Hypertension

NERVOUS SYSTEM

IL (Interleukin)-17A Acts in the Brain to Drive Neuroinflammation, Sympathetic Activation, and Hypertension

Yiling Cao,* Yang Yu,* Baojian Xue,* Ye Wang, Xiaolei Chen, Terry G. Beltz, Alan Kim Johnson, Shun-Guang Wei

ABSTRACT: IL (Interleukin)-17A is a key inflammatory mediator contributing to chronic tissue inflammation. The present study sought to determine whether IL-17A plays a role in regulating neuroinflammation, hemodynamics, and sympathetic outflow in normal and hypertensive animals. In urethane-anesthetized rats, intravenous injection of IL-17A induced dramatic and prolonged increases in blood pressure, heart rate, and renal sympathetic nerve activity, which were significantly attenuated by an IL-17RA (IL-17 receptor A) siRNA in the hypothalamic paraventricular nucleus (PVN). Either intracerebroventricular or PVN microinjection of IL-17A also elicited a similar excitatory response in blood pressure, heart rate, and renal sympathetic nerve activity. Intravenous injection of IL-17A upregulated the mRNA level of IL-17A, IL-17F, and IL-17RA in the PVN. Additionally, intravenous injection of IL-17A activated brain-resident glial cells and elevated the gene expression of inflammatory cytokines and chemokines in the PVN, which were markedly diminished by PVN microinjection of IL-17RA siRNA. Pretreatments with microglia or astrocyte inhibitors attenuated the increase in blood pressure, heart rate, and renal sympathetic nerve activity in response to PVN IL-17A. Moreover, intracerebroventricular injection of IL-17A activated TGF (transforming growth factor)-β activated kinase 1, p44/42 mitogen-activated protein kinase, and transcriptional nuclear factor κB in the PVN. IL-17A interacted with tumor necrosis factor-α or IL-1β synergistically to exaggerate its influence on hemodynamic and sympathetic responses. Central intervention suppressing IL-17RA in the PVN significantly reduced angiotensin II–induced hypertension, neuroinflammation, and sympathetic tone in the rats. Collectively, these data indicated that IL-17A in the brain promotes neuroinflammation to advance sympathetic activation and hypertension, probably by a synergistic mechanism involving the interaction with various inflammatory mediators within the brain.
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Key Words: angiotensin II ■ autonomic nervous system ■ blood pressure ■ cytokines ■ hemodynamics ■ paraventricular hypothalamic nucleus

IL (Interleukin)-17 secreted mainly by a unique subset of activated CD4+ T cells, the T helper 17 cells,[1] is a critical inflammatory mediator bridging innate immunity and adaptive immune responses.[2] Initially identified as cytotoxic T-lymphocyte-associated antigen 8, this molecule was subsequently renamed IL-17 because of its strong cytokine-like activity.[3] Following the discovery of other homologs, IL-17 became the founding member of the IL-17 family as IL-17A and was joined by 5 members identified from IL-17B to 17F.[4] IL-17A exists in the form of homodimer or heterodimer with IL-17F to activate a receptor complex composed of IL-17 receptor A and C (IL-17RA and IL-17RC),[5,6] which triggers multiple signaling molecules like TAK1 (TGF [transforming growth factor]-β activated kinase 1) to evoke transcriptional activation of MAPK (mitogen-activated protein kinase), NF-κB (nuclear factor κB), and CCAAT/enhancer-binding proteins.[7,8] IL-17A is the best-studied cytokine in the family and has been shown to activate macrophages, T cells, dendritic cells, and endothelial cells to prompt inflammatory states by amplifying the expression of numerous inflammatory cytokines and chemokines.

IL-17A has been implicated in the cause of several inflammatory and autoimmune disorders, including

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Novelty and Significance

What Is New?

- Systemic IL (interleukin)-17A acts upon the brain to promote neuroinflammation, hemodynamics, and sympathetic activation.
- IL-17A-prompted neuroinflammation and sympathetic activation contribute to Ang (angiotensin) II–induced hypertension.

What Is Relevant?

- IL-17A-induced neuroinflammation and sympathetic activation have important implications for the inflammatory mechanisms in hypertension.
- Interventions suppressing IL-17A/IL-17RA axis in the brain have the potential to ameliorate hypertension.

Summary

IL-17A provokes neuroinflammation by amplifying the expression of various inflammatory mediators in the brain to promote autonomic nervous system activation, probably through a synergistic mechanism by which IL-17A interacts with other cytokines to potentiate its inflammatory effects.

Nonstandard Abbreviations and Acronyms

BBB	blood-brain barrier
BP	blood pressure
CNS	central nervous system
CSF	cerebrospinal fluid
GFAP	glial fibrillary acidic protein
HR	heart rate
IκB-α	NF-κB inhibitor kappa B-α
IL	interleukin
IL-17RA	IL-17 receptor A
MAP	mean arterial pressure
MAPK	mitogen-activated protein kinase
MBP	mean BP
MCP-1	monocyte chemoattractant protein 1
MIP-1α	macrophage inflammatory protein 1α
NF-κB	nuclear factor κB
PVN	paraventricular nucleus
RSNA	renal sympathetic nerve activity
SDF-1	stromal cell-derived factor 1
TAK1	TGF-β activated kinase 1
TNF	tumor necrosis factor

psoriasis, psoriatic arthritis, and multiple sclerosis.[9–11] Additionally, emerging evidence indicates that IL-17A also contributes to pathophysiology in cardiovascular diseases.[12] Notably, patients with psoriasis are at greater risk for cardiovascular diseases, which points to IL-17A as a potential mechanistic link.[13] IL-17A is a critical mediator for tissue damage and repair, which can be detected in the heart as early as 1-hour postmyocardial infarction or ischemia/reperfusion injury.[14] Moreover, IL-17A was reported to be upregulated in several hypertensive models and involved in the regulation of blood pressure (BP).[15,16] However, how IL-17A contributes to the pathogenesis in these diseases remains unclear.

Sympathetic nervous system activation plays a vital role in cardiovascular dysfunction.[17,18] Work over the past decade has demonstrated that inflammation-driven sympathetic overactivity and neurohumoral activation have clear peripheral manifestations that contribute significantly to the development of hypertension and heart failure.[19–21] While the initially identified effect of IL-17A in periphery on the promotion of chronic inflammation that causes pressor responses and end-organ damages has been appreciated,[22] the role of IL-17A in the brain in mediating neuroinflammation and sympathetic outflow to enhance hypertension remains to be investigated.

We previously reported that circulating cytokines act within the brain to augment BP by prompting sympathetic excitation.[23] In this regard, IL-17A is of particular interest in activating the automimic nervous system because of its distinctive ability to function as a key inflammatory regulator rather than an effector cytokine. IL-17A has been reported to access the brain by disrupting the integrity of blood-brain barrier (BBB),[24–26] and its receptors, IL-17RA and IL-17RC are richly expressed in the central nervous system (CNS).[27,28] Furthermore, IL-17A displays a strong synergism when interacting with other cytokines to exaggerate its inflammatory effects.[29] Thus, investigation of a role for IL-17A in the brain is of great significance in elucidating the neuroinflammatory and sympathetic mechanisms in hypertension.

In the current study, we sought to determine whether IL-17A acts within the brain, particularly in the hypothalamic paraventricular nucleus (PVN), a cardiovascular/autonomic brain region, to promote neuroinflammation and sympathetic excitation, and whether IL-17A plays an essential role in Ang (angiotensin) II–induced hypertension. We further examined a synergistic mechanism by which IL-17A interacts with other cytokines to potentiate its inflammatory effects on sympathetic outflow. Finally, we identified the potential signaling molecules in the CNS that are activated by IL-17A.

METHODS

The authors declare that all supporting data are available within the article and its Data Supplement. The detailed methods are described in the Data Supplement.

Animals

The experimental protocols in this study were approved by the University of Iowa Institutional Animal Care and Use Committee and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments were performed on male adult Sprague-Dawley rats (300–350) purchased from Envigo/Harlan Sprague-Dawley (Indianapolis, IN). The animals had ad libitum access to standard rat chow and tap water. These rats were housed in a temperature- (23±2 °C) and light-controlled (12:12-hour light-dark cycle) animal care facility on the University of Iowa Health Science Campus. All efforts were made to minimize animal usage and suffering.

