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The cerebrovascular dysfunction induced by slow pressor doses of angiotensin II precedes the development of hypertension

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Capone C, Faraco G, Park L, Cao X, Davisson RL, Iadecola C. The cerebrovascular dysfunction induced by slow pressor doses of angiotensin II precedes the development of hypertension. Am J Physiol Heart Circ Physiol 300: H397–H407, 2011. First published August 12, 2010; doi:10.1152/ajpheart.00679.2010.—Hypertension alters cerebrovascular regulation and increases the brain’s susceptibility to stroke and dementia. We investigated the temporal relationships between the arterial pressure (AP) elevation induced by “slow pressor” angiotensin II (ANG II) infusion, which recapitulates key features of human hypertension, and the resulting cerebrovascular dysfunction. Minipumps delivering saline or ANG II for 14 days were implanted subcutaneously in C57BL/6 mice (n = 5/group). Cerebral blood flow was assessed by laser-Doppler flowmetry in anesthetized mice equipped with a cranial window. With ANG II (600 ng·kg⁻¹·min⁻¹), AP started to rise after 9 days (P < 0.05 vs. saline), remained elevated at 11–17 days, and returned to baseline at 21 days (P > 0.05). ANG II attenuated the cerebral blood flow increase induced by neural activity (whisker stimulation) or endothelium-dependent vasodilators, an effect observed before the AP elevation (7 days), as well as after the hypertension subsided (21 days). Nonpressor doses of ANG II (200 ng·kg⁻¹·min⁻¹) induced cerebrovascular dysfunction and oxidative stress without elevating AP (P > 0.05 vs. saline), whereas phenylephrine elevated AP without inducing cerebrovascular effects. ANG II (600 ng·kg⁻¹·min⁻¹) augmented neocortical reactive oxygen species (ROS) with a time course similar to that of the cerebrovascular dysfunction. Neocortical application of the ROS scavenger manganic-(I)-meso-tetrakis(4- benzoic acid)porphyrin or the NADPH oxidase peptide inhibitor gp91ds-tat attenuated ROS and cerebrovascular dysfunction. We conclude that the alterations in neurovascular regulation induced by slow pressor ANG II develop before hypertension and persist beyond AP normalization but are not permanent. The findings unveil a striking susceptibility of cerebrovascular function to the deleterious effects of ANG II and raise the possibility that cerebrovascular dysregulation precedes the elevation in AP also in patients with ANG II-dependent hypertension.

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raise the possibility that cerebrovascular dysfunction is an early event also in human hypertension.

METHODS

Methods for surgical preparation of mice, topical application of drugs, recording field potentials, blood pressure measurement, and monitoring CBF using laser-Doppler flowmetry have been described in detail in previous publications (3, 12, 19, 21, 22, 29) and are briefly summarized here.

General Surgical Procedures

All procedures were approved by the Institutional Animal Care and Use Committee of Weill Cornell Medical College. All studies were conducted in male C57BL/6J mice (age, 2 to 3 mo; and 20–30 g body wt), obtained from Jackson Laboratories (Bar Harbor, ME).

Minipumps implantation. Osmotic minipumps containing vehicle (saline), ANG II, or phenylephrine (PE) were implanted subcutaneously in mice (n = 5/group) under isoflurane anesthesia. Concentrations and delivery rates of ANG II (600 ng·kg\(^{-1}·\text{min}^{-1}\)) and PE (3 \(\mu\)g·kg\(^{-1}·\text{min}^{-1}\)) were adjusted to produce comparable levels of blood pressure elevation. In some experiments, a concentration of ANG II that does not increase blood pressure (200 ng·kg\(^{-1}·\text{min}^{-1}\)) was used. Systolic blood pressure was monitored daily in awake mice using tail-cuff plethysmography, as previously described (4, 21, 22). At different times after pump implantation (3, 7, 14, 16, 18, 21, and 28 days), the mice were anesthetized and instrumented for assessment of cerebrovascular reactivity by laser-Doppler flowmetry as described in Monitoring CBF.

