ROLE OF VASCULAR EXTRACELLULAR SUPEROXIDE DISMUTASE IN HYPERTENSION

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Abstract

Prior studies indicate that superoxide (O$_2^•-$) is important in modulation of blood pressure, but have not specifically identified the cell types or organs involved. We created mice with loxP sites flanking the extracellular superoxide dismutase (SOD3) gene. These mice were crossed with mice expressing inducible Cre-recombinase driven by the smooth muscle myosin heavy chain promoter allowing tissue specific deletion of SOD3. Deletion of SOD3 increased vascular O$_2^•-$ and reduced vascular NO levels as detected by ESR. Despite these changes in NO and O$_2^•-$, we did not observe increases in vascular inflammation caused by angiotensin II. Moreover, deletion of vascular SOD3 did not augment hypertension in response to angiotensin II. In additional studies, we also deleted SOD3 from the circumventricular organs by intracerebroventricular injection of an adenovirus encoding Cre-recombinase. While this raised blood pressure and augmented the hypertension caused by angiotensin II, these responses were not further increased by vascular deletion of SOD3. These data suggests that the extracellular superoxide dismutase in vascular smooth muscle is not involved in the genesis of angiotensin II-induced hypertension and further emphasize the role of central SOD3 in modulation of blood pressure.

Keywords

Superoxide dismutase; blood pressure; inflammation; vasculature; central nervous system

Introduction

Reactive oxygen species, and in particular the superoxide anion (O$_2^•-$), play a critical role in the genesis of hypertension via mechanisms that are incompletely understood. Experimental hypertension is ameliorated by treatment with membrane targeted forms of superoxide dismutase (SOD) or by SOD-mimetics, 1,2 and mice lacking the NADPH oxidase subunit p47$^{phox}$ have reduced hypertensive responses to either angiotensin II or DOCA-salt challenge. 3, 4 In keeping with a role of O$_2^•-$ in hypertension, others and we have found that mice lacking the extracellular SOD (SOD3) have augmented hypertension in response to

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Conflict of Interest
None.
chronic angiotensin II infusion, renal clipping and DOCA-salt challenge.\textsuperscript{5, 6} The precise cause of excessive hypertension in these animals remains unclear. Superoxide produced in various sites of the central nervous system modulates sympathetic outflow, and intracerebroventricular (ICV) injection of an adenovirus that expresses superoxide dismutase blunts the hypertensive response to either local or systemically administered angiotensin II.\textsuperscript{7} In the kidney, \(O_2^{•−}\) enhances tubular sodium reabsorption.\textsuperscript{8, 9} Finally, \(O_2^{•−}\) is a major determinant of vasomotor tone. Superoxide reacts rapidly with endothelium-derived nitric oxide, and directly promotes vasoconstriction.\textsuperscript{10} Superoxide has also been implicated in vascular smooth muscle hypertrophy and remodeling in response to angiotensin II.\textsuperscript{11} Thus, deletion of SOD3 in the vascular smooth muscle could increase systemic vascular resistance and thus enhance experimental hypertension.

To understand the mechanisms by which \(O_2^{•−}\) promotes hypertension, we have produced mice that allow targeted deletion of SOD3 using Cre-lox technology. In a recent study, we used these animals to show that SOD3 in the circumventricular organs (CVO) modulates baseline blood pressure and the hypertensive response to angiotensin II, likely by augmenting sympathetic outflow.\textsuperscript{12} We also found that this central intervention markedly enhances the vascular inflammation, as characterized by macrophage and T cell accumulation, during induction of experimental hypertension. Of interest, deletion of SOD3 in the CVO caused a striking increase in vascular \(O_2^{•−}\) production. This study emphasized that central oxidant injury can promote vascular oxidant stress and inflammation. It remained possible however, that the augmented hypertension caused by angiotensin II following CVO SOD3 deletion might have been in part due to increased vascular \(O_2^{•−}\).