Experimental Protocols

1. Acute study in normal animals: Under anesthetized condition with urethane, rats underwent electrophysiological recordings of renal sympathetic nerve activity (RSNA), BP, and heart rate (HR) in the groups outlined below. At the conclusion of the experiments (5–6 hours after IL-17A was injected), brain tissue, blood, and cerebrospinal fluid (CSF) were collected for molecular, immunofluorescent, or biochemical assay.
   i. Rats were treated with intravenous injections of either IL-17A (1000 ng/kg, n=6) or saline (0.9%) serving as a vehicle (n=6) control. Venous blood and CSF were collected for the measurement of IL-17A concentration by ELISA before these animals were decapitated to collect the brain tissue for assessment of PVN mRNA levels of IL-17A, IL-17F, IL-17RA, IL-17RC, TNF (tumor necrosis factor)-α, IL-1β, IL-6, IL-18, MCP-1 (monocyte chemoattractant protein 1/CCL2), MIP-1α (macrophage inflammatory protein 1α/CCL3), IL-8 (CXCL-8), and SDF-1 (stromal cell-derived factor 1/CXCL12). In a separate group (n=6 for intravenous vehicle or IL-17A), rats were transcardially perfused with 4% paraformaldehyde. The brains were collected for immunofluorescent staining of CD11b (microglia marker) and GFAP (glial fibrillary acidic protein; a specific cell marker for PVN astrocytes) in the PVN.
   ii. Rats underwent bilateral PVN microinjections of an adeno-associated virus serotype 9 (AAV9) harboring an IL-17RA siRNA (n=6) or a scrambled siRNA (n=6) 2 weeks before intravenous injection of IL-17A (1000 ng/kg). At the end of experiment, these rats were euthanized to collect brain tissues to determine the PVN mRNA levels of cytokines and chemokines indicated above.
   iii. Rats received intracerebroventricular injections of either IL-17A (100 ng/kg, n=6) or artificial CSF serving as a vehicle (n=6). These rats were euthanized to collect brain tissues for Western blot-based measurement of phosphorylated (p-) and the total levels of TAK1, p44/42 MAPK, NF-κB p65 subunit, and IκB-α (NF-κB inhibitor kappa B-α) in the PVN.
   iv. Rats received bilateral PVN microinjections of either IL-17A (25 ng/kg per side in 0.2 μL artificial CSF, n=6) or artificial CSF serving as a vehicle (n=6). Some rats were pretreated with microglia inhibitor minocycline (5 μg/kg, n=6) or astrocyte inhibitor fluorocitrate (1 nmol, n=6) before bilateral PVN microinjection of IL-17A (25 ng/kg per side). At the end of the experiment, PVN was microinjected with Pontamine sky blue (0.05 μL/side) to verify the site of microinjection.
   v. Rats received intracerebroventricular injections of either IL-17A (20 ng/kg, n=6) or TNF-α (20 ng/kg, n=6) alone. Some rats were intracerebroventricularly injected with a combination (n=6) of IL-17A (20 ng/kg) plus TNF-α (20 ng/kg) or pretreated with TNF-α inhibitor SPD304 (5 μg/kg, n=6) before intracerebroventricular injection of IL-17A (20 ng/kg) plus TNF-α (20 ng/kg).
2. Chronic study in Ang II–induced hypertension: Under ketamine plus xylazine (100+10 mg/kg) anesthesia, normal rats underwent 3 surgical procedures for embedding telemetric transmitters, bilateral PVN microinjection of IL-17RA siRNA and implantation of osmotic minipumps. These animals were divided into 3 treatment groups: (i) PVN microinjection of a scrambled siRNA + saline (n=6); (ii) PVN microinjection of a scrambled siRNA + Ang II (150 ng/kg per minute, n=6); and (iii) PVN microinjection of an IL-17RA siRNA + Ang II (150 ng/kg per minute, n=6). At the conclusion of the experiments (≈4 weeks after the initial surgery), rats were treated with ganglionic blocker hexamethonium bromide (30 mg/kg, IP) to assess the sympathetic tone and then euthanized by decapitation to collect brain tissues and blood for the assessments of mRNA levels of IL-17A, TNF-α, and IL-1β in the PVN and plasma norepinephrine level by ELISA.

Statistical Analysis

Electrophysiological recording data of sympathetic nerve activity and hemodynamics were analyzed with CED Spike2 software (Cambridge Electronic Design). The changes (Δ) of mean arterial pressure (MAP, mm Hg), HR in beats per minute (bpm), and integrated RSNA (mV·s) sampled over 10-minute intervals after injection of IL-17A by intravenous, intracerebroventricular, or PVN routes were compared with baseline values averaged over 10-minute intervals immediately preceding each intervention. Integrated voltage of RSNA was reported as a percentage change from baseline control. Systemic vascular resistance was expressed as mm Hg·s/mL as described previously.[30] The phosphorylated forms of TAK1, p44/42 MAPK, NF-κB p65, and IκB-α were normalized to their total levels of the proteins, respectively, while the total forms of these proteins were normalized to β-actin. The mRNA levels of cytokines and chemokines were corrected by β-actin and expressed as fold changes compared with vehicle control. IL-17A levels in the plasma and CSF were reported as pg/mL. The immunofluorescent intensity of CD11b and GFAP was expressed as arbitrary units. MAP and HR in chronic study through telemetry were presented as mean daily values averaged from daytime and nighttime measurements. Difference scores for MAP and HR were calculated for each animal based on the mean of the

5-day baseline subtracted from the mean of the final 5 days of treatments. All values are expressed as mean±SEM. The significance of differences among groups was analyzed with 1-way or 2-way ANOVA followed by Tukey multiple comparison test. An unpaired Student *t* test was used for comparison of the difference in 2 groups. $P<0.05$ was considered to indicate statistical significance.

RESULTS

Systemic IL-17A Increases Hemodynamic and Sympathetic Responses in Normal Rats

In rats treated with intravenous vehicle, the baseline mean BP (MBP: 91.6±1.9 mm Hg), HR (331±10 bpm), or integrated RSNA (8.8±0.8 mV·s) did not change significantly during the entire recording period (Figure 1A and 1E). However, in rats treated with intravenous IL-17A, significant and long-lasting excitatory responses in MAP (Δ15.2±1.4 mm Hg), HR (Δ49.8±5.1 bpm), and RSNA (138.1±15.9% change) were observed, beginning within 120 minutes of injection and peaking 5 to 6 hours afterwards (Figure 1B and 1E). These pressor and excitatory responses in MBP, HR, and RSNA remained elevated throughout the recording period.

IL-17RA Knockdown in the PVN Reduces Hemodynamic and Sympathetic Responses to Systemic IL-17A

The role of the PVN in mediating peripheral IL-17A-driven hemodynamic and sympathetic responses was examined by genetic knockdown of its receptor with an IL-17RA siRNA. In rats pretreated with a scrambled siRNA in bilateral PVN, intravenous injection of IL-17A led to significant elevations in MBP (Δ14.8±1.2 mm Hg), HR (Δ47.1±4.9 bpm), and RSNA (127.4±12.2% changes; Figure 1C and 1E). The time course and amplitude of these excitatory responses to IL-17A closely resembled those observed in rats receiving IL-17A alone. However, in rats pretreated with an IL-17RA siRNA in bilateral PVN, intravenous IL-17A-elicited increases in MBP (Δ6.3±1.1 mm Hg), HR (Δ23.5±4.2 bpm), and RSNA (55.7±10.2% changes) were significantly attenuated (Figure 1D and 1E).

The expression of IL-17RA in the PVN was detected (Figure S1A in the Data Supplement) by immunofluorescent staining. The confocal images exhibited that IL-17RA was expressed in dorsal parvocellular, medial parvocellular, ventrolateral parvocellular, and posterior magnocellular regions of PVN, the 4 commonly recognized subdivisions. The transfection potential of the virus and the efficacy of IL-17RA siRNA in the PVN of rats were verified before the study (Figure S1B and S1C in the Data Supplement).