Radiotelemetry. In some mice, arterial pressure was monitored by radiotelemetry (Data Sciences International) using a catheter implanted in the thoracic aorta via the left common carotid artery (3). Because of the carotid cannulation, these mice were not used in experiments involving CBF monitoring. These experiments with telemetry were used to select the time points at which to assess CBF reactivity. The changes in blood pressure in mice in which CBF was measured were confirmed by tail-cuff plethysmography, as previously described (4, 21, 22).

General Surgical Procedures

Surgery for CBF experiments. Mice were anesthetized with isoflurane in a mixture of \(\text{N}_2\) and \(\text{O}_2\) (induction, 5%; and maintenance, 2%). The trachea was intubated and the mice were artificially ventilated with an oxygen-nitrogen mixture. The \(\text{O}_2\) concentration in the mixture was adjusted to provide an arterial \(\text{P}_{\text{O}_2}\) of 120–130 mmHg (4) (see supplemental Table S1; note: supplemental material may be found posted with the online version of this article). One of the femoral arteries was cannulated for recording MAP and blood samples. Rectal temperature was maintained at 37°C using a thermospatially controlled rectal probe connected to a heating pad. End-tidal \(\text{CO}_2\), monitored by a \(\text{CO}_2\) analyzer (Capstar-100, CWE), was maintained at 2.6–2.7% to provide a \(\text{P}_{\text{CO}_2}\) of 33–36 mmHg (4) (see supplemental Table S1). After surgery, isoflurane was discontinued and anesthesia was maintained with urethane (750 mg/kg ip) and chloralose (50 mg/kg ip). Throughout the experiment, the level of anesthesia was monitored by testing corneal reflexes and motor responses to tail pinch.

To minimize the confounding effects of anesthesia on vascular reactivity, the time interval between the administration of urethane-chloralose and the testing of CBF responses was kept consistent among the different groups of mice studied.

Monitoring CBF

The parietal region was exposed through a small craniotomy (2 × 2 mm), the dura was removed, and the site was superfused with a modified Ringer solution (37°C, pH 7.3–7.4). CBF was continuously monitored at the site of superfusion with a laser-Doppler probe (Perimed) positioned stereotaxically on the cortical surface. The outputs of the flowmeter and blood pressure transducer were connected to a data acquisition system (PowerLab) and saved on a computer for off-line analysis. CBF values were expressed as percent increases relative to the resting level. Zero values for CBF were obtained after the heart was stopped by an overdose of isoflurane at the end of the experiment. Although laser-Doppler flowmetry is not quantitative, it monitors relative changes in CBF quite accurately (see Ref. 14 for a review).