In the present study, we sought to specifically examine the role of vascular \(O_2^{•−}\) in hypertension by deleting SOD3 in vascular smooth muscle cells. Using a vascular smooth muscle-targeted Cre-recombinase that could be induced in adult animals, we were able to enhance \(O_2^{•−}\) levels specifically in the vascular smooth muscle. We compared the effects of vascular vs. CVO SOD3 deletion and further examined the effect of simultaneous deletion of SOD3 in these two sites. We find that while vascular smooth muscle SOD3 deletion increases vascular \(O_2^{•−}\) production, it does not affect blood pressure either at baseline or during angiotensin II infusion, and has only modest effects on vascular inflammation.

Material and Methods

Mice studied and protocols

Mice with lox\textsuperscript{P} sites flanking the SOD3 coding region (SOD3\textsuperscript{loxP/loxP} mice), previously created in our laboratory,\textsuperscript{13} were used in these studies. These animals have been backcrossed for more than 11 generation with C57Blk/6 mice. To delete SOD3 in vascular smooth muscle cells, we crossed SOD3\textsuperscript{loxP/loxP} mice with mice transgenic for Cre-recombinase driven by a tamoxifen inducible smooth muscle cell myosin-heavy-chain promoter (tg\textsuperscript{cre/SMMHC}).\textsuperscript{14} The resultant male SOD3\textsuperscript{loxP/loxP} \(\times\) tg\textsuperscript{cre/SMMHC} mice were studied at 3 months of age. To delete vascular SOD3 in these animals, tamoxifen (3 mg/20 g body weight) was injected daily for 5 days beginning at 3 months of age. As controls, we treated littermate SOD3\textsuperscript{loxP/loxP} mice that were negative for Cre-recombinase with tamoxifen in an identical fashion. In other experiments we deleted SOD3 in the CVO of SOD3\textsuperscript{loxP/loxP} mice by ICV injection of adenoviral Cre-recombinase (AdCre) as described previously.\textsuperscript{12} In yet other SOD3\textsuperscript{loxP/loxP} \(\times\) tg\textsuperscript{cre/SMMHC} mice, we initially performed ICV injections of AdCre and then administered IP injections of tamoxifen to permit deletion of both vascular smooth muscle and CVO SOD3. Two weeks following the final tamoxifen injection, osmotic minipumps (Alzet, Model 2002) were implanted for subcutaneous infusion of either angiotensin II (140 ng/kg/min) or vehicle. This low dose of angiotensin II was chosen because it causes minimal elevation of blood pressure in control mice. In

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additional mice, we infused a higher dose of angiotensin II (490 ng/kg/min) which induces severe hypertension in C57Bl/6 mice. Blood pressure was measured using either radiotelemetry as previously described, or in some cases non-invasively by using the tail cuff method. When tail cuff recordings were made, mice were accustomed to this procedure for 3 days before measurements were obtained. For telemetry studies, mice were allowed to recover for 10 days before recording blood pressure and osmotic minipumps containing either angiotensin II or vehicle for angiotensin II were inserted 13 days after telemetry implantation. All studies were performed according to a protocol approved by the Emory University Institutional Animal Care and Use Committee.

**Measurement of vascular reactivity, superoxide, hydrogen peroxide levels and nitric oxide**

Following sacrifice, the aortas were immediately removed. Isometric tension studies were performed using 2 mm aortic segments as described previously. Aortic O$_2$$^•−$ levels were measured using electron spin resonance (ESR) spectroscopy with CAT-1H as a spin probe. The SOD-inhibited amplitude of the ESR low field component of oxidized CAT-1H was used to quantify extracellular O$_2$$^•−$ production. In separate animals we also measured aortic O$_2$$^•−$ levels by monitoring the oxidation of dihydroethidium (DHE) to 2-hydroxyethidium with high-pressure liquid chromatography (HPLC). Values were normalized to 2 mm aortic segments. Aortic hydrogen peroxide was measured using the Amplex Red assay (Invitrogen) as previously described. Aortic and mesenteric nitric oxide (NO) was measured using the Fe[DETC]$_2$ spin trap and ESR. Values were normalized to 2 mm aortic segments or tissue wet weight of the mesenteric arteries.

