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Central and Peripheral Mechanisms of T lymphocyte Activation and Vascular Inflammation Produced by Angiotensin II-Induced Hypertension

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Abstract

Rationale—We have previously found that T lymphocytes are essential for development of angiotensin II-induced hypertension however the mechanisms responsible for T cell activation in hypertension remain undefined.

Objective—To study the roles of the central nervous system and pressure elevation in T cell activation and vascular inflammation caused by angiotensin II.

Methods and Results—To prevent the central actions of angiotensin II we created anteroventral third cerebral ventricle (AV3V) lesions in mice. The elevation in blood pressure in response to angiotensin II was virtually eliminated by AV3V lesions, as was activation of circulating T cells and the vascular infiltration of leukocytes. In contrast, AV3V lesioning did not prevent the hypertension and T cell activation caused by the peripheral acting agonist norepinephrine. To determine if T cell activation and vascular inflammation are due to central influences or are mediated by blood pressure elevation, we administered hydralazine (250 mg/L) in the drinking water. Hydralazine prevented the hypertension, and abrogated the increase in circulating activated T cells and vascular infiltration of leukocytes caused by angiotensin II.

Conclusions—We conclude that the central and pressor effects of angiotensin II are critical for T cell activation and development of vascular inflammation. These findings also support a feed forward mechanism in which modest degrees of blood pressure elevation lead to T cell activation, which in turn promotes inflammation and further raises blood pressure, leading to severe hypertension.

Brief summary—We have previously shown that T cells are important for the development of hypertension and others have shown that CNS lesions such as AV3V disruption prevent hypertension. We examined the relationship between central actions of angiotensin II, T cell activation and hypertension by determining how AV3V lesions affect T cell activation and hypertensive responses to angiotensin II and norepinephrine. Our data are compatible with a scenario in which modest degrees of pressure elevation, mediated either directly by norepinephrine or via central actions of angiotensin II, promote an inflammatory response that

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leads to severe hypertension. These studies provide new insight into how the central nervous system contributes to systemic inflammation in hypertension.

Keywords

Hypertension; vascular inflammation; T cells; central nervous system

INTRODUCTION

The central nervous system (CNS) plays an integral role in blood pressure regulation, primarily through sympathetic activation and mediation of various neurohumoral factors such as angiotensin II and vasopressin.^{1–4} Hormones such as these can access the circumventricular organs, which are adjacent to the cerebral ventricles and have a poorly formed blood brain barrier. The circumventricular organs implicated in blood pressure control include the organum vasculosum of the lamina terminalis (OVLT), the area postrema, the subfornical organ and the anteroventral third ventricle (AV3V) region.⁵ The AV3V region includes the median preoptic nucleus, the OVLT and the periventricular nucleus and has been shown to play an important role in several behavioral, neural and hormonal functions involved in body fluid and cardiovascular homeostasis.^{6, 7} Electrolytic lesions that disrupt the AV3V region have been shown to abolish virtually all of the central actions of angiotensin II including the drinking behavior, sympathetic outflow and vasopressin release.⁶ Electrolytic lesions of this brain region abolish the centrally mediated pressor response to central and peripheral infusions of angiotensin II as well as preventing and/or reversing several other forms of experimental hypertension.^{5, 6}

In addition to central mechanisms, substantial evidence suggests that inflammation can contribute to the pathophysiology of hypertension. For example, inflammatory cells accumulate in the kidney and vasculature of hypertensive animals and the prevention of this can lower blood pressure.⁸ Recently our laboratory has found that mice lacking T-lymphocytes are resistant to the development of both angiotensin II and DOCA-salt induced hypertension.⁹ Adoptive transfer of T, but not B cells restored hypertension in these animals. Despite the growing evidence for the role of T cells in hypertension, the mechanisms underlying T cell activation and vascular infiltration by T cells remain unclear. Given the importance of the central nervous system in regulating blood pressure, we hypothesized that the CNS could participate in activation of peripheral T cells and contribute to vascular inflammation in response to angiotensin II. We tested this hypothesis by producing electrolytic lesions of the AV3V region in mice and then examined properties of circulating T cells and the vascular accumulation of these cells in response to chronic angiotensin II infusion. We also examined the role of pressure elevation in T cell activation and vascular inflammation. Our findings provide a new understanding for how the central nervous system, in particular the circumventricular organs, can contribute to the promotion of systemic inflammation in hypertension.

MATERIALS AND METHODS

Animals

C57BL/6, RAG-1^{-/-} and ovalbumin (OVA)-specific, MHC class II-restricted α/β T cell receptor (TCR) transgenic mice (OT2) were obtained from Jackson laboratories.

Production of Hypertension

Using an osmotic minipump (Alzet, Model 2002) either angiotensin II ($490 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), norepinephrine ($3.8 \text{ ug} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), or vehicle was infused for 14 days. Blood

pressure was measured by radiotelemetry or tail cuff method, as previously described.⁹ When radiotelemetry was employed, the transmitters were implanted 1 week prior to the minipumps. In some experiments, hydralazine (250mg/L) was administered in the drinking water to prevent the development of hypertension.¹⁰ Adoptive transfer of T cells into RAG-1^{-/-} mice was performed using methods similar to those previously described⁹ and norepinephrine or vehicle infusion initiated 3 weeks later.