ROS Detection and Immunocytochemistry

ROS production was assessed by dihydroethidium (DHE) microfluorography as previously described (4, 11, 12). Although there are many methods to assess ROS, each with advantages and disadvantages, DHE fluorimicrography is particularly well suited to in situ ROS detection with cellular resolution (4, 11, 13, 29, 37). DHE (2 \(\mu\)mol/l; Molecular Probes) was topically superfused on the somatosensory cortex for a total of 60 min (see Experimental Protocols). The brain was removed and frozen, and coronal sections (thickness, 20 \(\mu\)m) were cut through the cortex underlying the cranial window using a cryostat. Sections were analyzed using a Nikon Eclipse E800 fluorescence microscope equipped with a custom filter set for the detection of DHE oxidation products (4, 11, 12, 29). Images were acquired with a digital camera (Coolsnap, Roper Scientific) and analyzed in a blinded manner using the IPLab software (Scanalytics), as described (4). Fluorescent intensities of all sections (20 per animal) were added, divided by the total number of pixels analyzed, and expressed in relative fluorescence units. In experiments in which the cellular site of the ROS signal was established, DHE (10 mg/kg) was injected into the jugular vein and, 60 min later, the mice (n = 4–6/group) were perfused transcardially with PBS followed by 4% paraformaldehyde in PBS. We used this approach because it does not require a criotomy and results in better preservation and fixation of the somatosensory cortex. A potential drawback, however, is that some of the fluorescence might be lost during the tissue processing for immunocytochemistry. To minimize the confounding effects of the loss in fluorescence, brain sections from saline- and ANG II–treated mice were processed in parallel and under identical conditions. Coronal brain sections (thickness, 20 \(\mu\)m) were cut through the somatosensory cortex using a cryostat. Sections were incubated with primary antibodies (glial fibrillary acidic protein, 1:200, Sigma-Aldrich; neuronal nuclei, 1:200, Chemicon International; and CD31, 1:200, BD Biosciences). Sections were then incubated with a Cy5-conjugated secondary antibody (1:200; Jackson Immunoresearch), mounted on slides and examined using a Leica confocal microscope (4). Identical confocal settings were used for the acquisition of all images. To obtain a semiquantitative assessment of the increase in ROS induced by ANG II infusion in neurons, astrocytes, and endothelial cells, fluorescence was quantified in the cells immunopositive for the different markers using ImageJ. Data were expressed in arbitrary fluorescence units.

Experimental Protocols

Effect of ANG II or PE on CBF responses to whisker stimulation, endothelium-dependent vasodilators, or adenosine. Mice were surgically prepared for CBF measurement at different time points after implantation of osmotic minipumps containing ANG II, PE, or vehicle (see Surgery for CBF experiments). After stabilization of MAP and blood gases (see supplementary Table S1), the whisker-barrel region of the somatosensory cortex was activated for 60 s by stroking the contralateral facial whiskers (4), and the evoked changes in CBF were recorded. CBF responses to acetylcholine (ACH; 10 \(\mu\)M), bradykinin (50 \(\mu\)M), the \(\text{Ca}^{2+}\) ionophore A-23187 (3 \(\mu\)M), and adenosine (400 \(\mu\)M) were also tested (4). These agents were selected because they produce...
vasodilation through different mechanisms. ACh induces CBF increases mediated by endothelial nitric oxide (NO) and muscarinic receptors (38), whereas bradykinin and A-23187 act via endothelial cyclooxygenase-1 products via receptor-dependent and -independent mechanisms, respectively (27). Adenosine is a smooth muscle relaxant whose action is independent of the endothelium (30). Agents were applied at concentrations previously determined not to be supramaximal (19). In experiments in which the ROS scavenger manganic(I-II)meso-tetrakis(4-benzoic acid) porphyrin (MnTBAP; 100 μM), the NADPH oxidase peptide inhibitor gp91ds-tat (1 μM), or a scrambled control peptide (1 μM) was used (33), the responses were tested before and after topical superfusion of these agents for 30 min (11, 12). We have previously determined that the concentrations of MnTBAP and gp91ds-tat used are effective in blocking ANG II-induced ROS production (11, 12).

Effect of ANG II on somatosensory field potentials evoked by whisker stimulation. Mice were anesthetized and surgically prepared as described in Surgery for CBF experiments. The electrocorticogram was recorded using bipolar recording electrodes consisting of Teflon-coated silver wires (ID 0.005 inches; Stoelting). Electrodes were positioned stereotaxically in the left somatosensory cortex (3 mm lateral and 1.5 mm caudal to bregma; depth of 0.6 mm) (22, 29). A metal screw inserted into the occipital bone served as a reference electrode. The electrocorticogram was recorded for five epochs each lasting 5 min and separated by a 20-min interval. To avoid the confounding effects of anesthesia on cortical electrical activity, the timing of the recordings relative to the administration of the anesthetic was identical for all animals. The signals were amplified, digitized, and stored on a computer for off-line analysis (PowerLab, AD Instruments). Spectral analysis of the electrocorticogram was performed using a software module.
embedded in PowerLab. Field potentials were recorded using an electrode placed in the somatosensory cortex contralateral to the activated whiskers. The somatosensory cortex was activated by electrical stimulation of the whisker pad (2 V; 0.5 Hz; and pulse duration, 1 ms). Ten stimulation trials were averaged using a data acquisition system and stored on a hard drive for off-line analysis.