**SOD activity assay**

SOD activity was measured using a commercially available kit (Cayman Chemicals Inc.). To distinguish between the different SOD isoforms, homogenized aortic tissue was centrifuged at 10,000 g to separate mitochondrial and extra-mitochondrial cell lysate. The mitochondrial fraction was treated with NaCN to inactivate possible contamination of copper/zinc containing SODs. The mitochondrial fraction was used to measure SOD2 activity. To differentiate between SOD1 and SOD3, Concanavalin A Sepharose beads (Amersham) were added the extra-mitochondrial fraction in batch and agitated for 10 minutes. Low speed centrifugation (500 g) was performed to remove SOD3 and the remaining SOD1 activity was measured in the supernatant. We have employed this method previously.

**Immunohistochemistry and flow cytometry**

After euthanasia, a stainless-steel cannula was placed in the right ventricle and the mice were initially perfused at 100 mmHg with saline and then with 10% formaldehyde. Aortas were harvested and embedded in paraffin. Primary antibodies used were a polyclonal anti-SOD3, as previously described. Primary antibodies were visualized using the DAKO LSAB+ System-HRP Kit (DAKO, Carpinteria, CA) according to the manufacturer’s protocol. Fluorescent cell sorting was performed on peripheral blood mononuclear cells and single cell aortic suspensions as previously described.

**Statistical analysis**

Data are presented as Mean ± SEM. Comparisons between groups were performed using analysis of variance and a Bonferroni post hoc test for comparison of selected groups. When only two comparisons were made, we employed a Student’s t-test and the reported p value represents a Bonferroni correction if more than one comparison was made. Blood pressure
was analyzed using two-way ANOVA with repeated measures. Data from FACS were analyzed with FlowJo software (Tree Star, Inc.). For data from aortic FACS we used two-way ANOVA to detect an interaction between the angiotensin II treatment and SOD3 deletion.

Results

Expression of SOD3 in aortic tissue

In initial experiments, we confirmed that 5 days of tamoxifen administration successfully deleted SOD3 in SOD3<sup>loxp/loxp</sup> × tg<sup>cre/SMMHC</sup> mice. Using Western Blots we found that vessels from mice treated with the vehicle for tamoxifen (Corn oil) expressed all three SOD isotypes at the predicted molecular sizes (Figure 1A). Two days following the final tamoxifen injection, SOD3 was undetectable by Western blot in both the aorta and mesenteric arteries of SOD3<sup>loxP/loxP</sup> × tg<sup>cre/SMMHC</sup> mice, while SOD1 and SOD2 levels were unchanged (Figures 1A – 1D). Western blots of the lung, heart and kidney showed that tamoxifen injection had no effect on SOD3 in these organs (Supplemental Figure 1, please see http://hyper.ahajournals.org). Activity assays for each of the respective SOD isotypes further confirmed that tamoxifen injection markedly reduced SOD3 activity in vessels of SOD3<sup>loxP/loxP</sup> ×tg<sup>cre/SMMHC</sup> mice (Figure 1E) but not in SOD3<sup>loxP/loxP</sup> mice lacking Cre-recombinase. Immunohistochemistry of aortic rings also showed that tamoxifen injection deleted SOD3 in the medial layer of SOD3<sup>loxP/loxP</sup> ×tg<sup>cre/SMMHC</sup> mice but not in adventitial tissue (Figure 1F).

Effect of VSMC SOD3 deletion on reactive oxygen species, endothelial function and nitric oxide production

As expected, deletion of SOD3 in vascular smooth muscle increased vascular O$_2^•$− production by two-fold at baseline as detected by ESR (Figure 2A) and by dihydroethidium and HPLC (Figure 2B). Infusion of low-dose angiotensin II did not further increase vascular O$_2^•$− production in either Cre-negative mice or SOD3<sup>loxP/loxP</sup> ×tg<sup>cre/SMMHC</sup> mice (Figures 2A and 2B). The vascular production of hydrogen peroxide was not affected by SOD3 deletion in vascular smooth muscle (Figure 2C).