AV3V Lesions

Mice were anesthetized with intraperitoneal ketamine and acepromazine (90 mg/kg and 1.8 mg/kg, respectively) and mounted in a stereotaxic frame with skull level between bregma and lambda. Using aseptic technique, a small hole was drilled in the skull, the mid-sagittal sinus retracted, and a 200 μ m diameter tungsten electrode (AM Systems), teflon-insulated except at the tip, was inserted at the midline 0.4 mm anterior to bregma and 4.8 mm ventral to the dura.¹¹ Lesions were produced by passing a 1.0 mA DC anodal current through the electrode for 3 seconds. Surgery for sham-operated mice was identical except that the electrode was lowered only 4.0 mm, and no current was applied. Because AV3V-lesioned animals do not drink water for several days after lesioning, mice were given a highly palatable 10% sucrose solution prior to and after lesioning to induce voluntary fluid intake and prevent dehydration. Following the lesion, AV3V-lesioned mice were weaned onto normal drinking water by gradually reducing the concentration of sucrose over a 2 week period.⁷

AV3V Lesion Verification

Electrolytic lesioning in and around the third ventricle can produce neuronal destruction of variable extent as shown by the topography detailed in previous studies employing AV3V-lesioned animals.^{5, 12–14} Mice included in the AV3V-lesioned groups were functionally and histologically characterized as having sustained complete AV3V lesions using criteria (drinking behavior and histology) that were independent of the dependent variables of interest in these studies (blood pressure, superoxide production, T-cell function). Mice were considered to have complete AV3V lesions if they met the following three criteria: (1) Failed to drink any water within 24 hr following surgery, (2) drank less than 1.0 ml of water within 2.0 hr following subcutaneous injection of 6.0% NaCl and (3) histological confirmation following the experimental protocol of complete destruction of the AV3V region, including the OVLT, median preoptic and periventricular nuclei (Figure 1). Approximately 50% of mice that received AV3V lesions met the above criteria. In the AV3V mice that met these criteria the 24 hr water intake was $0.08 \pm .03$ ml compared to 2.65 ± 0.27 ml in the Sham-operated group while the drinking volume in response to the hypertonic 6.0% NaCl thirst challenge was 0.56 ± 0.17 ml and 2.16 ± 0.13 ml in the AV3V-lesioned and Sham-operated mice respectively.

Superoxide measurements, fluorescent cell sorting and analysis of cellular inflammation

Vascular O₂⁻ production was measured by quantifying formation of 2-hydroxyethidium from dihydroethidium (25 μ M) by HPLC as described previously.¹⁵ Analysis of circulating T cells from the blood and inflammatory cells in vascular homogenates of the aorta was performed using fluorescent cell sorting as previously described.⁹ Antibodies used for staining were as follows: FITC anti-CD45 (30-F11); APC anti-CD4 (GK1.5); PerCP anti-CD8 (53–6.7); APC anti-CD3 (145-2C11); FITC CD44 (IM7); FITC CD69 (H1.2F3). After immunostaining, cells were resuspended in FACS buffer (0.5% bovine serum albumin in PBS) and analyzed immediately on a LSR-II flow cytometer with DIVA software (Becton Dickinson). Data were analyzed with FlowJo software (Tree Star, Inc.). The institutional Animal Care and Use Committee at Emory University approved all of the above experimental protocols.

Data presentation and statistical analysis

Summary data in manuscript are expressed as the mean \pm the SEM and values of $P < 0.05$ were considered statistically significant. Comparisons between groups of animals or treatments were made by ANOVA. The Bonferroni post hoc test was used to make comparisons.

RESULTS

Effects of AV3V ablation on blood pressure and vascular superoxide

In sham-operated mice, angiotensin II infusion produced a progressive rise in blood pressure when measured either by tail cuff or radiotelemetry (Figure 2A–C). Infusion of vehicle alone had no effect on arterial pressure (Figure 2A). AV3V ablation did not affect baseline blood pressure, but markedly reduced the hypertension produced by peripheral infusion of angiotensin II compared to the sham-operated group (Figure 2A–C). Angiotensin II infusion in AV3V-lesioned mice produced a small rise in blood pressure after 7 and 14 days compared to sham vehicle (Panel A). In other groups of mice, direct measurement of arterial pressure by radiotelemetry largely confirmed the results obtained using the tail cuff method. AV3V lesions virtually eliminated increases in systolic and diastolic blood pressure normally produced by peripheral infusion of angiotensin II (Figure 2B and 2C).

Previous studies from our laboratory have shown that chronic angiotensin II infusion increased vascular $O_2^{\cdot -}$ production.^{9, 16} We confirmed these results in C57BL/6 mice without CNS lesions as shown in Figure 2D. Chronic infusion of angiotensin II in sham-operated mice elevated aortic superoxide levels to values similar to those without CNS lesions. In contrast, vascular superoxide levels of mice given angiotensin II were significantly reduced by AV3V lesioning, and were not different from mice that did not receive angiotensin II infusion (Figure 2D).