Systemically Administered IL-17A Increases IL-17A Concentration in CSF and Upregulates mRNA Expression of IL-17A, IL-17F, and IL-17RA in the PVN

Compared with vehicle, intravenous injection of IL-17A markedly increased the levels of IL-17A in plasma and CSF (Figure 2A) 5 hours after the injection in normal rats. The IL-17A concentration in the CSF was positively correlated ($P<0.01$, $R^2=0.76$) with the increased level of IL-17A in the plasma (Figure 2B).

Analysis by real-time polymerase chain reaction revealed that the mRNA levels of IL-17A and its close homolog IL-17F were significantly elevated in the PVN 5 hours after intravenous injection of IL-17A (Figure 2C). The level of the IL-17RA mRNA was also higher in the PVN of rats treated with intravenous IL-17A than in counterparts treated with vehicle. The IL-17RC mRNA showed a trend towards an increase, but this did not reach statistical significance (Figure 2C). Intravenous injection of IL-17A also upregulated the mRNA level of IL-17A in other brain nuclei including the subfornical organ and arcuate nucleus, but not in the cerebral cortex (Figure S2A in the Data Supplement).

We further examined the effects of peripheral or central injection of IL-17A on systemic vascular resistance. Compared with vehicle, intravenous or intracerebroventricular injections of IL-17A significantly augmented the systemic vascular resistance 5 hours after the injection (Figure S2B in the Data Supplement).

Systemic IL-17A Activates Brain-Resident Glial Cells and Upregulates the Expression of Inflammatory Mediators in PVN

Confocal images revealed that the immunoreactivity (arbitrary units) of CD11b (Figure S3A in the Data Supplement) and GFAP (Figure S3B in the Data Supplement) in the PVN was more intense 5 hours after intravenous injection of IL-17A versus vehicle, indicating the activation of microglia and astrocytes in the PVN by circulating IL-17A.

Furthermore, real-time polymerase chain reaction analysis showed that the mRNA levels of inflammatory cytokines TNF-α, IL-1β, IL-6, IL-18 (Figure 2D), and chemokines MCP-1/CCL2, MIP-1α/CCL3, IL-8/CXCL-8, and SDF-1/CXCL12 (Figure 2E) in the PVN were significantly elevated in rats treated with intravenous IL-17A versus vehicle. However, intravenous IL-17A-upregulated expressions of these inflammatory cytokines and chemokines were obviously decreased in the rats pretreated with an IL-17RA siRNA in the PVN when compared with the rats pretreated with a scrambled siRNA (Figure 2D and 2E).

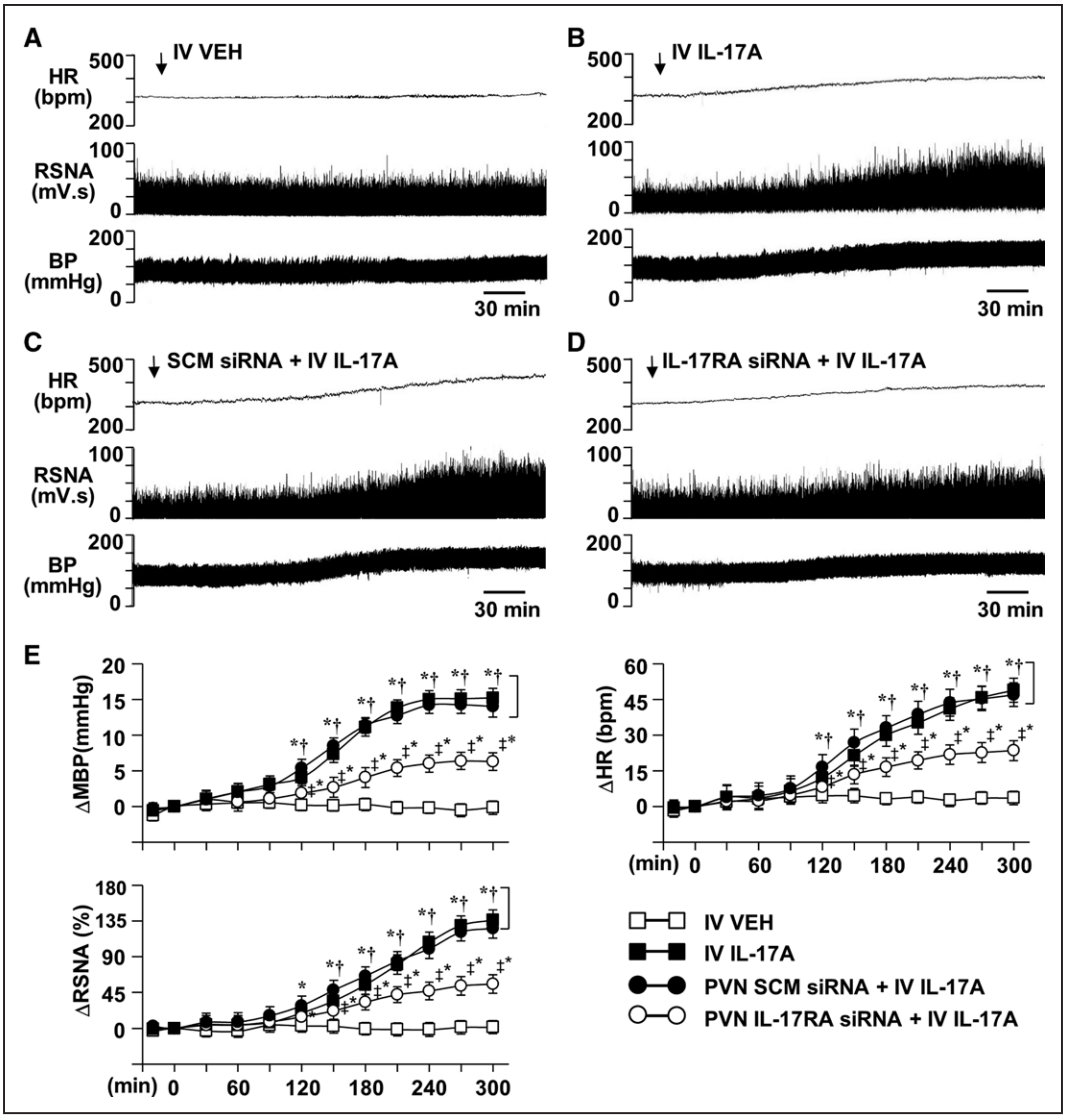

Figure 1. Effects of systemically administered IL (interleukin) -17A on hemodynamic and sympathetic responses. Representative tracings showing the effects of intravenous (IV) injection of vehicle (VEH; **A**), IL-17A (**B**) in normal rats or the rats pretreated with bilateral hypothalamic paraventricular nucleus (PVN) microinjection of a scrambled (SCM) siRNA (**C**) or IL-17RA siRNA (**D**) on blood pressure (BP, mm Hg), heart rate (HR, beats/min), and renal sympathetic nerve activity (RSNA, mV·s, integrated). **E**, Quantification of the change (Δ) from baseline over time for the mean BP (MBP), HR (bpm), and RSNA (%) in these 4 treatment groups. Values are expressed as mean±SEM (n=6 in each group). *P<0.05, compared with baseline. †P<0.05, vs VEH. ‡P<0.05, IL-17RA siRNA vs SCM siRNA.

Effects of Centrally Administered IL-17A on Hemodynamic and Sympathetic Responses

Intracerebroventricular injection of IL-17A increased its level in the CSF (Figure S2C in the Data Supplement) and elicited a dramatic and persistent increase in MAP (Δ21.7±2.8 mm Hg), HR (Δ80.1±7.9 bpm), and RSNA (234.3±18.3% change; Figure S4 in the Data Supplement). The pressor and excitatory responses in MBP, HR, and RSNA to intracerebroventricular IL-17A began within 20 to 30 minutes and peaked at 4 to 5 hours (Figure S4B and S4D in the Data Supplement) after the injection and remained at the higher level for the rest of recording period. Noticeably, the onset of the responses to intracerebroventricular IL-17A was more rapid than that of the responses to intravenous IL-17A. Intracerebroventricular injection of an equal volume of vehicle did not affect baseline MBP (89.2±2.1 mm Hg), HR (319±9 bpm), or integrated RSNA (8.1±1.0 mV·s; Figure S4A and S4D in the Data Supplement).