An increase in vascular O$_2^•$− can decrease vascular nitric oxide (NO) bioavailability and impair endothelium-dependent vasodilatation. In keeping with this, deletion of SOD3 caused modest reduction in endothelium-dependent vasodilatation in animals treated with low dose angiotensin II compared to controls. In contrast, endothelium-independent vasodilatation to sodium nitroprusside was not altered (Figure 2D and E). In mice lacking SOD3 we observed a dramatic reduction of NO bioavailability at baseline in aortic and mesenteric tissue (Figure 2F and G). After high-dose angiotensin II infusion NO levels were not further decreased in aortic tissue (Figure 2F). In mesenteric arteries, however, angiotensin II tended to decrease NO in mice lacking SOD3, but this difference did not achieve statistical significance (Figure 2G).

Effect of vascular SOD3 deletion on blood pressure

Previous studies from our laboratory and others have shown that global embryonic deletion of SOD3 predisposes to hypertension, and that this is recapitulated by deletion of SOD3 in the central nervous system. In the present study, we sought to determine the role of SOD3 in the vasculature in modulation of blood pressure. Using telemetry recordings of blood pressure, we found that low dose angiotensin II had only a modest effect on blood pressure in both the cre-negative SOD3<sup>loxP/loxP</sup> mice and in the SOD3<sup>loxP/loxP</sup> ×tg<sup>cre/SMMHC</sup> mice and that these responses were not different between the two groups of animals (Figures 3A and 3B). In additional experiments, we infused a higher dose of angiotensin II (490 ng/kg/
min) for two weeks. This dose, which is known to cause severe hypertension in control mice, increased blood pressure to a similar extent in SOD3^{loxP/loxP} \times \text{tg}^{smmhc/cre} mice and in SOD3^{loxP/loxP} mice without Cre-recombinase (Figure 3C). Additional experiments were performed in Cre-positive mice without loxP sites flanking SOD3. These animals demonstrated similar blood pressures at baseline and in response to angiotensin II as the Cre-negative mice, indicating that the presence of the Cre-transgene alone had no effects on blood pressure modulation (data not shown).

The above findings are in contrast to our prior findings with deletion of SOD3 in the CVO, which caused an elevation of blood pressure at baseline and a striking increase in the hypertensive response to this identical dose of angiotensin II. We considered the possibility that central and vascular oxidative stress might synergistically increase blood pressure. To test this possibility, we performed an additional group of experiments in which we deleted SOD3 either in the vascular smooth muscle or in both the CVO and the vascular smooth muscle simultaneously and then examined the hypertensive response to angiotensin II. The results of these experiments are shown in figure 3D. These studies confirmed that deletion of vascular smooth muscle SOD3 had no effect on blood pressure at baseline and did not alter the hypertensive response to angiotensin II. Further, the simultaneous deletion of SOD3 in the CNS and vasculature led to a hypertensive phenotype that was identical to that we observed previously with deletion of SOD3 in the CVO alone (Supplemental Figure 2, please see http://hyper.ahajournals.org).

**Effect of vascular SOD3 deletion on peripheral inflammation**

Previously we have found that deletion of SOD3 in the CVO causes a striking increase in vascular inflammation, as characterized by infiltration of T cells and total leukocytes. In the present study we examined aortic leukocyte accumulation following vascular smooth muscle SOD3 deletion and compared T cell infiltration and activation with mice lacking SOD3 in the CVO.

