Protection Against Ischemia and Improvement of Cerebral Blood Flow in Genetically Hypertensive Rats by Chronic Pretreatment With an Angiotensin II AT₁ Antagonist

Takeshi Ito, MD; Haruki Yamakawa, MD; Claudia Bregonzio, PhD; José A. Terrón, PhD; Alicia Falcón-Neri, BSc; Juan M. Saavedra, MD

Background and Purpose—Pretreatment with angiotensin II AT₁ receptor antagonists protects against cerebral ischemia. We studied whether modulation of cerebral blood flow (CBF) and morphometric changes in brain arteries participated in this protective mechanism.

Methods—We pretreated adult spontaneously hypertensive rats with equally antihypertensive doses of candesartan (0.1 or 0.3 mg/kg per day), nicardipine (0.1 mg/kg per day), or captopril (3.0 mg/kg per day) for 3 or 28 days via subcutaneous osmotic minipumps followed by permanent left middle cerebral artery (MCA) occlusion distal to the origin of the lenticulostriate arteries. We measured CBF by autoradiography with 4-iodo-[N-methyl-¹⁴C]antipyrine 3 hours after operation and the areas of infarct and tissue swelling 24 hours after operation. Morphometric changes in the MCA were studied after antihypertensive treatment.

Results—Twenty-eight days of candesartan pretreatment decreased the infarct area by 31%; reduced the CBF decrease at the peripheral area of ischemia and the cortical volume of severe ischemic lesion, where CBF was <0.50 mL/g per minute; increased the MCA external diameter by 16%; and reduced the media thickness of the MCA by 23%. Captopril pretreatment for 28 days decreased the infarct area by 25%. Pretreatment with candesartan for 3 days or nicardipine for 28 days was ineffective.

Conclusions—Angiotensin II system inhibition protects against neuronal injury more effectively than calcium channel blockade. Protection after AT₁ receptor blockade is not directly correlated with blood pressure reduction but with normalization of MCA media thickness, leading to increased arterial compliance and reduced CBF decrease during ischemia at the periphery of the lesion. (Stroke. 2002;33:2297-2303.)

Key Words: cerebral blood flow ■ hypertension ■ peptides ■ receptors ■ stroke

Circulating and locally formed angiotensin II (Ang II) controls cerebral blood flow (CBF)¹ by AT₁ receptor stimulation in cerebral vessels and sympathetic nerves.¹⁻³ Brain Ang II and sympathetic systems are stimulated in spontaneously hypertensive rats (SHR),¹ producing increased vasoconstrictor tone and arterial thickness with smooth muscle proliferation, decreased vascular compliance, and decreased capacity of cerebral vessels to dilate during hypoperfusion.⁵⁻⁷ Blockade of Ang II formation by angiotensin-converting enzyme (ACE) inhibitors inhibits cerebrovascular tone, and CBF is maintained by compensatory small resistance artery vasoconstriction,⁸ improving tolerance to hypotension⁹,¹⁰ and increasing adaptation to the reduction in blood flow during stroke.⁶,⁷ In SHR, acute¹¹ or prolonged¹² Ang II AT₁ receptor inhibition shifts the cerebrovascular autoregulatory response to the left, in the direction of lower blood pressures, in a manner similar to that of ACE inhibitors. Chronic AT₁ blockade inhibits brain and cerebral artery AT₁ receptors¹²,¹³ and reduces cortical ischemia and tissue swelling produced by middle cerebral artery (MCA) occlusion with reperfusion.¹² We proposed that increased capacity of cerebral arteries to dilate increased collateral flow, reduced CBF loss in the periphery of the zone of ischemia, and contributed to the neuroprotective effect of AT₁ antagonists.¹² To clarify this mechanism, we pretreated SHR with the AT₁ antagonist candesartan followed by permanent distal MCA occlusion,¹⁴ a model resulting in reproducible ipsilateral cortical infarct volumes. We quantified CBF after ischemia and MCA morphometry after prolonged treatment with antihypertensive drugs and compared the effects of short-term and long-term AT₁ receptor blockade with those of long-term ACE inhibition and calcium channel blockade, at doses resulting in similar reductions of blood pressure.
Materials and Methods

Animals
Adolescent, 8-week-old male SHR weighing 190 to 240 g were purchased from Taconic Farms, Germantown, NY, housed under standard conditions, and fasted 16 hours before surgery with free access to water. The National Institute of Mental Health Animal Care and Use Committee approved all procedures. Osmotic minipumps were implanted subcutaneously to deliver candesartan (0.1 or 0.3 mg/kg per day) or vehicle (0.1N Na₂CO₃) for 3 or 28 days or nicardipine (0.1 mg/kg per day) or captopril (3.0 mg/kg per day) for 28 days to groups of 6 to 7 animals each. We measured systolic arterial blood pressure in conscious rats by the tail-cuff method before treatment and on the 27th day of treatment. In an additional experiment, we measured systolic arterial blood pressure before and 1, 3, 7, and 14 days after treatment. During surgery, we measured mean intra-arterial blood pressure (MABP) after induction of anesthesia, continuously during the operation, and immediately after MCA occlusion.