Centrally Administered IL-17A Induces Transcription Activation in the PVN

Western blot analysis showed that intracerebroventricular injection of IL-17A substantially raised the expression of phosphorylated (p-) p44/42 MAPK and p-TAK1 in the PVN when compared with intracerebroventricular

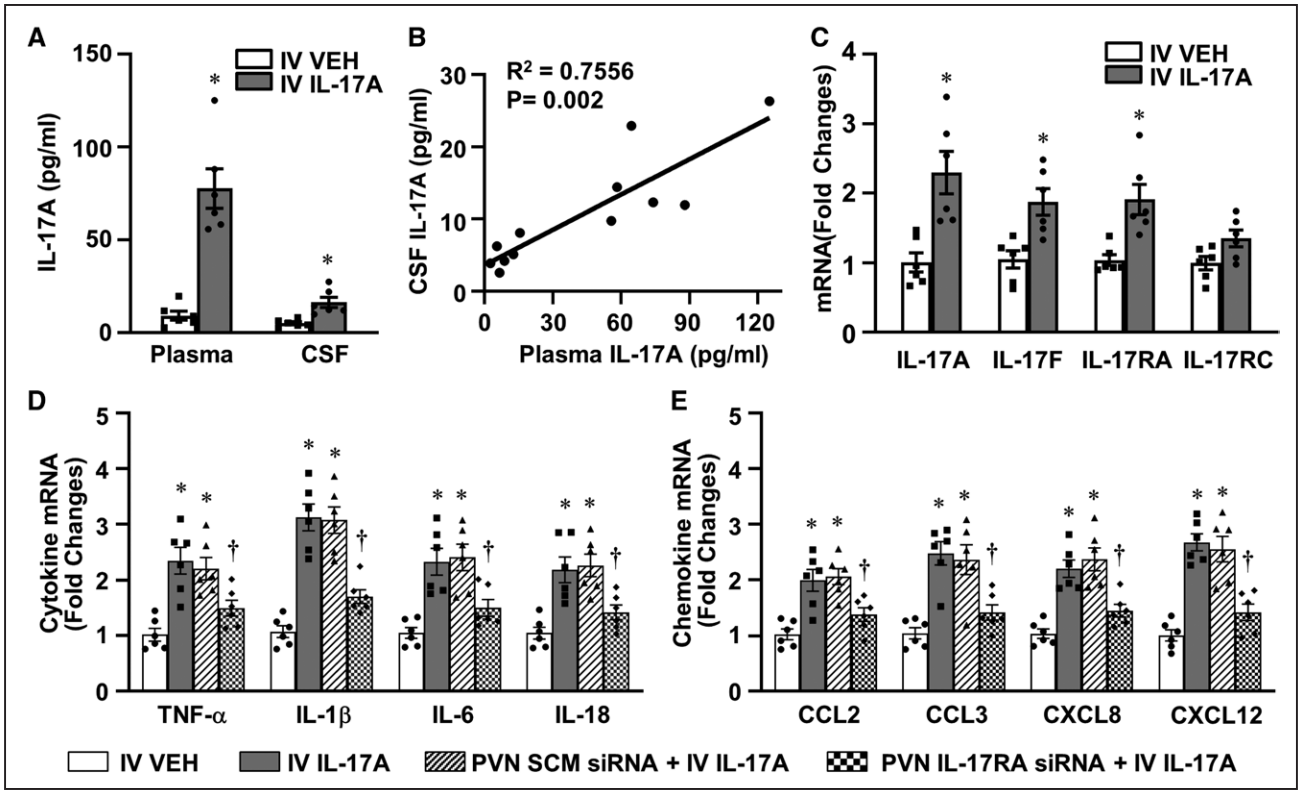

Figure 2. Effects of systemically administered IL (interleukin) -17A on neuroinflammation in the brain.
A, IL-17A level in plasma and cerebrospinal fluid (CSF) in normal rats treated with intravenous (IV) vehicle (VEH) or IL-17A. **B**, A linear regression indicating the correlation of IL-17A concentration between plasma and CSF. **C**, Quantitative analysis by real-time polymerase chain reaction (PCR) showing the mRNA level of IL-17A, IL-17F, IL-17 receptor A and C (IL-17RA, RC) in the hypothalamic paraventricular nucleus (PVN) after IV VEH or IL-17A. **D** and **E**, Quantitative analysis by real-time PCR showing the cytokine (**D**) and chemokine (**E**) mRNA levels of TNF (tumor necrosis factor)-α, IL-1β, IL-6, IL-18, MCP-1 (monocyte chemoattractant protein 1/CCL2), MIP-1α (macrophage inflammatory protein 1α/CCL3), IL-8 (CXCL-8), and SDF-1 (stromal cell-derived factor 1/CXCL12) in the PVN following the 4 treatments indicated in Figure 1. Values are expressed as mean±SEM (n=6 in each group). *P<0.05, vs VEH; †P<0.05, IL-17RA siRNA vs SCM siRNA.

injection of vehicle (Figure 3A and 3B). The total form of the proteins did not change significantly in response to intracerebroventricular IL-17A. Intracerebroventricular injection of IL-17A also elevated NF-κB activity in the PVN as indicated by increased expression of p-p65 NF-κB and decreased expression of total level of IκB-α (Figure 3C and 3D). The total protein level of NF-κB p65 subunit was not changed. Compared with vehicle, the elevated expression of p-IκB-α by intracerebroventricular IL-17A indicated the rapid degradation of IκB-α to boost NF-κB activity (Figure 3C and 3D).

Effects of IL-17A in the PVN on Hemodynamic and Sympathetic Responses

To further determine the potential role of PVN as a key cardiovascular and autonomic brain region in mediating IL-17A-driven sympathetic excitation, we directly microinjected IL-17A bilaterally into the PVN. Like intravenous or intracerebroventricular IL-17A, PVN microinjection of IL-17A elicited prolonged increase in MBP, HR, and RSNA. Similar to the effects of intracerebroventricular IL-17A, these excitatory responses initiated within 20 to 30 minutes, ramped up to the peak changes in MBP (Δ16.6±2.3 mm Hg), HR (Δ64.5±9.0 bpm), and RSNA (198.5±18.7% changes) 4 to 5 hours after PVN microinjection (Figure 4B and 4E), and persisted for the duration of the experiment. Pretreatment with the microglia inhibitor minocycline[31] (Figure 4C and 4E) or the astrocyte inhibitor fluorocitrate[32] (Figure 4D and 4E) significantly attenuated the excitatory effects induced by bilateral PVN IL-17A on MAP, HR, and RSNA.

Bilateral PVN microinjection of an equal volume of vehicle did not significantly affect the baseline MBP (87.8±2.2 mm Hg), HR (317±8 bpm), or integrated RSNA (7.9±1.8 mV·s) in normal rats (Figure 4A and 4E).

