

# Cerebrovascular Nitrosative Stress Mediates Neurovascular and Endothelial Dysfunction Induced by Angiotensin II

Helene Girouard, Laibaik Park, Josef Anrather, Ping Zhou, Costantino Iadecola

**Objective**—Angiotensin II (AngII) disrupts the regulation of the cerebral circulation through superoxide, a reactive oxygen species (ROS) generated by a nox2-containing NADPH oxidase. We tested the hypothesis that AngII-derived superoxide reacts with nitric oxide (NO) to form peroxynitrite, which, in turn, contributes to the vascular dysfunction.

**Methods and Results**—Cerebral blood flow (CBF) was monitored by laser Doppler flowmetry in the neocortex of anesthetized mice equipped with a cranial window. AngII ( $0.25 \pm 0.02 \mu\text{g/kg/min}$ ; intravenous for 30 to 45 minutes) attenuated the cerebral blood flow (CBF) increase produced by topical application of the endothelium-dependent vasodilator acetylcholine ( $-43 \pm 1\%$ ) and by whisker stimulation ( $-47 \pm 1\%$ ). AngII also increased the nitration marker 3-nitrotyrosine (3-NT) in cerebral blood vessels, an effect dependent on NO and nox2-derived ROS. Both the cerebrovascular effects of AngII and the nitration were attenuated by pharmacological inhibition or genetic inactivation of NO synthase. The nitration inhibitor uric acid or the peroxynitrite decomposition catalyst FeTPPS abolished AngII-induced cerebrovascular nitration and prevented the cerebrovascular effects of AngII.

**Conclusions**—These findings provide evidence that peroxynitrite, formed from NO and nox2-derived superoxide, contributes to the deleterious cerebrovascular effects of AngII. Inhibitors of peroxynitrite action may be valuable tools to counteract the deleterious cerebrovascular effects of AngII-induced hypertension. (*Arterioscler Thromb Vasc Biol.* 2007;27:303-309.)

**Key Words:** 3-nitrotyrosine ■ cerebral blood flow ■ gp91<sup>phox</sup> ■ laser Doppler flowmetry ■ NADPH oxidase ■ peroxynitrite ■ reactive oxygen species

Hypertension has profound effects on cerebrovascular structure and function.<sup>1</sup> For example, hypertension induces hypertrophy and remodeling of cerebral blood vessels, and promotes vascular inflammation and atherosclerosis.<sup>2-4</sup> In addition, hypertension disrupts the regulation of the cerebral circulation, resulting in alterations of fundamental cerebrovascular responses, such as the increase in cerebral blood flow (CBF) produced by endothelium-derived relaxing factors or neural activity.<sup>1</sup> Disruption of these critical homeostatic mechanisms impairs the ability of cerebral blood vessels to safeguard cerebral perfusion and increases the susceptibility of the brain to stroke and dementia.<sup>5</sup>

Angiotensin II (AngII) is a critical factor in the central and peripheral mechanisms of hypertension and its administration reproduces the main features of the cerebrovascular effects of hypertension.<sup>6,7</sup> The mechanisms by which AngII exerts its effect on the cerebral circulation are not fully understood. AngII, mainly through its AT1 receptors, activates a nox2-containing NADPH oxidase, a multiunit enzyme present in cerebrovascular cells, leading to production of the reactive oxygen species (ROS) superoxide in cerebral blood vessels.<sup>8-12</sup> However, the downstream mediators by which AngII-derived ROS mediate the cerebrovascular dysfunction remain unclear.

ROS can alter vascular regulation through multiple pathways.<sup>13</sup> However, one of the major pathogenic factors is peroxynitrite, a potent oxidant and nitrating agent derived from the reaction of nitric oxide (NO) with superoxide.<sup>14</sup> Peroxynitrite can impair the regulation of the cerebral circulation by inducing nitrosative damage of critical proteins involved in vascular regulation.<sup>15,16</sup> Furthermore, formation of peroxynitrite can reduce the bioavailability of NO leading to alterations of vascular responses dependent on NO.<sup>17</sup> Although there is evidence that AngII induces nitration in peripheral organs and blood vessels,<sup>18,19</sup> it is unknown whether such nitration occurs also in the brain and is mechanistically linked to the cerebrovascular effects of AngII.

In this study we sought to determine whether peroxynitrite participates in the deleterious effects of AngII on cerebrovascular regulation. To this end, we investigated whether AngII leads to nitrosative stress in cerebral blood vessels and whether such alteration is linked to the disruption of the CBF increase produced by the endothelium-dependent vasodilator acetylcholine (ACh) or neural activation. Furthermore, we examined whether AngII-induced nitration depends on NO and nox2-derived radicals, and whether agents that inhibit the

Original received September 5, 2006; final version accepted November 3, 2006.