Effects of AV3V ablation on T cell activation and vascular inflammation

Consistent with previous observations,^{17–19} angiotensin II infusion in sham-operated mice increased the percentage of circulating helper (CD4+) T cells that expressed the early activation marker CD69 (Figure 3A) and the tissue homing marker CD44^{high} (Figure 3B). This increase in activated CD4+ T-cells was abolished by AV3V lesioning (Figure 3A and 3B). In sham-operated mice, angiotensin II infusion promoted vascular infiltration by inflammatory leukocytes as evidenced by aortic accumulation of total CD45+ leukocytes (Figure 3C) and CD3+ T-cells (Figure 3D). Similar to the results observed for circulating lymphocytes, vascular accumulation of inflammatory cells was completely prevented by the AV3V lesion. These data indicate that angiotensin II-induced activation of circulating T cells and infiltration of vascular tissue by inflammatory cells cannot be ascribed to a direct action of the circulating hormone on peripheral tissues, but instead must be mediated by a CNS action of angiotensin II that is dependent on the AV3V region.

Effect of AV3V ablation on norepinephrine induced hypertension, T cell activation and vascular inflammation

Systemic infusion of the peripherally-acting vasoconstrictor norepinephrine was employed as a positive control for any potential non-specific or detrimental effects of the AV3V lesion on cardiovascular function. As shown in Figure 4A, AV3V lesions had no effect on hypertension produced by norepinephrine. When immune cell activation and vascular inflammation were measured, similar increases in circulating T cells expressing the early activation marker CD69 and the tissue homing marker CD44^{high} were observed in both AV3V-lesioned and sham-operated animals (Figure 4B–C). Moreover, norepinephrine infusion also promoted vascular inflammation assessed by aortic accumulation of CD45+

leukocytes and CD3⁺ T cells, irrespective of whether mice had sustained AV3V lesions or not (Figure 4C–E). These results indicate that AV3V lesions do not impair the production of hypertension and T cell activation produced by a peripherally-acting stimulus. We further confirmed that, as in the case of angiotensin II, T cells largely mediate the hypertensive response to norepinephrine, as the blood pressure elevation to this catecholamine was markedly reduced in RAG-1^{-/-} mice and restored by adoptive transfer of T cells (Figure 4F).

Effect of preventing hypertension with hydralazine on vascular superoxide, T cell activation and vascular inflammation

Results from the previous experiments using angiotensin II and norepinephrine infusion to produce hypertension suggest that immune cell activation may be more dependent on elevated arterial pressure per se, rather than the specific stimulus that produces it. To determine whether the reduction in T cell activation and vascular inflammation following AV3V ablation and angiotensin II infusion was a consequence of preventing hypertension, we administered hydralazine via the drinking water during angiotensin II infusion. Following 2 weeks of angiotensin II infusion, hydralazine eliminated the elevation in blood pressure to angiotensin II (Figure 5A) as well as vascular superoxide production (Figure 5B). Hydralazine also prevented the activation of circulating T cells (Figure 5C–D) as well as vascular accumulation of inflammatory cells (Figure 5E–F). We also examined the effect of hydralazine administration during norepinephrine infusion. As in the case of angiotensin II, hydralazine prevented the increase in arterial pressure, T cell activation and infiltration during norepinephrine induced-hypertension (Online Figure I).

The effects of hydralazine on T cell activation and vascular inflammation could be related to its anti-hypertensive actions, or alternatively to a direct effect on T cells that might prevent their activation. To distinguish between these possibilities, we performed studies using OT2 mice. OT2 mice are transgenic for a T cell receptor that induces production of CD4⁺ activated T cells when the receptor is activated by the peptide ovalbumin 323-339. OT2 mice were injected on day 1 intraperitoneally with ovalbumin (OVA) 323-339 peptide (0.5µg/µl) or vehicle (Al(OH)₃ (2.5µg/µl)) and hydralazine was administered in the drinking water during the 7 days. Blood pressure was not significantly changed between groups (OVA: 122 ± 5.7; Al(OH)₃ 122 ± 7.6 mmHg) (Hydralazine + OVA: 113 ± 1.3 mmHg; Hydralazine + Al(OH)₃ 113 ± 10.1 mmHg). In control mice, OVA markedly enhanced T cell activation as evidenced by an increase in CD69 and in CD44^{high} expressed on CD4⁺ cells (Figure 6A and 6B) compared to OT2 mice treated with vehicle Al(OH)₃. Treatment of OT2 mice with hydralazine for one week did not diminish T-cell activation evoked by OVA (Figure 6A and 6B). These results indicate that hydralazine does not impair T cell activation in response to a non-hypertensive stimulus.