Vascular smooth muscle deletion of SOD3 had no effect on vascular T cell infiltration in mice either before or after infusion of low-dose angiotensin II (Figure 4A). In addition, angiotensin II had no effect the vascular levels of CD44^{high} T cells, while deletion of SOD3 in the CVO caused a 2-fold increase in the vascular levels of these cells in hypertensive mice (Figure 4B). Similarly, deletion of SOD3 from the CVO and vascular smooth muscle caused an increase in CD44^{high} cells that was similar to that observed in animals in which SOD3 was deleted in the CVO only (Figure 4B).

**Discussion**

In prior studies, our laboratory and others have shown that mice lacking SOD3 are predisposed to excessive hypertension in response to either angiotensin II or DOCA-salt challenge. We have attempted to define in which tissues SOD3 modulates blood pressure. Previously we showed that deletion of SOD3 from the CVO leads to hypertension and causes peripheral inflammation as characterized by vascular infiltration of T cells and other leukocytes. In the present paper, we find that surprisingly, deletion of SOD3 specifically in the vascular smooth muscle, despite increasing vascular O_2^{−} levels, had no effect on blood pressure, either at baseline or in response to angiotensin II, and does not augment the inflammatory response to angiotensin II. Moreover, deletion of SOD3 in both the CVO and the vascular smooth muscle had no greater effect than deletion in the CVO alone. These findings indicate that SOD3 in the CNS likely plays a more important role in modulation of blood pressure than SOD3 in the vasculature.
There are several reasons to suspect that an increase in vascular $O_2^{\cdot-}$ might promote hypertension. Superoxide inactivates endothelium-derived NO and therefore could indirectly enhance vasoconstriction. Indeed, in the present study, we found that SOD3 deletion reduced ambient levels of NO as detected by ESR and that angiotensin II modestly impairs endothelium-dependent vasodilatation in mice lacking vascular smooth muscle SOD3. In addition, $O_2^{\cdot-}$ has been implicated as an endothelium-derived vasoconstrictor agent and could enhance formation of vasoconstrictor isoprostanes. Superoxide can alter vascular smooth muscle calcium handling, predisposing to vasoconstriction. These factors could increase systemic vascular resistance. Despite these potential roles of vascular $O_2^{\cdot-}$, we observed no change in blood pressure at baseline or in response to angiotensin II in animals lacking vascular smooth muscle SOD3. In keeping with our findings, vascular $O_2^{\cdot-}$ is increased in experimental models of type 1-diabetes, obesity and atherosclerosis, and in these conditions blood pressure is not consistently increased.

Our present findings also emphasize the importance of oxidative events in the CNS, and in particular the CVO in regulation of blood pressure. In our prior study, we showed that deletion of SOD3 in the CVO caused an elevation of baseline blood pressure and markedly enhances the hypertension caused by low-dose angiotensin II. In the present study, we also showed that CVO deletion of SOD3 increases blood pressure and vascular inflammation when combined with vascular smooth muscle deletion of SOD3, but to the same extent we previously observed for deletion of SOD3 in the CNS alone. Of interest, we previously showed that CVO SOD3 deletion also increases vascular smooth muscle $O_2^{\cdot-}$ production, in a fashion similar to vascular smooth muscle deletion of this antioxidant. In the present studies, we infused angiotensin II at rates of 140 and 490 ng/kg/day. In normal mice the 140 ng/kg day dose of angiotensin II has minimal effect on blood pressure. It is possible that vascular smooth muscle deletion of SOD3 could have augmented the hypertensive response to a higher dose of angiotensin II, however we found that the higher dose caused an increase in blood pressure which is identical in with vascular smooth muscle deletion of SOD3 vs control animals. Moreover, the low-dose angiotensin II employed in the present studies caused severe hypertension in mice lacking CVO SOD3. Thus, it is reasonable to conclude that the vascular smooth muscle deletion of SOD3 does not augment hypertension in response to either dose of angiotensin II.