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*Arterioscler Thromb Vasc Biol.* is available at <http://www.atvbaha.org>

DOI: 10.1161/01.ATV.0000253885.41509.25

biological effects of peroxynitrite can counteract the deleterious cerebrovascular actions of AngII.

## Materials and Methods

### Mice

All procedures were approved by the Institutional Animal Care and Use Committee of Weill Cornell Medical College. Studies were conducted in C57BL/6J mice (The Jackson Laboratory, Bar Harbor, Me), or in transgenic mice lacking nox2,<sup>20</sup> neuronal NO synthase (nNOS),<sup>21</sup> or endothelial NO synthase (eNOS).<sup>22</sup> Null mice were back-crossed to the C57BL/6J strain for several generations. All mice were male and 2 to 3 months old.

### General Surgical Procedures

Mice were anesthetized with isoflurane (maintenance 2%), intubated, and artificially ventilated (SAR-830; CWE Inc).<sup>8,9</sup> The femoral vessels were cannulated for recording or arterial pressure, blood sample collection, and AngII administration. Rectal temperature was maintained at 37°C. After surgery, anesthesia was maintained with urethane (750 mg/kg; intraperitoneal) and chloralose (50 mg/kg; intraperitoneal).<sup>8,9</sup>

### Monitoring of CBF

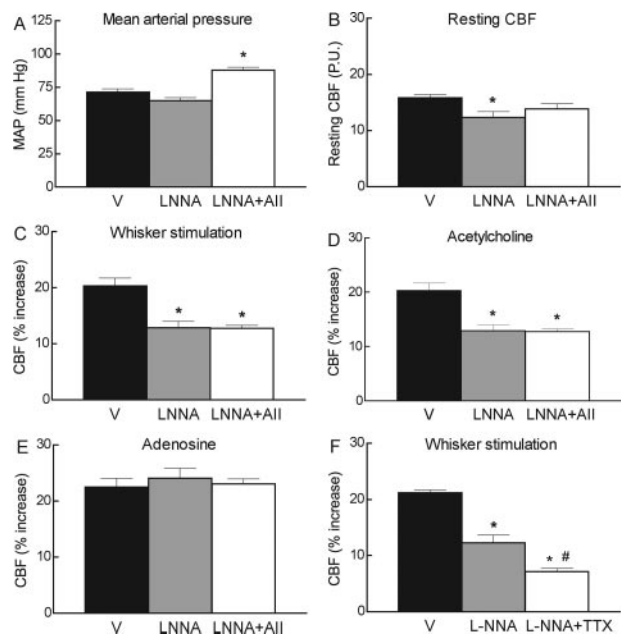
The parietal cortex was exposed (2×2 mm), the dura removed, and the site superfused with a modified Ringer's solution (37°C; pH: 7.3 to 7.4).<sup>8,9</sup> CBF was monitored in the window using a laser Doppler flowmeter (Vasamedic). The outputs of the flowmeter and blood pressure transducer were connected to a computerized data acquisition system (MacLab). CBF was expressed as percentage increases relative to the resting level.<sup>8,9</sup> Zero values were obtained after the heart was stopped by an overdose of isoflurane.

### 3-Nitrotyrosine and CD31 Immunohistochemistry

Mice were perfused transcardially with saline and their brains were removed and frozen. Brain sections (thickness: 14 μm) were cut through the region exposed by the cranial window using a cryostat, and collected at 100-μm intervals. To assure uniformity of the immunolabel, sections from all treatment groups, including controls (see Experimental Protocol), were processed together. Sections were post-fixed in ethanol and incubated with purified rabbit anti-mouse 3-nitrotyrosine (1:200; Upstate, Millipore Corporation) followed by a fluorescein isothiocyanate goat anti-rabbit secondary antibody (1:200; Molecular Probes). For simultaneous visualization of 3-NT and the endothelial marker CD31 sections were incubated with a rat anti-mouse CD31 monoclonal antibody (1:100; BD Biosciences), followed by a Texas Red goat anti-rat secondary antibody (1:200; Molecular probes). The specificity of the immunolabel was tested by processing sections without the primary antibody or after preadsorption with the antigen. Brain sections were examined with a fluorescence microscope (Eclipse E800; Nikon) equipped with Texas Red and fluorescein isothiocyanate filter sets. Images were acquired with a computer-controlled digital monochrome camera (Coolsnap; Robert Scientific). Image acquisition was performed with the same fluorescence settings in all cases. Because systemic administration of AngII increases ROS only in cerebral blood vessels,<sup>9</sup> analyses focused on cerebrovascular 3-NT immunoreactivity, using CD31 as a vascular marker. The quantification of 3-NT immunoreactivity in the different conditions studied (5 mice/group) was performed using IPLab software (Scanalytics). After background subtraction of the camera dark current, pixel intensities of 3-NT fluorescent signal over CD31-positive vessels were quantified. Ten sections per mouse were studied. For each section, fluorescence intensities were divided by the total number of pixels, averaged, and expressed as relative fluorescence units.