IL-17A Interacts with TNF-α to Strengthen Hemodynamic and Sympathetic Responses

To determine whether IL-17A synergizes with other cytokines to potentiate its effects on hemodynamics and sympathetic outflow, we performed a combinatorial injection of IL-17A with TNF-α via the intracerebroventricular route. We found that a relatively low dose of IL-17A (20 ng/kg, Figure 5A and 5E) or TNF-α (20 ng/

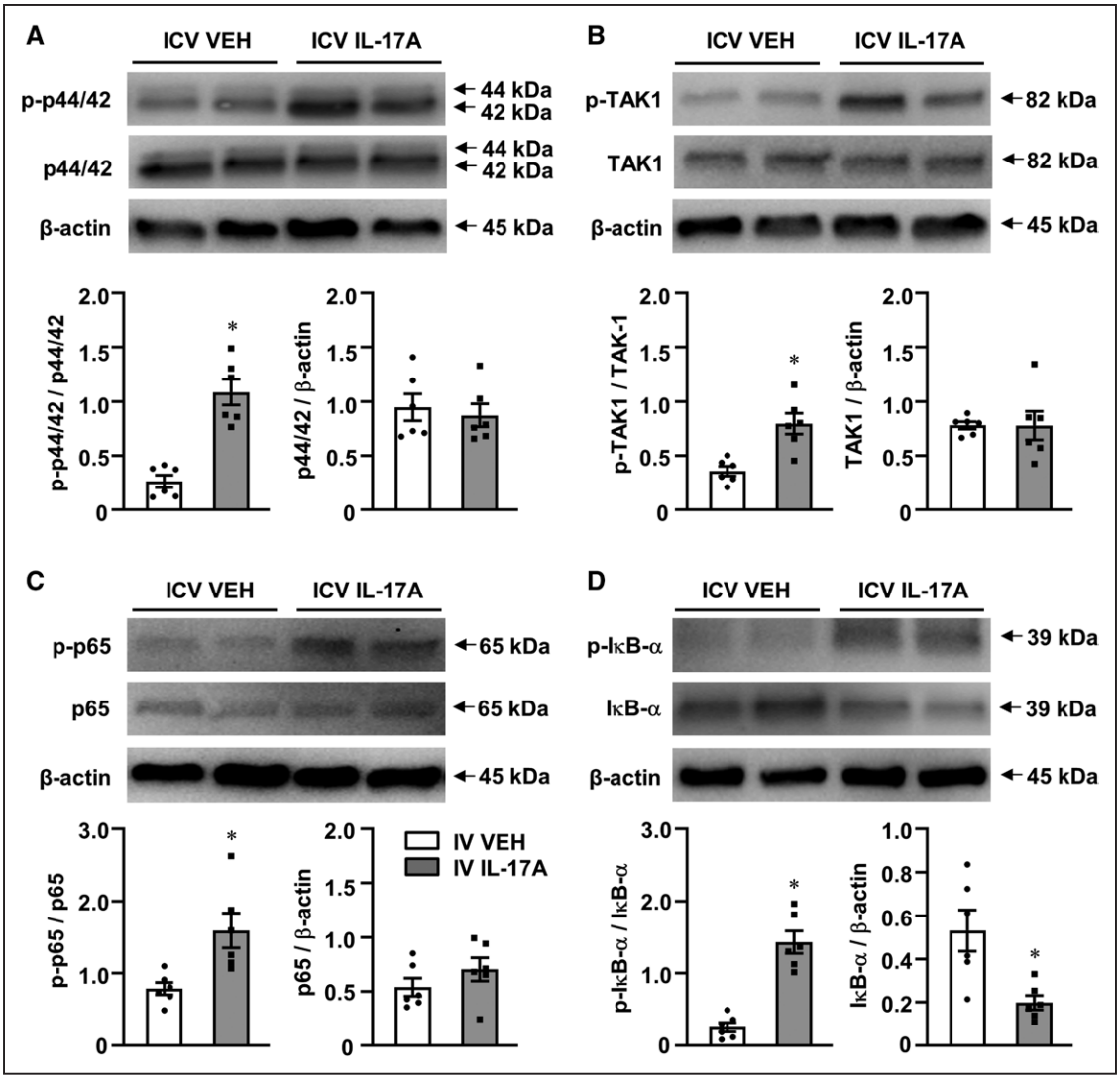

Figure 3. Effects of centrally administered IL (interleukin) -17A on the activation of transcription factors in the brain. Western blot analysis of phosphorylated (p-) and total form of p44/42 MAPK (mitogen-activated protein kinase; **A**), TAK1 (TGF [transforming growth factor]-β activated kinase 1; **B**), NF-κB (nuclear factor κB) p65 subunit (**C**), and IκB-α (NF-κB inhibitor kappa B-α; **D**) in the hypothalamic paraventricular nucleus (PVN) of rats following intracerebroventricular (ICV) injection of vehicle (VEH) or IL-17A. Representative bands are shown above the bar groups. The phosphorylated form of the proteins is normalized to their total levels and the total form of the proteins is normalized to β-actin. Values are mean±SEM (n=6 for each group). **P*<0.05, vs VEH.

kg, Figure 5B and 5E) injected alone did not significantly alter MBP, HR, and RSNA. However, when the combined at the same dose (20 ng/kg), intracerebroventricular injection of IL-17A plus TNF-α elicited a substantial increase in MBP (Δ10.3±0.8 mm Hg), HR (Δ43.6±4.0 bpm), and RSNA (65.6±6.9% changes; Figure 5C and 5E). Pretreatment with a TNF-α inhibitor SPD304 (Figure 5D and 5E) prevented the pressor and sympathetic responses by intracerebroventricular injection of IL-17A plus TNF-α. Also, in combination with cytokine IL-1β, IL-17A exhibited a similar effect to amplify BP, HR, and RSNA (Figure S5 in the Data Supplement), suggesting a synergistic and reinforced effect of IL-17A in the brain on hemodynamics and sympathetic outflow when interacting with other inflammatory mediators like TNF-α and IL-1β.

Effects of Knockdown of IL-17RA in the PVN on Ang II–Induced Hypertension

To test the role of IL-17A in Ang II–induced hypertension, IL-17RA in the PVN was knocked down with its siRNA in Ang II–infused animals. Bilateral PVN microinjection of scrambled siRNA plus subcutaneous infusion of saline did not alter baseline MBP and HR (Figure 6A and 6B). However, a 2-week Ang II infusion resulted in a significant increase in MBP (Figure 6A) in the rats treated with PVN microinjection of a scrambled siRNA (Δ44.4±7.1 mm Hg). The elevated MBP induced by Ang II infusion was significantly attenuated by PVN microinjection of IL-17RA siRNA (Δ22.8±5.5 mm Hg; Figure 6A). However, Ang II infusion induced a mild but nonsignificant decrease in HR in rats treated with PVN microinjection