Our present findings do not exclude a role of intracellular $O_2^{\cdot-}$ in the vascular smooth muscle in modulation of blood pressure. Deletion of SOD3 would be expected to predominantly increase extracellular $O_2^{\cdot-}$, however extracellular $O_2^{\cdot-}$ can affect the activity of the cytoplasmic superoxide dismutase (SOD1) in other models. Moreover, changes in the extracellular redox state can induce striking signaling events within the cell. In keeping with these considerations, we found that SOD3 deletion increased $O_2^{\cdot-}$ measured by HPLC detection of 2-hydroxyethidium, which predominantly reflects intracellular $O_2^{\cdot-}$. Thus, while we cannot exclude a role of intracellular $O_2^{\cdot-}$, our data suggest that vascular smooth muscle $O_2^{\cdot-}$ in general has minimal effects on blood pressure.

Our studies also do not exclude a role of vascular hydrogen peroxide ($H_2O_2$) on blood pressure regulation. Deletion of SOD3 would be expected to increase vascular $O_2^{\cdot-}$, but not $H_2O_2$. In keeping with this, we found no change in vascular $H_2O_2$ as measured by the Amplex red assay. $H_2O_2$ can promote vascular smooth muscle hypertrophy and thus could promote vascular remodeling characteristic of hypertension. Indeed, mice overexpressing thioredoxin, which eliminates $H_2O_2$, are protected against hypertension. In contrast, mice lacking the $H_2O_2$ scavenging enzyme glutathione peroxidase do not develop excessive hypertension when given angiotensin II. Nevertheless, we cannot exclude a role for vascular $H_2O_2$ in the modulation of blood pressure based on our present findings.
We and others have also shown an important role for inflammatory cells in hypertension and have shown that hypertension causes infiltration of both macrophages and activated T cells into the perivascular fat. A potential signal for this could be oxidative stress, which could increase vascular expression of adhesion molecules and chemokines that signal inflammatory cell accumulation. Surprisingly, we did not observe an increase in vascular inflammation following deletion in vascular smooth muscle SOD3, despite an increase in vascular $\mathbf{O}_2^•−$ levels. In contrast, deletion of SOD3 in the CVO enhanced vascular inflammation in response to angiotensin II, as reflected by total vascular T cells and CD44$^{\text{high}}$ T cells. These findings again reflect the importance of CNS signaling in vascular inflammation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Literature


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Significance and Perspective