### Detection of ROS

ROS production was assessed by hydroethidine microfluorography as previously described.<sup>8,9,23</sup> This method was chosen because it has been extensively validated in vascular preparations and is suitable to



**Figure 1.** Effect of L-NNA on the cerebrovascular dysfunction induced by AngII (All). \* $P < 0.05$  from vehicle (V); # $P < 0.05$  from L-NNA; analysis of variance;  $n = 5$ /group. TTX indicates tetrodotoxin.

detect vascular ROS production in situ.<sup>8,9,23,24</sup> Hydroethidine (2 μmol/L; Molecular Probes) was topically superfused on the somatosensory cortex for 30 minutes. At the end of the superfusion, the brain was removed and frozen (−30°C). Brain sections (thickness: 20 μm) were cut serially in a cryostat through the region of the cranial window and collected at 100-μm intervals. ROS production was assessed using methods described in previous publications.<sup>8,9,23</sup> Data were expressed in relative fluorescence units.

### Experimental Protocols

CBF recordings were started after arterial pressure and blood gases were in a steady state (Figure 1; supplemental Figure I and supplemental Table I, available online at <http://atvb.ahajournals.org>). All pharmacological agents were dissolved in Ringer's solution unless otherwise indicated.

#### Effect of AngII on CBF Responses to Whisker Stimulation, Ach, or Adenosine

First, cerebrovascular responses were tested while the cranial window was superfused with vehicle (Ringer's solution). The whisker-barrel cortex was activated for 60 seconds by stroking the contralateral facial whiskers,<sup>25</sup> and the evoked changes in CBF were recorded. Next, the endothelium-dependent vasodilator ACh (10 μmol/L; Sigma) or the endothelium-independent vasodilator adenosine (50 μmol/L; Sigma) was superfused topically for 5 minutes. After testing CBF responses, saline or AngII (AngII acetate; Sigma) was administered intravenously. The AngII infusion was adjusted to elevate MAP by 20 to 25 mm Hg gradually over 10 to 15 minutes until a stable increase was obtained. At this time, the infusion rate was  $0.25 \pm 0.02$  μg/kg per minute, which produces elevations in plasma AngII within the upper range of that produced by endogenous activation of the renin-angiotensin system in rodents.<sup>26</sup> CBF responses to whisker stimulation, Ach, or adenosine were tested again after 30 to 45 minutes of AngII infusion.

#### 3-NT Immunoreactivity and ROS Production Induced by AngII

Wild-type mice or mice lacking eNOS, nNOS, or nox2 were prepared as described. AngII was infused intravenously, and 30 to 45 minutes later mice were euthanized for assessment of 3-NT immu-

noreactivity. For assessment of ROS production, hydroethidine was superfused on the cranial window for 30 minutes before starting the infusion of AngII. At the end of the AngII infusion, brains were processed for ROS production. In other studies, the effect of the following agents on AngII-induced ROS production or 3-NT immunoreactivity was tested in wild-type mice: nitro-L-arginine (L-NNA) (1 mmol/L; topical superfusion; Sigma), a nonselective NOS inhibitor<sup>27</sup>; 7-nitroindazole (7-NI) (50 mg/kg, in peanut oil; intraperitoneally; Cayman Chemicals), a nNOS inhibitor<sup>28</sup>; NS398 (100  $\mu$ mol/L; topical superfusion; Cayman Chemicals), a selective COX-2 inhibitor<sup>25</sup>; uric acid (UA) (100  $\mu$ mol/L; topical superfusion; Sigma), a peroxynitrite scavenger and inhibitor of nitration<sup>29–31</sup>; 5,10,15, 20-tetrakis(4-sulfonatophenyl)porphyrinato iron (III) (FeTPPS) (20  $\mu$ mol/L; topical superfusion; Calbiochem), a peroxynitrite decomposition catalyst.<sup>32–34</sup> L-NNA, 7-NI, NS398, UA, FeTPPS, and vehicle were administered 30 minutes before AngII.