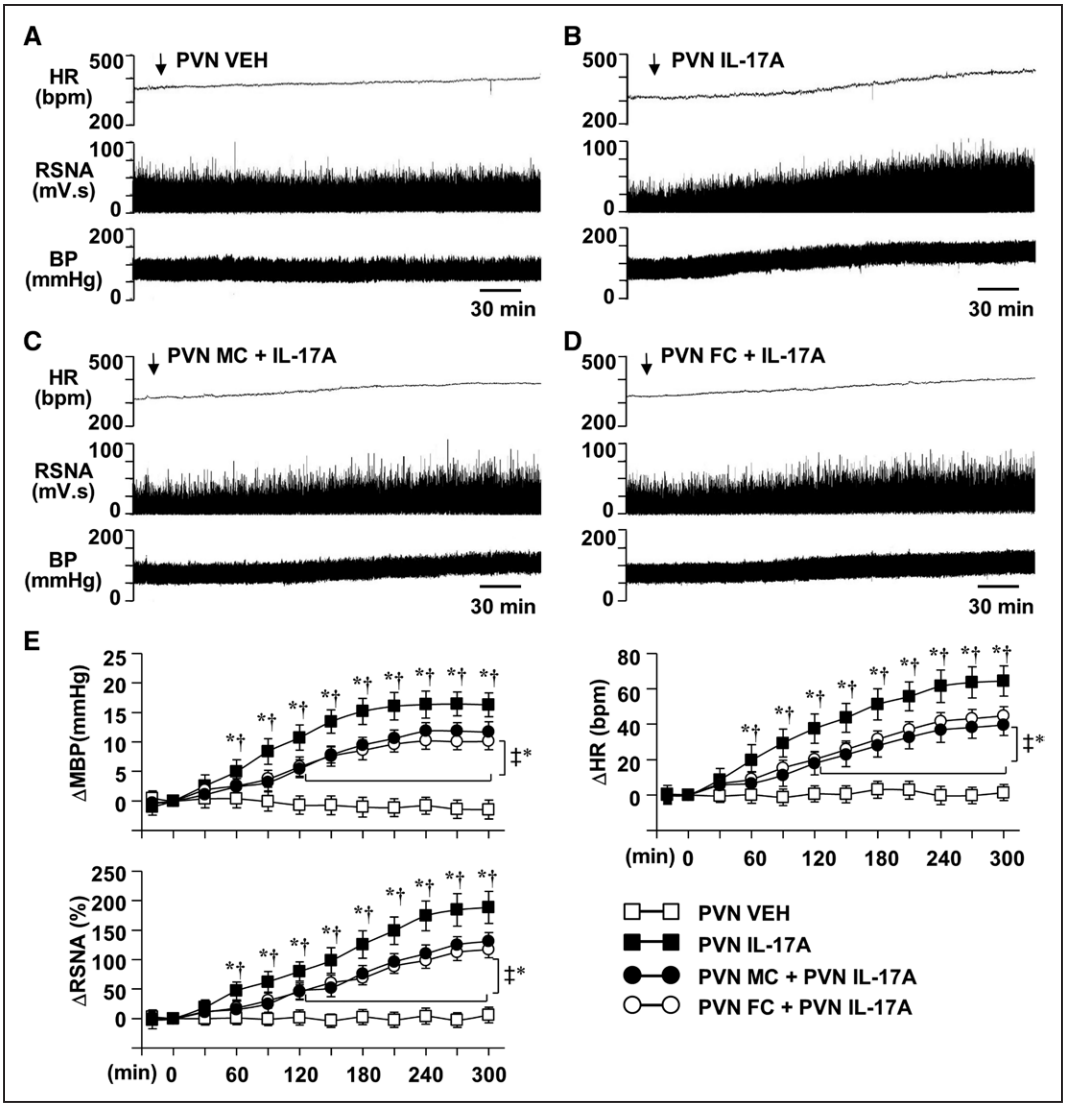

Figure 4. Effects of IL (interleukin) -17A in hypothalamic paraventricular nucleus (PVN) on hemodynamic and sympathetic responses.

Representative tracings showing the effects of bilateral PVN microinjection of vehicle (VEH, **A**) or IL-17A (**B**) in normal rats or the rats pretreated with bilateral PVN minocycline (MC; **C**) or fluorocitrate (FC; **D**) on blood pressure (BP, mm Hg), heart rate (HR, beats/min), and renal sympathetic nerve activity (RSNA, mV·s, integrated). **E**, Quantification of the change (Δ) from baseline over time for mean BP (MBP), HR, and RSNA (%) in these 4 treatment groups. Values are expressed as mean±SEM (n=6 in each group). *P<0.05, compared with baseline. †P<0.05, compared with VEH. ‡P<0.05, compared with PVN IL-17A.

of either scrambled or IL-17RA siRNA (Δ−14.5±3.2 versus Δ−9.2±2.9 beats/min; Figure 6B).

Compared with the rats treated with saline, a 2-week Ang II infusion significantly elevated the mRNA levels of inflammatory cytokines IL-17A, TNF-α, and IL-1β in the PVN in rats pretreated with bilateral PVN microinjection of a scrambled siRNA. However, the upregulated mRNA levels of these inflammatory mediators were substantially lessened in the rats pretreated with an IL-17RA siRNA in the PVN (Figure 6C).

At the end of 2-week infusion of Ang II or saline, hexamethonium bromide, a ganglionic blocker, was injected in these rats to assess the sympathetic tone. We found that hexamethonium bromide-elicited drop of MBP in Ang II–infused rats pretreated with PVN IL-17A siRNA was lower (Δ−33.4±3.4 versus Δ−55.5±8.6 mm Hg) than that in Ang II–infused rats pretreated with a scrambled siRNA (Figure 6D), indicating that IL-17A-induced sympathetic tone contributes to Ang II–induced hypertension. Additionally, ELISA analysis revealed that the augmented plasma norepinephrine level induced by Ang II infusion in the rats treated with a scrambled siRNA was also significantly attenuated in rats pretreated with PVN IL-17A siRNA (Figure 6E).

DISCUSSION

IL-17A was initially identified as a key inflammatory mediator due to its intrinsic ability to orchestrate immune

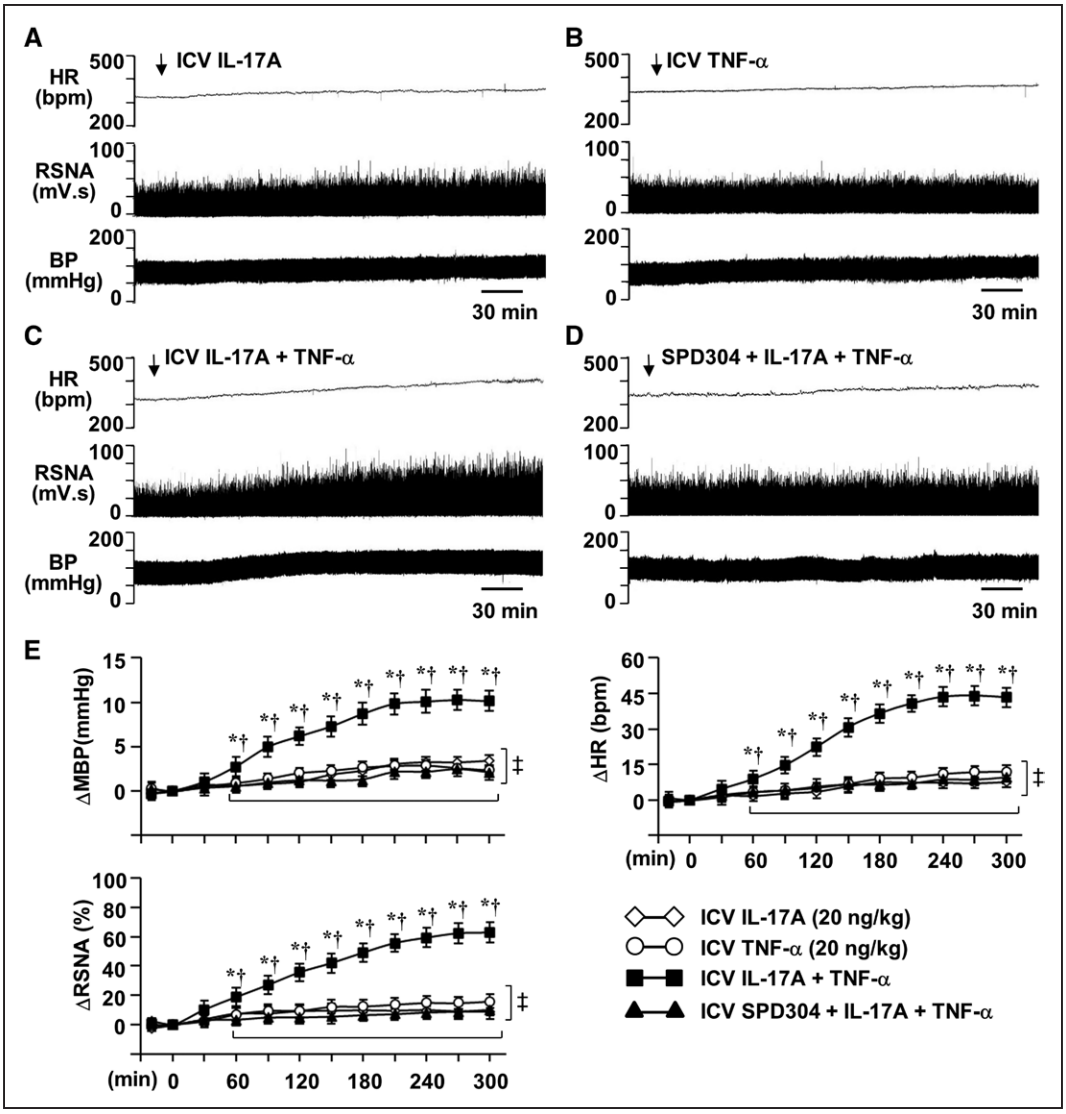

Figure 5. Synergistic effects of IL (interleukin) -17A with TNF (tumor necrosis factor)-α in the brain on hemodynamic and sympathetic responses.