Fig. 2. Stability of MAP and increases in CBF over time in mice implanted with osmotic minipumps loaded with saline. A: MAP measured through a femoral catheter during the CBF experiments. B–D: increases in CBF induced by whisker stimulation or topical application of ACh or adenosine. $P > 0.05$ (ANOVA and Tukey’s test; $n = 5$group).

ROS measurement. The protocol for these experiments was identical to that of the CBF studies. After stabilization of MAP and blood gases, DHE was superfused on the cranial window or

Fig. 3. Elevation of MAP with phenylephrine (PE) does not attenuate the CBF responses. A: time course of the systolic blood pressure elevation by tail-cuff plethysmography induced by infusion of PE (3 $\mu$g·kg$^{-1}$·min$^{-1}$) for 14 days. SAP, systolic arterial pressure. *$P < 0.05$ from saline; $n = 5$group. B: increases in MAP induced by PE in anesthetized mice in which CBF was monitored. C–E: despite the MAP increase, PE does not attenuate the increase in CBF induced by whisker stimulation, ACh, or adenosine. $P > 0.05$ from vehicle; $n = 5$group.
administered intravenously. The brain was removed 60 min later, and ROS were determined as described in ROS Detection and Immunocytochemistry.

Data Analysis

Data in the text and figures are expressed as means ± SE. Two-group comparisons were analyzed by the two-tailed Student’s t-test. Multiple comparisons were evaluated by the analysis of variance and Tukey’s test. Probability values of <0.05 were considered statistically significant.

RESULTS

Slow Pressor ANG II Infusion Elevates Blood Pressure and Induces Cerebrovascular Dysfunction

Using telemetric blood pressure measurements, we found that slow pressor ANG II infusion (600 ng·kg⁻¹·min⁻¹) for 14 days induces a delayed increase in MAP that started at day 9 and reached a plateau between days 11 and 17 (Fig. 1A). As the osmotic minipumps emptied, MAP started to decline and returned to normal by day 21, where it remained until the end of the study (day 28) (Fig. 1A). In separate mice, in which cerebrovascular reactivity was studied, ANG II infusion elevated MAP (Fig. 1B) and attenuated the increase in CBF induced by whisker stimulation and by the endothelium-dependent vasodilators ACh, bradykinin, and A-23187 (Fig. 1, C–F). In contrast, the increase in CBF induced by the smooth muscle relaxant adenosine was not affected (Fig. 1G). The cerebrovascular dysfunction was first observed at 7 days before the increase in MAP occurred and persisted after MAP returned to baseline at 21 days (Fig. 1, C–F). The stability of the increases in CBF in mice implanted with minipumps delivering saline was tested at 3, 7, and 14 days, and responses were stable in

A: systolic blood pressure measured by tail-cuff plethysmography during infusion of nonpressor doses of ANG II (200 ng·kg⁻¹·min⁻¹; n = 5/group).
B: MAP measured through a femoral catheter during the CBF experiments in mice infused with ANG II (200 or 600 ng·kg⁻¹·min⁻¹) for 14 days. C–G: ANG II infusion (200 and 600 ng·kg⁻¹·min⁻¹) attenuate CBF responses to whisker stimulation, ACh, bradykinin, A-23197, but not adenosine. *P < 0.05 from vehicle; #P < 0.05 from ANG II-200 and vehicle (ANOVA and Tukey’s test; n = 5/group).
Nonpressor Doses of ANG II Induce Cerebrovascular Dysfunction

The data presented in the previous section suggest that ANG II may induce cerebrovascular dysfunction independently of the elevation in MAP. Therefore, we examined whether a dose of ANG II that does not elevate MAP is able to induce cerebrovascular dysfunction. Nonpressor doses of ANG II (200 ng·kg$^{-1}$·min$^{-1}$) did not elevate MAP (Fig. 4, A and B) but did induce a cerebrovascular dysfunction that was comparable with that produced by slow pressor ANG II doses (600 ng·kg$^{-1}$·min$^{-1}$) (Fig. 4, C–G).