### Effect of L-NNA, 7-NI, NS398, UA, FeTPPS, or TPPS on the Cerebrovascular Actions of AngII

Cerebrovascular responses to whisker stimulation, Ach, and adenosine were tested while the window was superfused with vehicle. Then, L-NNA, 7-NI, NS398, UA, FeTPPS, or the inactive decomposition catalyst TPPS (20  $\mu$ mol/L; Porphyrin Products) was administered and cerebrovascular responses were tested 30 minutes later. AngII was then infused and cerebrovascular responses were tested again after 30 to 45 minutes. In some experiments the sodium channel inhibitor tetrodotoxin (tetrodotoxin; 3  $\mu$ mol/L; topical superfusion) was administered after L-NNA or 7-NI, and CBF responses to whisker stimulation were tested.

### Cerebrovascular Effects of AngII in nNOS- and eNOS-Null Mice

Mice were surgically prepared for systemic administration of AngII while CBF was recorded. Responses to whisker stimulation, ACh, and adenosine were tested before and 30 to 45 minutes after AngII administration. Wild-type mice served as controls.

### Data Analysis

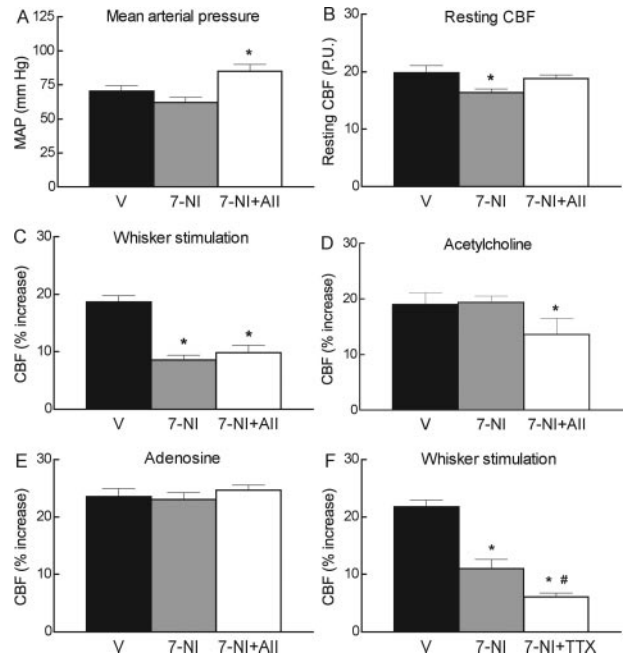
Data are expressed as mean  $\pm$  SEM. Two-group comparisons were analyzed by the 2-tailed *t* test. Multiple comparisons were evaluated by ANOVA and Tukey or Dunnett tests, as appropriate. Differences were considered statistically significant for  $P < 0.05$ .

## Results

### NOS Inhibition Prevents the Cerebrovascular Effects of AngII

Systemic administration of AngII elevated MAP (vehicle:  $75 \pm 3$ ; AngII:  $95 \pm 1$  mm Hg;  $P < 0.05$ ; analysis of variance;  $n = 5$ /group) and attenuated the increase in CBF evoked by whisker stimulation ( $-47 \pm 1\%$ ;  $P < 0.05$ ) or by topical application of ACh ( $-43 \pm 1\%$ ;  $P < 0.05$ ). AngII did not alter resting CBF (vehicle:  $18.2 \pm 0.7$ ; AngII:  $18.0 \pm 0.5$  perfusion units;  $P > 0.05$ ) or the increase in CBF produced by topical superfusion of adenosine (vehicle:  $21 \pm 1\%$ ; AngII:  $23 \pm 1\%$ ;  $P > 0.05$ ). The cerebrovascular effects of AngII were not caused by a deterioration of the preparation because MAP, resting CBF, and CBF reactivity remained stable throughout the experiment (Figure 1).