DISCUSSION

The purpose of this study was to investigate the potential mechanisms by which angiotensin II increases blood pressure and T cell activation. Anteroventral third cerebral ventricle (AV3V) ablation of the forebrain, which is known to prevent the central actions of angiotensin II, was found to prevent activation of circulating T cells and to inhibit the vascular inflammation caused by this octapeptide. In contrast, AV3V lesions did not alter the effect of norepinephrine on these parameters. AV3V lesioning also prevented the increase in vascular O₂⁻ production caused by angiotensin II infusion. The anti-inflammatory effects of AV3V lesioning could have been due to a reduction of neurohumoral stimuli, or due to prevention of pressure-induced tissue damage. Hydralazine, which prevented the hypertensive response to angiotensin II infusion, also prevented T cell activation, vessel infiltration of leukocytes as well as vascular superoxide production. In

contrast, hydralazine did not block T cell activation in response to a non-hypertensive stimulus in OT2 transgenic mice. This suggests that the reduction in T cell activation and vascular infiltration caused by hydralazine during angiotensin II and norepinephrine infusion were likely a result of its blood pressure lowering effects rather than any direct actions it has on T cells. Our results suggest that angiotensin II-mediated hypertension is caused by central mechanisms and that T cell activation is likely dependent on mechanical stimuli caused by elevations of arterial pressure induced by these central signals.

Prior to the present study, it was unknown whether T cell activation and vascular infiltration in angiotensin II-induced hypertension was due to the direct effects of angiotensin II on T cells or whether T cell activation was mediated by its CNS actions. To address this question, we created lesions of the AV3V region which encompasses the organum vasculosum of the lamina terminalis (OVLT), the ventral portion of the median preoptic nucleus (MnPO), and the preoptic periventricular nuclei.⁵ The AV3V region receives input from other circumventricular structures such as the subfornical organ, which transduces peripheral angiotensin signals and sends them to the AV3V, thus contributing to the development of hypertension.^{20, 21} These regions of the brain have a poorly formed blood brain barrier and are capable of responding to circulating hormones such as angiotensin II^{22, 23} and modulating its central actions, which include drinking behavior, vasopressin secretion, sympathetic activity and norepinephrine synthesis.^{5, 24} Our studies confirm these effects in that the increase in blood pressure following systemic angiotensin II infusion was attenuated after AV3V ablation. Interestingly, AV3V lesioning also prevented activation of circulating T cells and vascular inflammation caused by angiotensin II. Thus, these results indicate that the central actions of angiotensin II contribute to its pro-inflammatory effects.

To demonstrate that the reduction of T cell activation after AV3V lesioning was not a non-specific effect of this intervention, we performed additional studies in which we infused norepinephrine. We reasoned that one of the major effects of AV3V lesioning was to abolish sympathetic outflow to the periphery and therefore that this could be bypassed by administration of norepinephrine, a peripherally acting vasoconstrictor stimulus. The degree of blood pressure elevation, T cell activation and vascular infiltration of CD45+ and CD3+ cells in response to norepinephrine was similar in the absence and presence of AV3V lesioning. These results demonstrate that AV3V lesioning does not non-specifically block the inflammatory response to all hypertensive stimuli and verifies the specificity for the central actions of angiotensin II. Moreover, these experiments with norepinephrine demonstrate that other vasoconstrictor stimuli are capable of mediating vascular inflammation during hypertension.

Angiotensin II is known to promote sympathetic outflow and therefore one interpretation of our results is that immune cell activation is dependent on sympathetic activation and catecholamine release. T cells possess adrenergic receptors, and sympathetic nerves richly innervate lymphatic tissues.²⁵ Angiotensin II could therefore stimulate T cells via activation of the sympathetic nervous system. These considerations are in keeping with the findings of Ganta et al, who showed that angiotensin II administration in the lateral cerebral ventricle increased splenic sympathetic nerve activity, which in turn causes increased mRNA expression of various proinflammatory cytokines in splenocytes. Splenic sympathetic denervation abrogated these responses, thus clearly linking the central effects of angiotensin II to peripheral immune activation and cytokine production.²⁶

Given the above considerations, it is possible that the consequent release of central mediators in response to angiotensin II, such as catecholamines could directly lead to T cell activation in the absence of blood pressure elevation. To address this, we performed studies using hydralazine to prevent hypertension during angiotensin II and norepinephrine infusion.

The results of these studies suggest that pressure elevation per se could lead to T cell activation and vascular infiltration. In contrast to AV3V lesioning, hydralazine activates the sympathetic nervous system,²⁷ in part via baroreflex-mediated mechanisms, but in the present study, completely prevented T cell activation. These results would seem to exclude a direct effect of sympathetic nerves on T cell activation, and would favor an effect of pressure per se in T cell activation.