Blood pH, PaO₂, and PaCO₂ were determined as described. Plasma glucose levels were determined with blood glucose test strips (Accu-Check ADVANTAGE, Boehringer Mannheim Corporation) immediately before and immediately after MCA occlusion. From a total of 75 rats, 6 died during surgery, 3 were hyperglycemic, and 10 showed abnormal PaCO₂ measurements. Animals presenting abnormal measurements were evenly distributed among all groups and were not included in the study.

Permanent Occlusion of Distal MCA
We used a modified method to electrocoagulate and transect the left MCA 2 mm proximal to the inferior cerebral vein, distal to the origin of the lenticulostriate arteries, on day 28 of treatment. Animals were anesthetized with 3% halothane in 70% nitrous oxide and 3% oxygen under spontaneous respiration. Rectal temperature was maintained with the use of a heating pad between 36.5°C and 37.5°C. All animals showing abnormalities in rectal temperature were excluded from the study.

Measurement of Volume of Injury
We determined the infarct volume after 24 hours of ischemia with the 2,3,5-triphenyltetrazolium chloride (TTC) method with image scanning and computerized microdensitometry after correction for brain swelling. Tissue swelling was measured by subtracting the volume of the nonaffected hemisphere from the volume of the affected hemisphere divided by the volume of the nonaffected hemisphere.

Measurement of CBF
We measured CBF in cortical and subcortical areas of interest in awake animals treated with 0.3 mg/kg per day candesartan, 0.1 mg/kg per day nicardipine, or vehicle for 28 days, 3 hours after MCA occlusion, by a modified quantitative autoradiographic technique using 4-iodo-[N-methyl-¹⁴C]antipyrine. We quantified local tissue concentrations of ¹⁴C by autoradiography. The brain areas were divided into 3 groups: those with CBF <0.25 mL/g per minute, those with CBF >0.25 but <0.5 mL/g per minute, and those with CBF >0.5 mL/g per minute.

Results

Systolic Blood Pressures in Conscious Animals During Drug Pretreatment

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<td>163±6</td>
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<td>180±3</td>
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<td>172±3</td>
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<td>...</td>
<td>...</td>
<td>91±3*</td>
<td>113±5*</td>
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<td>147±2</td>
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<td>102±8†</td>
<td>...</td>
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<tr>
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<td>134±5*</td>
<td>123±6*</td>
<td>120±6*</td>
<td>112±3*</td>
<td>124±3*</td>
</tr>
<tr>
<td>Nicardipine 0.1 mg/kg per day</td>
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<td>138±6*</td>
<td>126±6*</td>
<td>122±4*</td>
<td>113±4*</td>
<td>125±5*</td>
</tr>
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</table>

Values are expressed in millimeters of mercury. *P<0.05, treatment groups vs vehicle and pretreatment. †P<0.05, candesartan 0.3 mg/kg per day vs nicardipine and captopril.

Morphometry of MCA
Morphometric analysis of MCA wall was performed as reported previously in artery sections with an external diameter of 100 to 150 μm, at the level just above the inferior horn of the lateral ventricles (horizontal sections corresponding to H 4.2 [plate 84]). Results were pooled from 3 consecutive 20-μm-thick sections for each animal, and each animal was measured individually. The arterial external diameter and the media thickness of the vessel wall were calculated as external circumference/π and (external circumference−internal circumference)/2π, respectively.

Statistical Analysis
Results were expressed as mean±SEM and analyzed by 1-way ANOVA and post hoc analysis for significance with the Tukey multiple comparisons test.

Physiological Variables
After 27 days of treatment, all drugs decreased systolic blood pressure similarly (Table). The comparison between 27 days of treatment with candesartan and nicardipine was repeated 3 times. The combined measures for all groups were 110±3 (n=17) and 121±3 (n=15) mm Hg for candesartan 0.3 mg/kg per day and nicardipine 0.1 mg/kg per day, respectively (P>0.15). The effects of captopril and nicardipine treatments were also identical; a significant decrease in blood pressure was noted as early as the first day after treatment (Table).

When measured intra-arterially during surgery, all treatments had significantly decreased MABP, and values were not different when measured before and immediately after MCA occlusion (results not shown). Immediately before MCA occlusion, blood glucose, blood pH, PaO₂, and PaCO₂ were within normal limits, and there were no significant differences between the groups of drug-treated and vehicle-treated SHR (results not shown).