Topical superfusion with the non-selective NOS inhibitor L-NNA, as anticipated, reduced resting CBF and the CBF increase produced by whisker stimulation or ACh<sup>28,35</sup> (Figure 1B to 1D). The CBF response to adenosine was not altered (Figure 1E). With AngII infusion, L-NNA did not affect the MAP increase, but it prevented the attenuation of the CBF responses to whisker stimulation or ACh (Figure 1A, 1C, 1D). In contrast, superfusion with tetrodotoxin attenuated the



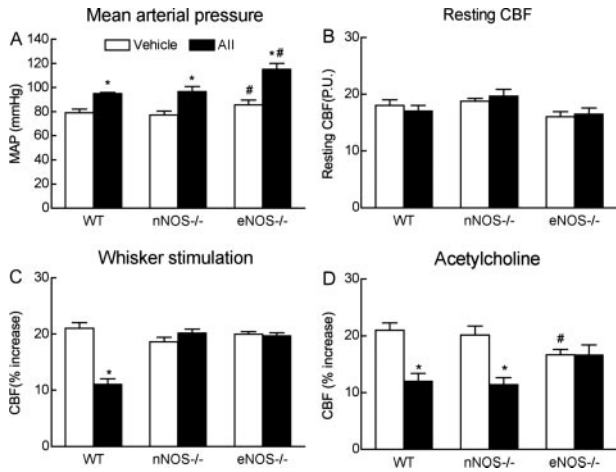
**Figure 2.** Effect of 7-NI on the cerebrovascular dysfunction induced by AngII (All). \* $P < 0.05$  from vehicle (V); #  $P < 0.05$  from 7-NI; analysis of variance;  $n = 5$ /group.

increase in CBF evoked by whisker stimulation after L-NNA (Figure 1F), suggesting that the lack of effect of AngII was not caused by the fact that L-NNA maximally suppressed the vasodilation produced by whisker stimulation. Similarly, atropine (10  $\mu$ mol/L; topical application) attenuated further the increase in CBF elicited by ACh after L-NNA (L-NNA+AngII:  $12.8 \pm 0.5\%$ ; L-NNA+atropine:  $3.9 \pm 1\%$ ;  $n = 5$ /group;  $P < 0.05$  from L-NNA+AngII). The nNOS inhibitor 7-NI attenuated resting CBF and the response to whisker stimulation, but not ACh, attesting to the specificity of its action (Figure 2B to 2D). 7-NI did not affect the elevation in MAP induced by AngII and prevented the attenuation of the CBF increase produced by whisker stimulation (Figure 2A, 2C, 2D). In contrast, 7-NI did not influence the attenuation of the response to ACh by AngII (Figure 2D). Tetrodotoxin was able to reduce the CBF response to whisker stimulation after 7-NI (Figure 2F).

As a control, we also examined the effect of the COX-2 inhibitor NS398 on the AngII-induced cerebrovascular dysfunction. NS398, like 7-NI (Figure 2), attenuated the CBF response evoked by whisker stimulation and not ACh<sup>25</sup> (supplemental Figure II). However, unlike 7-NI, NS398 did not prevent the effect of AngII on the CBF response to whisker stimulation (supplemental Figure II).

### NOS-Deficient Mice Are Protected from the Cerebrovascular Effects of AngII

The CBF response to whisker stimulation did not differ among wild-type, eNOS-null, and nNOS-null mice, whereas the response to ACh was slightly attenuated in eNOS-null mice (Figure 3A to 3D).<sup>36–38</sup> As suggested by 7-NI (Figure 2), AngII did not attenuate the CBF increase evoked by whisker stimulation in nNOS-null mice, but it reduced the CBF response to ACh (Figure 3C and 3D). In eNOS-null



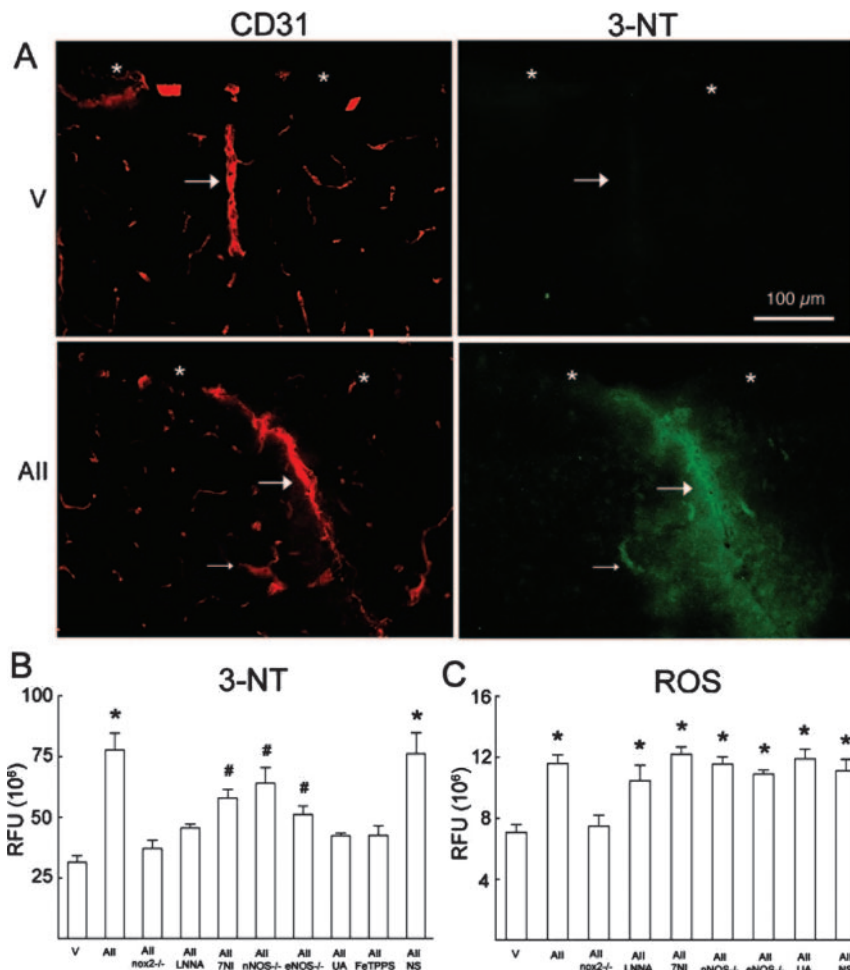
**Figure 3.** Effect of AngII (All) on the cerebrovascular regulation in nNOS, eNOS-null mice, and in wild-type (WT) controls. \* $P < 0.05$  from vehicle, paired  $t$  test; #  $P < 0.05$  from corresponding WT mice; analysis of variance;  $n = 5$ /group.