Spontaneous or Evoked Neocortical Neural Activity Is Not Altered During Slow Pressor ANG II Hypertension

Neural activity is a powerful determinant of cerebrovascular reactivity (7). To determine whether the alterations in cerebrovascular function were associated with alterations in neural activity, we examined the effect of slow pressor doses of ANG II on the electrocorticogram and on the field potentials induced in the somatosensory cortex by whisker stimulation. As illustrated in Fig. 5, ANG II infusion for 14 days elevated MAP (vehicle, 79 ± 2; and ANG II, 88 ± 1 mmHg; $P < 0.05$; $n = 4$ group) but did not affect the amplitude of the field potentials or the frequency distribution of the electrocorticogram. In contrast, the anesthetic isoflurane attenuated the field potentials and the electrocorticogram at all frequencies (Fig. 5, A and B), providing a positive control for the sensitivity of the monitoring system. Therefore, the cerebrovascular dysfunction induced by ANG II is not associated with alterations in spontaneous or evoked neural activity in the neocortex in which CBF was measured.

ANG II Induces Oxidative Stress in Somatosensory Cortex

ANG II is well known to induce ROS production (10). However, it is not known whether slow pressor ANG II infusion increases ROS in the cerebral cortex and, if so, whether the increase parallels the elevation in blood pressure.

DISCUSSION

We have demonstrated that slow pressor doses of ANG II induce a marked disruption in major cerebrovascular regulatory mechanisms, leading to the attenuation of the increases in CBF induced by neural activity and by endothelium-dependent mechanisms, leading to the attenuation of the increases in CBF induced by neural activity and by endothelium-dependent mechanisms, leading to the attenuation of the increases in CBF induced by neural activity and by endothelium-dependent mechanisms, leading to the attenuation of the increases in CBF induced by neural activity and by endothelium-dependent mechanisms, leading to the attenuation of the increases in CBF induced by neural activity and by endothelium-dependent mechanisms, leading to the attenuation of the increases in CBF induced by neural activity and by endothelium-dependent mechanisms, leading to the attenuation of the increases in CBF induced by neural activity and by endothelium-dependent mechanisms, leading to the attenuation of the increases in CBF induced by neural activity and by endothelium-dependent mechanisms, leading to the attenuation of the increases in CBF induced by neural activity and by endothelium-dependent mechanisms, leading to the attenuation of the increases in CBF induced by neural activity and by endothelium-dependent mechanisms.
vasodilators. The cerebrovascular dysfunction preceded the MAP elevation induced by ANG II and was still present after MAP returned to baseline at the end of the infusion. We also found that nonpressor doses of ANG II produce cerebrovascular alterations comparable with those observed with slow pressor doses, whereas the elevation of MAP with PE fails to alter cerebrovascular reactivity. These observations indicate that the elevation of MAP is not necessary or sufficient to alter cerebrovascular regulation. The cerebrovascular dysfunction was paralleled by an increase in ROS, which also preceded the onset of slow pressor hypertension and persisted beyond the normalization of MAP. Interestingly, immunolabel with cell markers suggested that the increase in ROS occurred not only in cerebral vessels but also in neocortical neurons and, possibly, astroglia, consistent with the extravascular effects of circulating ANG II. Collectively, these data demonstrate that slow pressor ANG II infusion has profound effects on the regulation of the cerebral circulation, which precede the onset of hypertension, persist after the hypertension subsides, and are mediated by oxidative stress.