We have previously found that hydralazine inhibits the NADPH oxidase.²⁸ The NADPH oxidase has been identified in T cells²⁹, and its blockade might have prevented T cell activation. Hydralazine also opens calcium sensitive potassium channels,³⁰ which are present in T cells³¹ and therefore may have direct effects on T cell function. To determine if hydralazine directly inhibits T cell activation in response to non-hypertensive stimuli, we studied OT2 transgenic mice. These animals express a T cell receptor that leads to CD4+ cell activation in response to ovalbumin peptide administration. We found that hydralazine did not block T cell activation caused by ovalbumin peptide administration in these animals, suggesting that it does not prevent T cell activation by stimuli that do not cause hypertension. These findings support the notion that the effect of hydralazine during angiotensin II infusion was not due to a direct effect of the drug on T cells, but more likely due to its prevention of hypertension. We are not completely able, however, to delineate a direct effect of neurohumoral mediators, such as catecholamines, from mechanical stimuli caused by pressure elevation. Our findings are nevertheless compatible with a scenario in which the central effects of angiotensin II lead to pressure elevation, which in turn promotes T cell activation and vascular inflammation.

These results should not be taken to indicate that inflammation, and in particular T cell activation is simply a result of hypertension and does not contribute to blood pressure elevation. As we previously demonstrated, in the absence of T cells, the hypertensive response to angiotensin II and DOCA-salt challenge is minimal⁹. Our current findings suggest however that a modest increase in blood pressure may lead to an inflammatory response that further increases and sustains blood pressure in a feed-forward fashion (Online Figure III). This might explain the propensity of pre-hypertension to progress to overt hypertension. These considerations stress the benefit of lowering blood pressure by virtually any means to prevent and reverse this mechanism.

NOVELTY AND SIGNIFICANCE

What is known

- We have previously shown that T cells are essential for development of hypertension.
- The circumventricular organs in the central nervous system are key targets for the peptide angiotensin II and are essential in the regulation of blood pressure.

What new information does this article contribute

- Central effects of angiotensin II are required for T cell activation and peripheral vascular inflammation in the setting of hypertension caused by this octapeptide.
- Modest elevations of blood pressure mediated by central stimuli promote inflammation, which leads to severe hypertension in a feed-forward fashion.

There is growing evidence for the role of T cells in hypertension, however the mechanisms underlying T cell activation and vascular infiltration by T cells remain unclear. The circumventricular organs are critical for the regulation of blood pressure and therefore we hypothesized that the CNS could participate in activation of peripheral T

cells and contribute to vascular inflammation in response to angiotensin II. Lesioning of the anteroventral third ventricle (AV3V) is known to prevent hypertension caused by angiotensin II. In the present study, we found that T cell activation and vascular inflammation caused by angiotensin II infusion were completely blocked by AV3V lesioning. The increase in blood pressure and T cell activation to peripherally acting norepinephrine was unaffected by AV3V ablation. Administration of an anti-hypertensive drug attenuated the elevation in blood pressure following angiotensin II infusion and also prevented the increase in T cell activation and vascular inflammation. Our findings are compatible with a pathway in which central stimuli such as angiotensin II cause modest elevations of blood pressure which leads to T cell activation, and ultimately more severe hypertension. This study provides new understanding of the link between central signals, peripheral inflammation and hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

Al(OH)₃	Aluminum hydroxide
ANG II	Angiotensin II
AC	Anterior commissure
AV3V	Anteroventral third ventricle
CNS	Central nervous system
HYD	Hydralazine
NE	Norepinephrine
OC	Optic chiasm
OVLT	Organum vasculosum of the lamina terminalis
OVA	Ovalbumin

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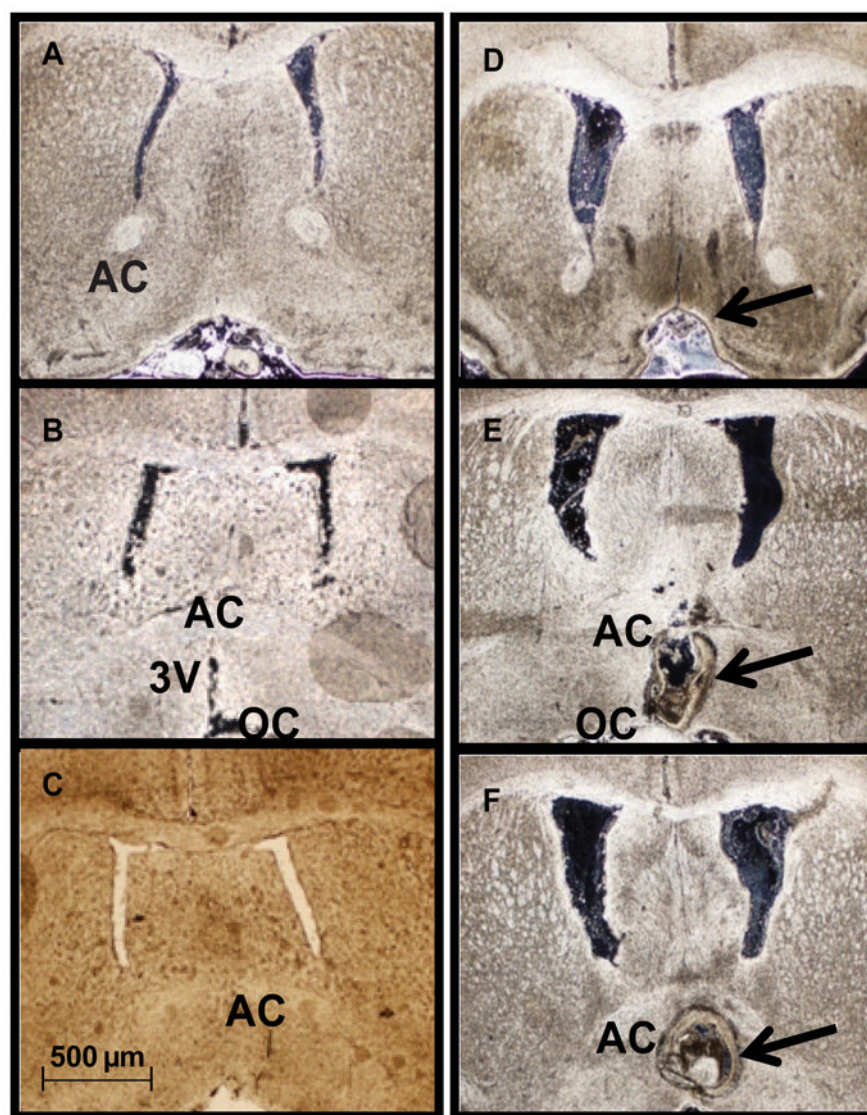