mice, AngII elevated MAP more than in wild-type controls, but it failed to attenuate CBF responses both to whisker stimulation and ACh (Figure 3A to 3D). CBF responses to adenosine were not altered (supplemental Figure III). These findings provide further evidence that NO, particularly eNOS-derived NO, is required for the cerebrovascular dysfunction induced by AngII.

### AngII Induces 3-NT Immunoreactivity in Cerebral Blood Vessels

Superoxide derived from *nox2* is required for the cerebrovascular dysfunction induced by AngII.<sup>8,9</sup> Because NO reacts with superoxide to form peroxynitrite, we used the nitration marker 3-NT to determine whether AngII induces cerebrovascular nitration and, if so, whether NO and *nox2*-derived superoxide are involved. AngII induced abundant 3-NT immunoreactivity restricted to CD31-positive vascular profiles (Figure 4A). 3-NT immunoreactivity was observed in vessels with the morphological characteristics of pial arterioles and in smaller vessels, presumably capillaries (Figure 4A). In some instances, a halo of 3-NT immunoreactivity was observed in the neuropil surrounding the blood vessels (Figure 4A). AngII-induced 3-NT immunoreactivity was not observed in *nox2*-null mice and was abolished by treatment of wild-type mice with L-NNA (Figure 4B and supplemental Figure IV). The immunoreactivity was attenuated in nNOS- or eNOS-null mice, and in wild-type mice treated with 7-NI (Figure 4B). Furthermore, 3-NT immunoreactivity was abolished by topical application of the nitration inhibitor UA or by the peroxynitrite decomposition catalyst FeTPPS, but was not affected by the COX-2 inhibitor NS398 (Figure 4B and supplemental Figure IV).

As before,<sup>8,9</sup> systemic administration of AngII increased ROS production in cerebrocortical vessels (Figure 4C). The



**Figure 4.** Effect of AngII (All) on cerebrovascular 3-NT immunoreactivity and ROS production. A, 3-NT immunoreactivity is observed CD31-positive pial arterioles (large arrows) and capillaries (small arrows). The asterisks identify the brain surface. B, Quantification of vascular 3-NT immunoreactivity. C, Vascular ROS production assessed by hydroethidine fluorimicrography. \* $P < 0.05$  from vehicle (V). # $P < 0.05$  from vehicle and All; analysis of variance. Data are from 10 sections/mouse and 5 mice/group. NS indicates NS398.

ROS increase was not observed in *nox2*-null mice (Figure 4C), confirming that ROS are produced by *nox2*.<sup>8,9</sup> However, L-NNA, 7-NI, NS398, and UA did not affect AngII-induced ROS production (Figure 4C). Similarly, the ROS production was not attenuated in nNOS- or eNOS-null mice (Figure 4C). Thus, AngII-induced ROS production is not altered in nNOS- and eNOS-null mice, and in wild-type mice treated with L-NNA, 7-NI, or UA (Figure 4C).