The findings of the present study cannot be attributed to alterations in the physiological parameters of the mice, because MAP was monitored and body temperature and blood gases were carefully controlled. Furthermore, the dysfunction cannot be attributed to hypertension-induced structural or functional alterations in the vascular smooth muscle preventing vascular relaxation, because ANG II did not affect the increase in CBF induced by the smooth muscle relaxant adenosine. Although neural activity can have profound effects on cerebral blood vessels, the observation that ANG II does not affect the frequency profile of the electrocorticogram or the magnitude of somatosensory-evoked potentials indicates that the attenuation in functional hyperemia is not due to the suppression of neural activity. Furthermore, the fact that ANG II attenuates also

Fig. 6. ANG II increases reactive oxygen species (ROS) in the somatosensory cortex, and the ROS scavenger managonic(I-II)meso-tetrakis(4-benzoic acid)porphyrin (MnTBAP) reverses the cerebrovascular dysfunction. A: slow pressor dose of ANG II (600 ng·kg⁻¹·min⁻¹) increases ROS production at 7, 14, and 21 days. ROS return to baseline at 28 days. B: nonpressor dose of ANG II (200 ng·kg⁻¹·min⁻¹) also increases ROS at 14 days but to a lesser extent. *P < 0.05 from saline (ANOVA and Tukey’s test; n = 5/group). C–F: topical neocortical application of the ROS scavenger MnTBAP (100 μM) reverses the effect of ANG II on CBF responses to whisker stimulation, ACh, bradykinin, and A-23187. G: CBF response to adenosine is not affected. *P < 0.05 from saline and MnTBAP; n = 5/group.
nonneurally mediated responses, e.g., endothelium-dependent vasodilation, argues against a role of neural activity in the mechanisms of the effect. One limitation of the present study is that because of the cranial window, the CBF experiments had to be performed under anesthesia, which can have profound effects on MAP and CBF reactivity (18, 20, 35). However, this concern is mitigated by the fact that the time course of the MAP increase established by telemetry in awake mice was comparable with that of anesthetized mice, i.e., the increases in MAP at 14 but not 7 days of ANG II infusion. As for the CBF responses, the same conditions of anesthesia were used in saline- and ANG II-treated mice, minimizing the possibility that the observed differences in CBF responses were attributable to the anesthesia.

A new finding of the present study is that the cerebrovascular dysfunction and cerebral oxidative stress induced by ANG II precede the development of hypertension and persist even after the elevation in MAP has subsided. This observation suggests that cerebrovascular function is highly susceptible to the effects of circulating ANG II. Thus alterations in the regulation of CBF may be one of the early manifestations of the neurohumoral dysregulation that mediates the peripheral vascular effects of ANG II. This conclusion is also suggested by a study in which nonpressor doses of ANG II attenuated the response of the isolated basilar artery to ACh (6). Our finding that doses of ANG II not sufficient to elevate blood pressure induce marked cerebrovascular disruption also underlines the sensitivity of the cerebral circulation to ANG II-induced dysregulation. Because we did not assess vascular function in other arterial territories, we could not establish whether the regulation of cerebral vessels is more susceptible to the effects of ANG II than that of systemic vessels. However, cerebral blood vessels have a greater capacity to produce NADPH oxidase-derived ROS than systemic vessels (25), which could explain their increased vulnerability. Therefore, assuming that systemic resistance vessels contribute to the genesis of ANG II hypertension, the findings suggest that cerebral vessels are affected before the expression of the neurovascular and humoral changes driving the hypertension.