Figure 1. Coronal brain sections of anteroventral third ventricle (AV3V) lesioned and Sham-operated mice

Panels A–F depict brains from a representative single sham operated (AC) and AV3V lesioned (D–F) mouse. Coronal brain sections are at the level of 0.4 mm anterior to bregma according to the atlas of Paxinos and Franklin¹¹. Arrows point to the neuronal damage to the AV3V region. (AC, anterior commissure; OC, optic chiasm; 3V, third ventricle).

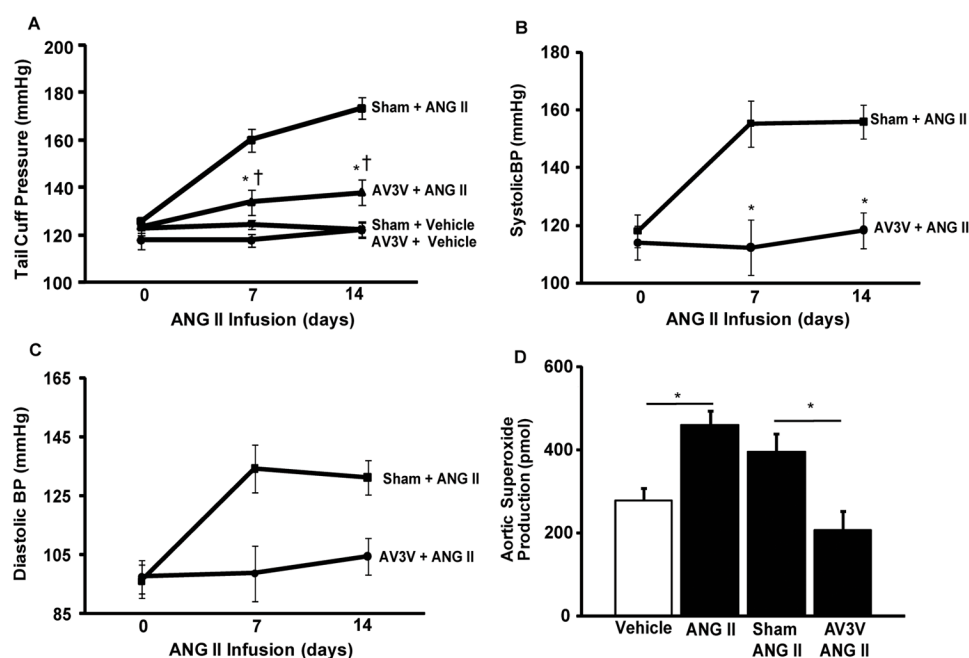


Figure 2. Effect of AV3V lesioning on hypertension and superoxide produced by angiotensin II
 Panel A: Blood pressure measured by tail cuff following 0, 7, and 14 days of angiotensin II (ANG II) infusion. AV3V + ANG II (n=13), Sham + ANG II (n=13), AV3V + Vehicle (n=4), Sham + Vehicle (n=7). Panels B and C: Systolic and diastolic blood pressure measured by radiotelemetry. AV3V + ANG II (n=3), Sham + ANG II (n=5). Panel D: Aortic superoxide levels in Vehicle and Sham + ANG II and AV3V-lesioned mice (n = 5–8 in each group). (AV3V + ANG II vs Sham + Vehicle †; AV3V + ANG II vs Sham ANG II *; $P < 0.05$) (Dashed Line represents Vehicle; Solid Line represents ANG II).