### Inhibitors of Nitration Prevent the Cerebrovascular Effects of AngII

These results suggest that AngII leads to formation of peroxynitrite in cerebral blood vessels, which, in turn, contributes to the cerebrovascular effects of AngII. To test this hypothesis we used UA and FeTPPS, agents that we have shown to block AngII-induced 3-NT immunoreactivity (Figure 4B). UA or FeTPPS did not influence resting CBF or the increase in CBF produced by whisker stimulation, ACh, or adenosine (Figures 5 and 6; supplemental Figure III). UA or FeTPPS did not affect the increase in MAP induced by AngII, but they prevented the attenuation in the CBF increase evoked by whisker stimulation or ACh (Figures 5 and 6). In contrast, the inactive peroxynitrite decomposition catalyst TPPS failed to prevent the cerebrovascular effects of AngII (supplemental Figure V).

### Discussion

We investigated whether the cerebrovascular dysfunction induced by AngII is mediated by peroxynitrite formed by NO and *nox2*-derived superoxide. We found that non-elective NOS inhibition or selective inactivation of eNOS in null mice prevents the cerebrovascular effects of AngII. Using the nitration marker 3-NT, we found that AngII induces extensive nitration of cerebral blood vessels and that such nitration is abolished by inhibition of NOS or in *nox2*-null mice. Next, we examined the effect of UA and FeTPPS, agents that inhibit the actions of peroxynitrite.<sup>29–34</sup> We found that both UA and FeTPPS block the nitration induced by AngII and prevent the associated cerebrovascular dysfunction. These novel observations, collectively, support the hypothesis that AngII administration induces *nox2*-derived superoxide, which reacts with NO to form peroxynitrite. Peroxynitrite, in turn, acts on cerebral blood vessels to attenuate responses to functional hyperemia and to the endothelium-dependent vasodilator ACh.

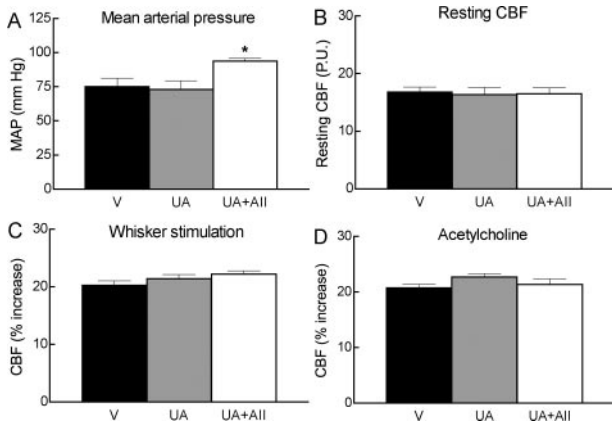
Peroxynitrite is not the only agent that can nitrate proteins.<sup>39</sup> For example, heme peroxidase enzymes, such as myeloperoxidase, can produce nitration in the presence of nitrite and H<sub>2</sub>O<sub>2</sub>.<sup>39–41</sup> However, this is not the case in our studies because the nitrosative stress induced by AngII depends on NO and on superoxide derived from *nox2*. However, the attenuation of nitration observed in NOS-null mice and in wild-type mice treated with L-NNA cannot be attributed to scavenging of ROS, because ROS production, assessed in parallel experiments, was not reduced. The fact that the ROS production was not reduced also rules out the possibility that eNOS uncoupling, a condition in which eNOS produces superoxide instead of NO,<sup>17</sup> is a source of ROS involved in the nitration and cerebrovascular dysfunction

induced by AngII. Similarly, the fact that UA attenuated 3-NT immunoreactivity without affecting ROS production, attest to the fact that this agent acts as a nitration inhibitor and not as a radical scavenger. Finally, the observation that the selective COX-2 inhibitor NS398 does not attenuate the ROS production induced by AngII rules out COX-2 as a significant source of ROS in this model.

We used NOS inhibitors and NOS-null mice to explore the sources of NO linked to the cerebrovascular effects of AngII. We found that although both nNOS and eNOS contribute to the vascular nitration, NO derived from eNOS is more critical for the cerebrovascular dysfunction. Thus, AngII attenuated CBF responses to ACh in nNOS-null mice, but it was completely devoid of cerebrovascular effects in eNOS-null mice. The lack of effect of AngII cannot be attributed to reduced sensitivity to AngII because the AngII-induced increases in MAP and ROS production were not attenuated in eNOS-null mice. Therefore, the findings indicate that eNOS-derived NO is both necessary and sufficient for the peroxynitrite production linked to the vascular dysfunction induced by AngII. The reasons why eNOS is more critical than nNOS in the cerebrovascular dysfunction remain to be determined. One possibility may be related to the fact that eNOS contributes to intramural vascular peroxynitrite production more than nNOS. Ongoing studies are examining this question.