We also found that the infusion of slow-pressor doses of ANG II induces oxidative stress not only in cerebral blood vessels but also in neurons and, possibly, astrocytes. This is in contrast to the effects of an acute infusion of pressor doses of ANG II, which induce oxidative stress predominantly in cerebral blood vessels (11, 12). In addition to the data with DHE, further evidence of an involvement of ROS in the effects of ANG II is provided by our observations that ROS scavenger MnTBAP and the NADPH peptide inhibitors gp91ds-tat abrogate the cerebrovascular dysfunction. Although our finding with gp91ds-tat suggests that NADPH oxidase is involved in
the ROS generation, the mechanisms by which circulating ANG II activates this enzyme in neurons and astrocytes remain to be defined. Circulating ANG II does not cross the blood-brain barrier, and its effects on vascular ROS production are thought to be mediated by the activation of vascular ANG II receptors linked to NADPH oxidase (21). One possibility is that ANG II, when infused chronically, acts at brain sites located outside the blood-brain barrier, i.e., the circumventricular organs. The subfornical organ (SFO), one of the circumventricular organs, is critically involved in the mechanisms of slow pressor ANG II hypertension (40), and it is conceivable that the increase in cortical ROS is mediated by neurohumoral mechanisms triggered by the SFO. In particular, the SFO projects heavily to the hypothalamic paraventricular nucleus, which can release vasoactive hormones, e.g., vasopressin, as well as activate neural projection that could reach the cerebral cortex multisynaptically (2, 34). Another possibility is that circulating ANG II, possibly through the SFO, induces endogenous ANG II production in the brain, resulting in a global increase in ROS. These issues need to be addressed in additional experiments in which the role of the SFO in the cerebrovascular dysfunction induced by ANG II is investigated.

ANG II administration attenuated the increase in CBF produced by neural activity, ACh, bradykinin, and A-23187, responses mediated by different mediators. The increase in CBF evoked by neural activity is mediated, in part, by NO derived from the neuronal NO synthase, whereas endothelial NOS-derived NO mediates the response to ACh (12, 38, 39). On the other hand, in the mouse microcirculation the CBF responses to bradykinin and A-23187 are mediated by cyclooxygenase-1 pathway (27). Therefore, alterations involving a single vasodilatatory mechanism cannot explain the diversity of the cerebrovascular effects of ANG II. Studies on acute ANG II administration have suggested that the cerebrovascular effects of ANG II are mediated by peroxynitrite, which can alter vascular responses through the nitration of proteins critical for vascular function (8, 12, 28). It remains to be determined whether a similar mechanism applies for the effects of chronic ANG II administration.

The vascular and neuronal oxidative stress observed with ANG II infusion has important implications for the central nervous system effects of ANG II-induced hypertension and, possibly, essential hypertension. Hypertension increases the susceptibility of the brain to ischemia and promotes cognitive dysfunction (15). Although the cerebrovascular alterations and related reduction in vascular reserves could play a role, neuronal and glial oxidative stress could also be involved. Inasmuch as the ANG II slow-pressor model recapitulates some of the features of essential hypertension (32), our data support the notion that preventive treatments should be instituted also in...
prehypertensive states (31), conditions in which the MAP elevation is below the threshold for hypertension (5). Indeed, dietary modifications and exercise may be beneficial for the cognitive effects observed in prehypertension (36), attesting to the reversibility of the brain dysfunction. In conclusion, we have demonstrated that slow pressor ANG II infusion induces a delayed elevation in MAP and an alteration of key regulatory mechanisms of the cerebral circulation. The cerebrovascular dysfunction, which is also observed with ANG II doses that do not elevate MAP, precedes the onset of hypertension and persists beyond the return of MAP to baseline but is not permanent. In contrast, an elevation of MAP with PE fails to alter cerebrovascular regulation. The cerebrovascular dysfunction induced by slow pressor ANG II is mediated by oxidative stress, which involves not only cerebral microvessels but also neurons and, possibly, astroglia. The findings indicate that slow pressor ANG II infusion targets cerebrovascular function before inducing hypertension, highlighting the vulnerability of cerebral blood vessels to the damaging effects of ANG II. Although the relevance of these experimental data to human hypertension remains uncertain, the data support early preventive interventions in patients with prehypertension, especially in high-risk individuals with other cardiovascular risk factors.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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