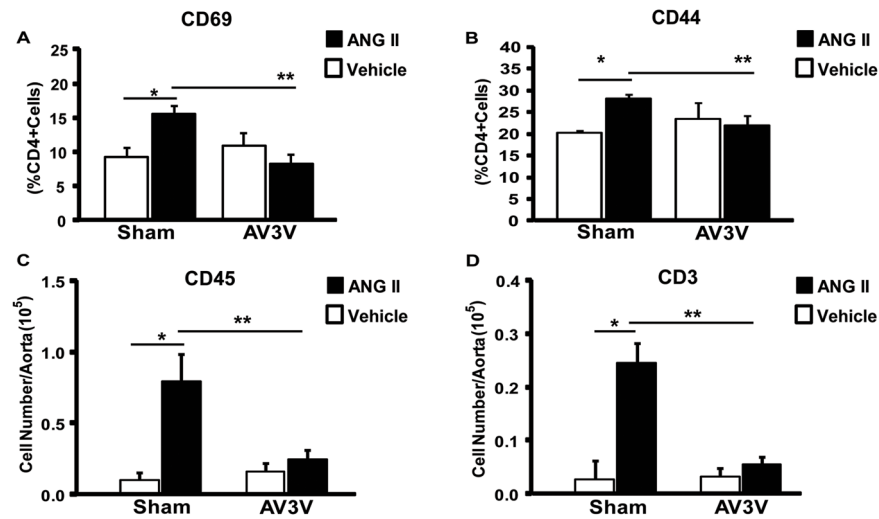


Figure 3. AV3V lesioning prevents angiotensin II-induced peripheral T cell activation and tissue infiltration

Percentage of circulating CD4⁺ lymphocytes expressing the early activation marker CD69 (Panel A) and tissue homing marker CD44 (Panel B) for sham-operated (n = 5–14) and AV3V-lesioned (n=5–11) mice. Total number of CD45⁺ leukocytes (Panel C) and CD3⁺ T cells (Panel D) in aortas of Sham-operated (n = 5–13) and AV3V-lesioned (n = 5–9) mice. Representative flow cytometric analysis of aortic CD45⁺ and CD3⁺ cells from sham-operated and AV3V lesioned groups (Panel E). Numbers in boxes represent a percent of the cell population that is positively labeled for the respective marker. (Vehicle vs ANG II **P*<0.05) (Sham + ANG II vs AV3V + ANG II ***P*<0.05).

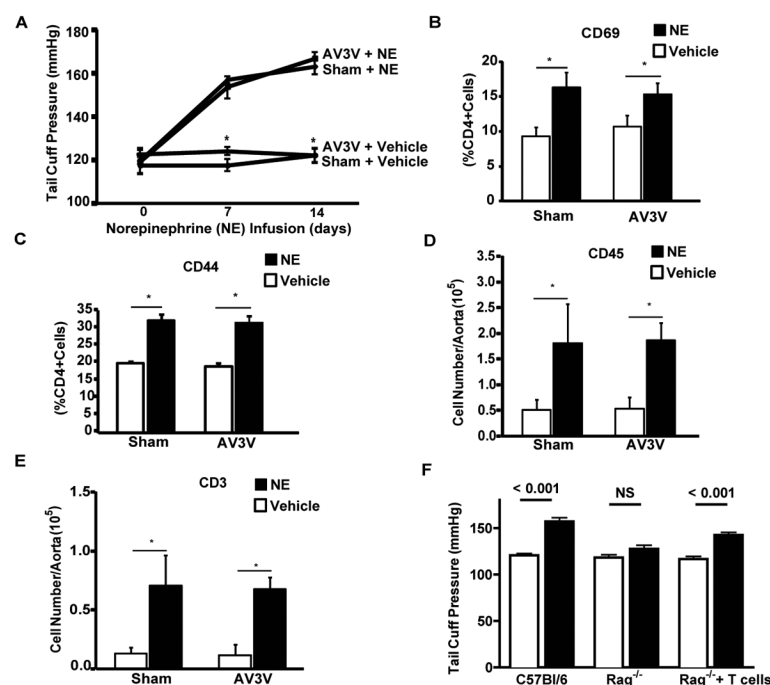


Figure 4. Norepinephrine (NE) induced hypertension promotes T cell activation and vascular inflammation independent of the AV3V region

Panel A: Blood pressure measured by tail cuff after 0, 7, 14 days of NE infusion (AV3V n=4–6; Sham n= 5–7). Panel B–C: Percentage of circulating CD4+ lymphocytes expressing the early activation marker CD69 and the T cell tissue homing marker CD44 (AV3V n = 4–6; Sham n=5). Panel D–E: Total number of CD45+ leukocytes and CD3+ T cells in aortas of sham-operated (n=5–7) and AV3V lesioned (n=6–7) mice after 14 day infusion with vehicle or NE. Panel F: Blood Pressure in C57Blk/6 and RAG1^{-/-} mice at baseline and following vehicle or norepinephrine infusion for 14 days. Some RAG1^{-/-} mice received adoptive transfer of T cells via retro-orbital injection (2×10^7 cells) and norepinephrine infusion begun 3 weeks later (n=5–9/group). (* $P < 0.05$ NE vs Vehicle) (AV3V and sham vehicle vs AV3V and sham NE ** $P < 0.05$).