Peroxynitrite, alone or in combination with oxidative stress, can alter vascular function through a wide variety of mechanisms.<sup>13</sup> Formation of peroxynitrite reduces the bioavailability of NO and attenuates NO-dependent responses.<sup>42</sup> Furthermore, peroxynitrite leads to nitration of tyrosine residues on proteins, altering their function. Thus, peroxynitrite alters several proteins involved in vascular regulation, including, for example, prostacyclin synthase, mitochondrial enzymes, soluble guanylyl cyclase, and smooth muscle contractile proteins.<sup>16,19,29,43</sup> In addition, peroxynitrite and its derivatives amplify oxidative stress and lead to single-strand breaks on DNA with important biological consequences. Consequently, peroxynitrite has profound cerebrovascular effects, including alterations of myogenic tone and attenuation of vasodilatory responses in cerebral arteries.<sup>15,16</sup>

Peroxynitrite is unlikely to mediate the cerebrovascular dysfunction induced by AngII by reducing the bioavailability of NO, because the peroxynitrite “scavenger” UA or the decomposition catalyst FeTPPS, agents that interfere with peroxynitrite action after its formation, prevent the cerebrovascular actions of AngII. Therefore, vascular responses could be re-established by these agents despite ongoing formation of peroxynitrite and, presumably, removal of NO. Our data support the hypothesis that peroxynitrite acts directly on cerebral blood vessels to selectively impair the vasodilation induced by endothelium-dependent vasodilators and whisker stimulation. Although peroxynitrite can alter contractile proteins in cerebrovascular muscles,<sup>16</sup> a global disturbance of vascular muscle function is unlikely to play a role because the CBF response to the smooth muscle relaxant adenosine is not affected by AngII. Another potential mechanism for the effect of peroxynitrite includes DNA damage, which leads to activation of the DNA repair enzyme poly-(ADP)ribose polymerase and vascular dysfunction.<sup>44</sup>



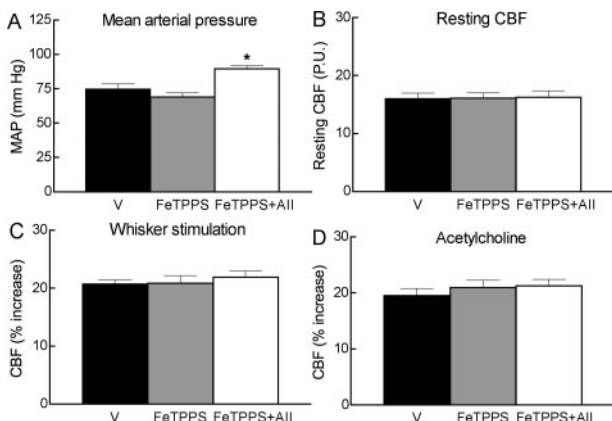
**Figure 5.** Effect of UA on the cerebrovascular dysfunction induced by AngII (All). \* $P < 0.05$  from vehicle (V); analysis of variance;  $n = 5$ /group.

Peroxyntirite-induced poly(ADP)ribose polymerase activation has been implicated in the dysfunction induced by aging in cerebral vessels,<sup>45</sup> and by AngII, aging, diabetes, and atherosclerosis in systemic vessels.<sup>46</sup> However, it remains to be determined whether poly(ADP)ribose polymerase is also involved in the cerebrovascular effects of AngII.

In conclusion, we have demonstrated that AngII induces nitration in cerebral blood vessels, which depends on NO and nox2-derived superoxide. Inhibition of NO production, especially endothelial-derived NO, blocks the deleterious vascular effects of AngII. Furthermore, inhibitors of peroxyntirite action prevent the cerebrovascular effects of AngII. The findings provide evidence that the effects of AngII on CBF responses to ACh and whisker stimulation require peroxyntirite, which is formed mainly from eNOS-derived NO and nox2-derived superoxide. Thus, inhibitors of peroxyntirite action may be valuable tools to counteract the deleterious cerebrovascular effects of AngII-induced hypertension.

### Sources of Funding

This work was supported by NIH grant HL18974. H.G. holds a fellowship from the Canadian Institute in Health Research. C.I. is the recipient of a Javits award from NIH/NINDS.



**Figure 6.** Effect of FeTPPS on the cerebrovascular dysfunction induced by AngII (All). \* $P < 0.05$  from vehicle (V); analysis of variance;  $n = 5$ /group.

### Disclosures

None.

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