This study extends prior research devoted to understanding how the extracellular superoxide dismutase, and by inference the superoxide anion contributes to hypertension. In prior studies, it has been shown that mice in with global deletion of SOD3 have augmented hypertension in response to either angiotensin II or DOCA-salt challenge. More recently, we found that deletion of SOD3 in the circumventricular organs caused an increase in baseline blood pressure and markedly enhanced the hypertension caused by low dose angiotensin II infusion. The present research now demonstrates that surprisingly, deletion of SOD3 in the vasculature has no effect on the hypertension caused by angiotensin II and does not augment the blood pressure elevation caused by elimination of SOD3 in the central nervous system. These data do not exclude a role of other vascular reactive oxygen species in the genesis of hypertension, but provide new insight into the relative roles of \( \text{O}_2^{•−} \) in the vasculature and the central nervous system in regulation of blood pressure. Therapeutic interventions directed toward the circumventricular organs rather than the vasculature might be more effective in treatment of hypertension.
Figure 1.
Selective deletion of SOD3 in the vasculature using Cre-lox technology. SOD3^{loxP/loxP} mice crossed with transgenic mice expressing inducible Cre-recombinase in the vascular smooth muscle were treated with either tamoxifen or its vehicle (corn oil). Western blots shown in panel A illustrate that expression of SOD1 and SOD2 is unchanged by tamoxifen administration. In contrast, SOD3 expression in the aorta was markedly reduced by tamoxifen but not vehicle injection. Panel B shows densitometric quantification of Western bands (n = 5). Panels C shows a Western blots illustrating the SOD3 expression in mesenteric arteries. The densitometric analysis is depicted in Panel D. Panel E shows activities of SOD1, SOD2 and SOD3 in vascular homogenates as determined enzymatically (n=5). Tamoxifen injection eliminated SOD3 activity in SOD3^{loxP/loxP} × tg^{cre/SMMHC} mice, but not in mice without cre-recombinase. The reported significance was obtained using a two-sided t test and reflects a Bonferroni correction for 3 comparisons. Panel F shows immunoreactivity for SOD3 (as indicated by brown staining) in SOD3^{loxP/loxP} mice with and without vascular smooth muscle Cre-recombinase following tamoxifen injection. Similar immunostaining results were obtained in three separate experiments.
Figure 2.
Levels of reactive oxygen species, vascular function and nitric oxide after vascular SOD3 deletion. SOD3<sup>loxP/loxP</sup> with and without the vascular smooth muscle inducible Cre-recombinase were treated with tamoxifen and one week later received osmotic minipumps for infusion of either vehicle or angiotensin II (140 ng/kg/min or 490 ng/kg/min). Panel A shows estimates of extracellular superoxide production as determined by the spin probe CAT1-H and electron spin resonance. Panel B shows estimates of vascular superoxide production as detected by formation of 2-hydroxyethidium from dihydroethidium, determined using HPLC. Panel C shows vascular production of hydrogen peroxide as determined using Amplex Red. Aortic rings were studied in organ chambers. Vessels were
contracted by phenylephrine and the relaxations evoked by either acetylcholine (Panel D) or sodium nitroprusside (Panel E) were studied. Estimates of nitric oxide production in different tissue as determined by the spin trap Fe[DETC]₂ and electron spin resonance is shown in Panels F and G. Panel F reflects aortic nitric oxide, while Panel G show nitric oxide production in mesenteric tissue. Two-way ANOVA was employed to compare groups in panels A–C and F–G, while two-way ANOVA with repeated measures was employed in panels D and E. In Panel D, *= 0.04 between cre negative sham and cre positive angiotensin II.
Figure 3.
The role of vascular SOD3 in regulation of blood pressure. Radiotelemeters were implanted in SOD3<sup>loxp/loxp</sup> mice with and without vascular inducible cre-recombinase. Osmotic minipumps were inserted on day five for infusion of angiotensin II (140 ng/kg/min). Panel A shows systolic pressures and panel B shows diastolic pressures recorded over two weeks following minipumps insertion. Values represent an n of 5 for each group. In separate experiments, blood pressure was measured non-invasively using the tail cuff method (Panel C). For the experiments shown in panel C, SOD3<sup>loxp/loxp</sup> mice with and without vascular smooth muscle cre-recombinase received a higher dose angiotensin II (490 ng/kg/min). For experiments in panel D mice underwent ICV injection of an adenovirus encoding Cre-recombinase (AdCre) and then received tamoxifen for 5 consecutive days. Osmotic minipumps for infusion angiotensin II (140 ng/kg/min) were then inserted three weeks after ICV injection. Blood pressure was compared using ANOVA with repeated measures. (ns = not significant)
Figure 4.
The role of vascular SOD3 on vascular inflammation in SOD3^{loxP/loxP} mice. Panel A shows CD3⁺ T cell infiltration in aortic tissue in SOD3^{loxP/loxP} mice with or without vascular inducible Cre-recombinase. Osmotic minipumps to deliver angiotensin II (140 ng/kg/min) or vehicle were inserted 2 weeks after the last tamoxifen injection. Some mice underwent ICV injection of adenovirus encoding Cre-recombinase (AdCre) and then received tamoxifen injections for 5 consecutive days; other mice only received ICV AdCre. Osmotic minipumps for infusion of angiotensin II (140 ng/kg/min) or vehicle were inserted 3 weeks after ICV injection. Panel B shows the infiltration of the T cell activation marker CD44 in aortic tissue after angiotensin II (140 ng/kg/min) or vehicle in the same groups of mice. Statistical
analysis was performed using two-way ANOVA with a Bonferroni post-hoc analysis. (CVO = circumventricular organs, VSMC = vascular smooth muscle cell)