NAME (1.5 mg/ml in drinking water). (B) Schematic outline of motor cortex and PVN in mouse brain. Coordinates are in reference to the Bregma based on coronal sections adapted from the atlas of Paxinos and Watson. (C) Iba1 staining of microglia in the PVN and motor cortex of normotensive mice or the mice treated with Ang II or L-NAME for 4 weeks. (D) Fractional area analysis of microglia in the PVN of above three groups. * P<0.05 vs. control by One-way ANOVA.
S2. Blood pressure responses to i.c.v. DT. (A) Systolic blood pressure was measured after i.c.v. saline or DT infusion into CD11b-DTR mice. The two groups have no difference observed. (B) Systolic blood pressure of C57BL/6J mice treated with or without s.c. Ang II infusion (1000 ng/kg/min). The group without Ang II infusion and one group with Ang II infusion received i.c.v. DT (1000 pg/g body weight) treatment. DT treatment did not alter the blood pressure patterns of C57BL/6J mice.