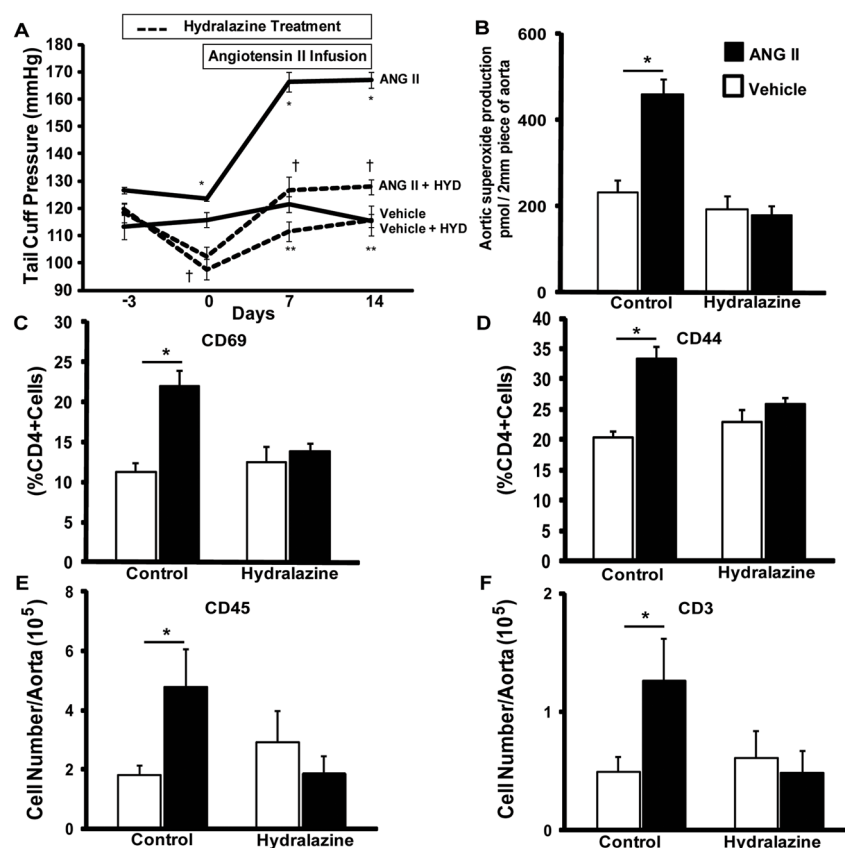


Figure 5. Hydralazine prevents angiotensin II-induced hypertension, increased superoxide production, T cell activation and vascular inflammation

Panel A: Blood pressure measurements during 2 weeks of angiotensin II (ANG II) infusion and hydralazine (HYD) treatment ($n = 8-10$). Panel B: Aortic superoxide levels following ANG II infusion and HYD treatment ($n = 5-11$ in each group). Panel C-D: Percentage of circulating CD4+ lymphocytes expressing the early activation marker CD69 and the T cell tissue homing marker CD44 following ANG II and HYD treatment ($n = 11-14$). Panel (E-F) Absolute numbers of total leukocytes (CD45+) and total T cells (CD3+) in collagenase digested whole aortas following ANG II infusion and HYD treatment ($n = 7-16$) (* $P < 0.05$ ANG II vs Vehicle) († $P < 0.05$ ANG II + HYD vs ANG II) (** $P < 0.05$ Vehicle + HYD vs ANG II + HYD).

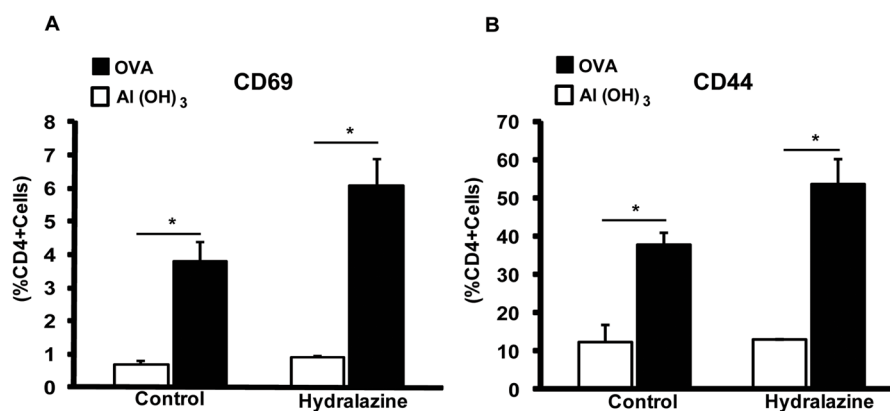


Figure 6. Hydralazine does not prevent circulating T cell activation in OT2 transgenic mice
 Panel (A) The percentage of circulating CD4+ lymphocytes expressing the early activation marker CD69 and Panel (B) T cell tissue homing marker CD44 in OT transgenic mice immunized with ovalbumin (OVA) and vehicle aluminum hydroxide (Al(OH)₃) (n = 3 OVA; n = 2 Al(OH)₃). Control represents mice that did not receive hydralazine. (**P* < 0.05 OVA vs Al(OH)₃ in control and hydralazine groups).