Representative tracings showing the effects of intracerebroventricular (ICV) injection of IL-17A (20 ng/kg, **A**), TNF-α (20 ng/kg, **B**) alone or combined injection (**C**) of IL-17A (20 ng/kg) and TNF-α (20 ng/kg) in normal rats or the rats pretreated with TNF-α inhibitor SPD304 (**D**) on blood pressure (BP, mm Hg), heart rate (HR, beats/min), and renal sympathetic nerve activity (RSNA, mV·s, integrated). **E**, Quantification of the change (Δ) from baseline over time for mean BP (MBP), HR, and RSNA (%) in the 4 treatment groups. Values are expressed as mean±SEM (n=6 in each group). **P*<0.05, compared with baseline. †*P*<0.05, compared with IL-17A or TNF-α alone. ‡P<0.05, SPD304+IL-17A plus TNF-α compared with IL-17A plus TNF-α.

responses to promote chronic inflammation in the peripheral tissues. In the current study, we discovered an important role for IL-17A in regulating neuroinflammation, hemodynamics, and sympathetic drive in normal animals and a potential role of IL-17A in the brain in Ang II–induced hypertension. This distinctive action of IL-17A on sympathetic outflow and neuroinflammation has not been previously appreciated. These findings indicate that IL-17A in the brain might be a critical inflammatory mediator that drives neurohumoral activation and pressor responses in various pathophysiological conditions like hypertension and heart failure.

We examined the effects of IL-17A on BP, HR, and RSNA in normal rats in 3 different levels of analysis from the periphery to the CNS. We found that IL-17A in the circulation, brain, or more specifically in the PVN, all of which induced dramatic and persistent increases in hemodynamics and sympathetic outflow. The significant change in sympathetic outflow observed in this study could potentially account for tissue damage and the impairment of the function of peripheral organs in the contexts of not only cardiovascular diseases but also inflammatory and autoimmune diseases, characterized by elevated IL-17A levels. Notably, the onset of pressor responses on BP, HR, and RSNA following systemic administration of IL-17A was significantly slower (≈120 versus 30 minutes) than that of these responses induced by direct central injection, suggesting that

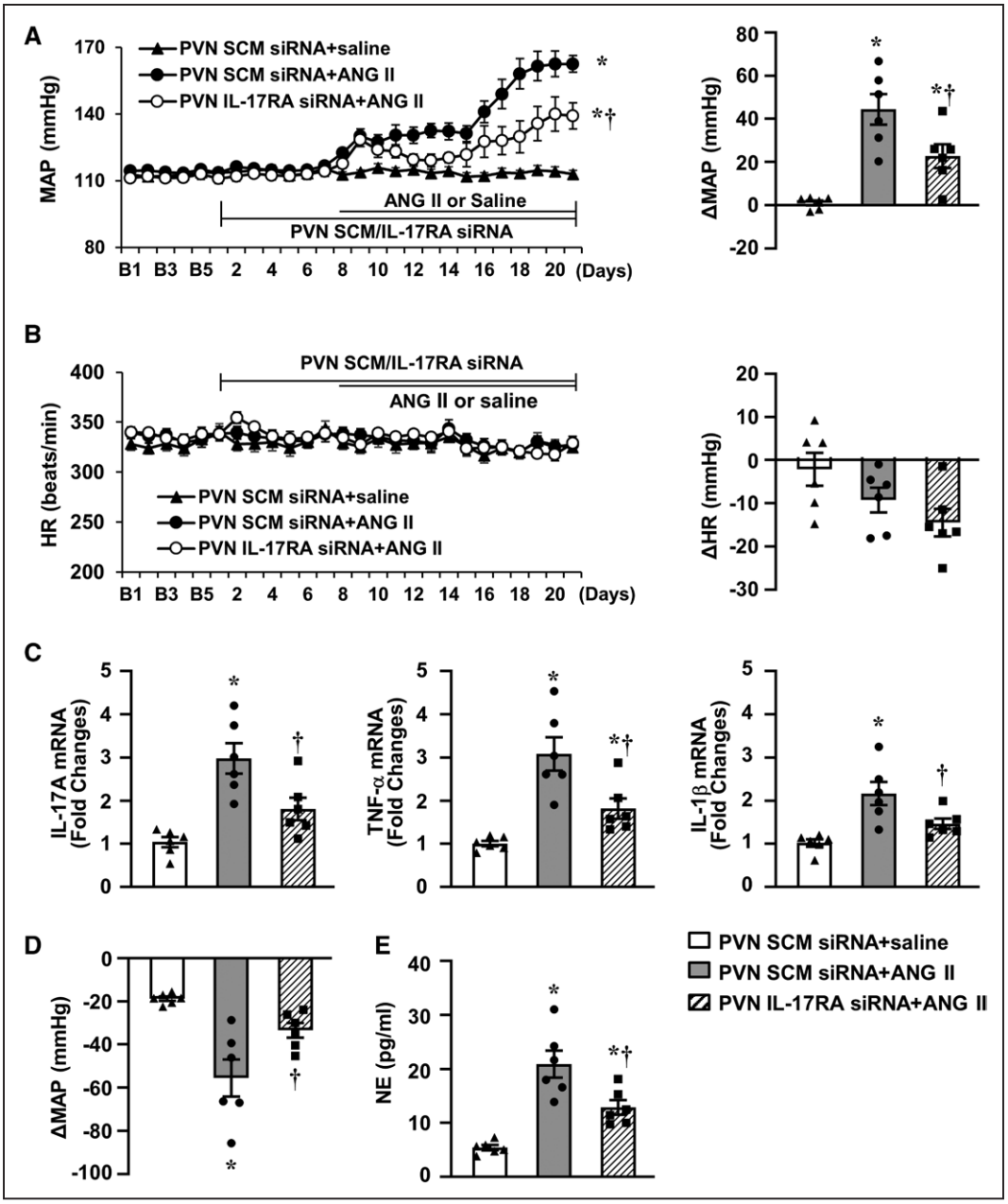

Figure 6. Effects of bilateral hypothalamic paraventricular nucleus (PVN) microinjection of IL-17RA (interleukin-17 receptor A) small interfering RNA (siRNA) or a scrambled (SCM) siRNA on Ang (angiotensin) II-induced hypertensive, or saline-infused control rats.

A, Daily mean blood pressure (MBP) and average change (Δ); (**B**) daily heart rate (HR) and average change (Δ); (**C**) mRNA levels of IL-17A, TNF (tumor necrosis factor)-α and IL-1β in the PVN; (**D**) maximum changes (Δ) in MBP in response to ganglionic blockade 2 wk after Ang II or saline infusion; and (**E**) plasma norepinephrine (NE) level. The average changes of MBP and HR in **A** and **B** are calculated based on the mean of the 5-d baseline subtracted from the mean of the final 5 d of treatment for each animal. Baseline recordings are denoted by B. Values are mean±SEM (n=6 for each group). **P*<0.05, vs baseline; †*P*<0.05, IL-17RA siRNA+Ang II vs SCM siRNA + Ang II.

circulating IL-17A might cross the BBB to act on the key brain regions controlling sympathetic nerve activity. This concept is supported by observations that CSF IL-17A levels increased after a recombinant IL-17A protein was administered systemically and correlated with plasma IL-17A levels, even with a possibility that the elevated CSF IL-17A levels may be partly a result of increased IL-17A gene expression within the brain.

The notion that systemic IL-17A can cross the BBB has been proposed by findings from studies of autoimmune diseases like psoriatic or rheumatoid arthritis characterized by elevated IL-17A in peripheral tissues, both of which have a high level of IL-17A detected in the CNS.[33] The BBB serves as an effective barrier to large molecules, such as cytokines, chemokines, and other neurochemicals in the circulation, preventing them from readily

accessing the brain. Thus, how IL-17A might reach the brain in this scenario is not fully understood. Although the current study did not address this mechanism specifically, several studies have indicated that IL-17A access the brain by disrupting the integrity of the BBB.[33] Using an in vitro model of BBB with human brain-derived microvascular endothelial cells, Kebir et al[26] demonstrated that IL-17A interrupts BBB by reducing the expression of 2 tight junction-associated molecules, occludin, and zonula occludens-1. In an in vivo mice model of experimental autoimmune encephalomyelitis, IL-17A was reported to disrupt the BBB probably through an oxidative stress-activated endothelial contractile mechanism involving a downregulation of occludin and integral adhesion molecule-1 protein located at the tight junctions on endothelial cells of BBB.[24] The impairment of the BBB eventually leads to lymphocyte infiltration into the brain, including that of IL-17 producing cells.[25] Infiltration of T helper 17 cells into the PVN has been reported in a stress-induced hypertensive model.[34] We should be aware that newly produced cytokines or chemokines by activated CNS-resident glial cells in response to increased IL-17A might cause further BBB damage to facilitate an inflex of IL-17A and infiltration of immune cells in this context. Alternatively, IL-17A may access the brain via circumventricular organs, the intrinsic brain structures that lack a BBB and allow rapid neurochemical exchange between CNS and the circulation. Our previous studies have demonstrated that the subfornical organ, a circumventricular organ in the lamina terminalis, mediates the central effects of blood-borne cytokines on hemodynamic and sympathetic responses.[23]

Increased sympathetic nerve activity is associated with peripheral vascular remodeling and altered vascular reactivity, contributing to hypertension. This study indicated that systemic vascular resistance was significantly elevated by IL-17A, implying that the raised BP should be regulated at least partly by this mechanism. It should be noted that although other cytokines also induce an excitatory response in BP, HR, and RSNA, such a magnitude of sympathetic excitation caused by IL-17A reported in this work has not been observed in other inflammatory mediators. This observation suggests that IL-17A might use a unique mechanism for which the synergistic feature of IL-17A is critical to promote sympathetic activation. Indeed, whereas intracerebroventricular injection of IL-17A or TNF-α individually at a low dose failed to induce an obvious increase in BP, HR, and RSNA, the combined injection of IL-17A with TNF-α at the same dose elicited excitatory responses that can be prevented by a TNF-α inhibitor. Many studies have supported synergy between IL-17A and other cytokines. In an mouse model of ischemic stroke, IL-17A secreted by T helper 17 and TNF-α produced by macrophages, act synergistically on astrocytes to enhance the production of CXCL1.[35] In the CNS parenchyma of multiple sclerosis patients, IL-17A was found to interact with other cytokines to exacerbate neuronal damage.[29] Collectively, our finding with the others suggests that many of the proinflammatory effects of IL-17A can be enhanced by working in synergy with other cytokines. Regarding the key role of IL-17A in hemodynamics and sympathetic drive, the synergistic feature of IL-17A is a novel finding that underscores its importance in the pathogenesis of cardiovascular disorders.

Another intriguing finding of the present study is that peripherally administered IL-17A boosts inflammation in the brain by inducing various inflammatory mediators in tissues inside the BBB in the PVN. The substantial activation of microglia and astrocytes of the PVN by systemic IL-17A implies that these CNS-resident cells might be the main producers for de novo-synthesized inflammatory cytokines and chemokines in the brain although we cannot exclude the possibility of other cellular elements, such as neurons, endothelial cells, and perivascular macrophages in the blood vessel. Inhibition of microglia or astrocytes diminishes IL-17A-caused excitatory response in BP, HR, and RNSA, suggesting that the centrally produced inflammatory mediators may also contribute to the sympathetic activation in response to IL-17A. IL-17A causes immune responses and tissue inflammation primarily as an inflammatory regulator to amplify a plethora of inflammatory mediators like cytokines, chemokines, adhesion molecules, and matrix metalloproteinases.[36,37] The newly produced cytokines and chemokines are both targets of IL-17A and stimulators of IL-17-producing cells, forming a positive-feedback loop to further potentiate the inflammation. This notion was supported by our observations that IL-17A in the brain can upregulate the expression of its close homolog IL-17F, as well as their cognate receptor IL-17RA. Taken together, acting in synergy with many other cytokines, IL-17A would be a powerful inducer of chronic inflammation in the CNS.

The present study revealed that IL-17RA is abundantly expressed in cardiovascular and autonomic brain regions and colocalized with both parvocellular presympathetic and magnocellular neuroendocrine neurons of the PVN. Moreover, the finding that a siRNA viral construct specifically suppressing the IL-17RA in the PVN interferes with the ability of peripheral IL-17A to increase BP, HR, and RSNA indicates that IL-17A in the brain, particularly in the PVN, plays a vital role in mediating pressor responses and sympathetic activation in the pathological conditions. Using a well-established Ang II–induced hypertensive rat model,[38] we found that subcutaneously infused Ang II promoted sympathetic outflow and elevated the expression of inflammatory cytokines in the brain. Elimination of the long-term effect of IL-17A by knocking down its receptor IL-17RA in the PVN significantly attenuated Ang II–elicited pressor response and reduced neuroinflammation and sympathetic tone

in this model of hypertension. These data suggest that IL-17A is a critical contributor to Ang II–induced hypertension, probably via a potential mechanism involving IL-17A-prompted autonomic activation and neuroinflammatory regulation in the CNS.

Although PVN has been shown to mediate the effect of IL-17A on neuroinflammation, hemodynamics, and sympathetic drive in normal and hypertensive rats, we cannot exclude roles for other brain nuclei in mediating the central effects of systemic IL-17A on sympathetic outflow, given the fact that IL-17RA is also presented in other brain nuclei and IL-17RA knockdown in PVN did not completely block IL-17A-elicited pressor and excitatory responses. In addition, systemic IL-17A also increases the mRNA level of IL-17A in other hypothalamic nuclei such as subfornical organ and arcuate nucleus that have direct or indirect neural projections to the PVN.

The neural and molecular mechanisms by which IL-17A promotes sympathetic activation and pressor response remain a mystery. Activation of the IL-17A/IL-17RA axis triggers a series of downstream signaling events that might modulate sodium and potassium ion channel activity in presympathetic neurons. In terms of the sustained increase in sympathetic and pressor responses to IL-17A, the potential mechanisms are more likely associated with long-term regulation of the expression of excitatory mediators on the neurochemical milieu in the brain. These might include molecular subunits of ion channel, components of renin-angiotensin system, reactive oxygen species, and biomarkers of endoplasmic reticulum stress. The current study demonstrated that central injection of IL-17A enhanced the phosphorylation of several signaling molecules that are closely associated with the transcriptional activation of MAPK and NF-κB, suggesting that these factors in the PVN are robustly involved in the inflammatory process of IL-17A on hemodynamics and sympathetic drive. Both MAPK and NF-κB have been reported to exert acute and chronic influences on sympathetic nerve activity under both physiological and pathophysiological conditions.[39,40] Alternatively, it is possible that signaling by IL-17A/IL-17RA affects the synthesis and release of certain key neurotransmitters to modulate neuronal activity. It has been reported that IL-17A can promote glutamate-induced neuronal excitation through decreasing glutamate uptake by astrocytes and increasing calcium-dependent glutamate release.[41] In multiple sclerosis, the high level of IL-17A in the CSF is escorted with an elevation of glutamate,[42] suggesting that IL-17A–induced sympathetic activation might be facilitated by this excitatory neurotransmitter mechanism as well.

PERSPECTIVES

Several limitations of this study deserve comment. First, although the potential of IL-17A in disrupting the BBB has been reported by others, direct evidence for peripherally administered IL-17A accessing the brain was not demonstrated in the present study. Second, while minocycline or fluorocitrate was effective in lowering the effect of IL-17A on MAP, HR, and RSNA, the specificity of both inhibitors targeting microglia and astrocytes was not verified. Third, the responses to a bolus injection of a recombinant IL-17A protein at a supraphysiological dose in normal animals may not truly reflect the pathophysiological action of endogenous IL-17A in vivo. However, these studies, particularly in the chronic experimental protocols in Ang II–induced hypertensive rats using an IL-17RA siRNA specifically knocking down IL-17A signaling in the PVN, do reveal a crucial role for IL-17A in mediating neuroinflammation, sympathetic drive, and hypertension and suggests a synergistic and feed-forward neuroinflammatory mechanism-driven by IL-17A in the CNS.

Further studies will need to examine the contribution of IL-17A to the neuroinflammatory mechanisms driving sympathetic activation in other experimental models of hypertension and heart failure. Although the data in the present study were obtained from animals, these new findings have important implications for human disease states characterized by high level of IL-17A. Specifically, these findings suggest that interventions decreasing IL-17A/IL-17RA signaling in the brain have the potential to reduce neuroinflammation and hold promise for the development of novel therapeutic agents capable of ameliorating sympathetic overactivity in cardiovascular diseases.

ARTICLE INFORMATION

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Disclosures

None.

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