Infarct Volume and Tissue Swelling
Permanent MCA occlusion produced an ischemic lesion of approximately 200 mm³ (Figure 1A and 1B) localized to parts of the frontal, occipital, temporal, and parietal cortex, and tissue swelling of approximately 20% of the volume of the ipsilateral hemisphere (Figure 1A and 1B). Candesartan treatment for 28 days significantly decreased the infarct size compared with vehicle-treated or nicardipine-treated SHR (Figure 1A and 1B). Inhibition of ACE with captopril also significantly reduced the size of the infarct (Figure 1A and 1B). Conversely, nicardipine was without effect (Figure 1A...
Figure 1. Effects of drug treatments on topography and size of infarction and degree of brain edema after distal permanent left MCA occlusion. A, Topography of infarct. Figures represent typical serial 2-mm-thick brain sections stained with TTC. Areas of infarct appear white in the figures. Scale bar is 1 cm. B, Quantification of infarct area volumes. The infarct area was measured at 6 to 7 levels in the anteroposterior plane. The results were combined for each animal to obtain the total infarct area volume. C, Quantification of tissue swelling. Groups were of 6 to 7 animals measured individually. Results are expressed as percentages. Groups are as follows: open bars, group treated with vehicle for 28 days; closed bars, group treated with candesartan 0.3 mg/kg per day for 3 days and groups treated with 0.1 mg/kg per day nicardipine for 28 days; vertical striped bars, group treated with 3.0 mg/kg per day captopril for 28 days; horizontal striped bars, group treated with 0.1 mg/kg per day nicardipine for 28 days. Values are mean ± SEM. *P < 0.05 compared with vehicle-treated group; †P < 0.05 compared with nicardipine-treated group.

Figure 2. Effect of candesartan on CBF at rest and after MCA occlusion. Horizontal dotted lines indicate 0.25, 0.50, and 1.25 mL/g per minute thresholds. Groups were of 6 to 7 rats, measured individually. A, Effect of candesartan on CBF of sham-operated rats. Values are from sham-operated rats shown. Vehicle-treated animals: □, right hemisphere; ▪, left hemisphere. Rats treated with candesartan, 0.3 mg/kg per day: ◆, right hemisphere; ○, left hemisphere. B, Effect of MCA occlusion on CBF. Values are from the ipsilateral hemisphere of sham-operated rats treated with vehicle (□) or from animals submitted to MCA occlusion, treated with vehicle (○). Groups were of 6 rats, measured individually. Note how in many cortical areas from the frontal, occipitotemporal, and parietal cortex, CBF decreased to below the 0.5 mL/g per minute threshold and in some cases below the 0.25 mL/g per minute threshold. Values are mean ± SEM. *P < 0.05 compared with sham-operated rats. C, Effects of AT1 receptor blockade on CBF after MCA occlusion. We measured CBF in the left hemisphere, ipsilateral to the lesion. Values are from animals with MCA occlusion, treated with vehicle, as in B (○), or from rats with MCA occlusion, pretreated with candesartan, 0.3 mg/kg per day (◆). Note how treatment with the AT1 receptor antagonist improved CBF in several cortical areas, reaching values above the 0.25 mL/g per minute threshold and in some cases above the 0.5 mL/g per minute threshold. CBF was measured 3 hours after the operation. Values are mean ± SEM. *P < 0.05 compared with vehicle-treated group. For A, B, and C, area numbers correspond to specific areas: 1, cingulate cortex; 2, frontal cortex; 3, sensory cortex; 4, cingulate cortex; 5, frontal cortex; 6, sensory cortex; 7, parietal cortex; 8, caudate putamen; 9, caudate putamen; 10, retrosplenial cortex; 11, frontal cortex; 12, sensory cortex; 13, parietal cortex; 14, hippocampus; 15, retrosplenial cortex; 16, occipital cortex; 17, occipital cortex; 18, temporal cortex; 19, hippocampus.
All antihypertensive drugs administered for 28 days significantly reduced tissue swelling (Figure 1A and 1C). To examine the effect of short-term AT₁ receptor blockade on the infarct size, we pretreated SHR with 0.3 mg/kg per day candesartan for 3 days. This treatment did not reduce the infarct size or the tissue swelling (Figure 1A, 1B, and 1C), although the systolic blood pressure was reduced to 69% of pretreatment levels (Table).

**Regional CBF**

In sham-operated SHR, candesartan pretreatment (0.3 mg/kg per day) did not modify local CBF compared with vehicle-treated animals (Figure 2A).

MCA occlusion produced substantial decreases in CBF in the ipsilateral (left) hemisphere (Figure 2B). In the contralateral hemisphere, MCA occlusion significantly decreased CBF only in the cingulate cortex (1.93±0.29 and 1.18±0.09 mL/g per minute for sham-operated rats treated with vehicle and rats treated with vehicle and subjected to MCA, respectively; P<0.05).

Candesartan (0.3 mg/kg per day for 28 days) substantially reduced the decrease in CBF that occurred after the operation (Figure 2C). In parts of the frontal, occipital, and temporal cortex, located in the periphery of the area of ischemia, where CBF decreased after MCA occlusion in vehicle-treated rats to levels <0.50 mL/g per minute, candesartan pretreatment maintained the CBF at a level above the 0.50 mL/g per minute threshold (Figure 2C). In other parts of the frontal, occipital, temporal, and parietal cortex, where the CBF dropped to a level <0.25 mL/g per minute after MCA occlusion in vehicle-treated rats, candesartan pretreatment maintained the CBF at above the 0.25 mL/g per minute threshold (Figure 2C). AT₁ receptor blockade completely reversed the loss in CBF after MCA occlusion in the contralateral cingulated cortex (1.18±0.09 mL/g per minute in vehicle-treated SHR and 1.79±0.08 mL/g per minute in candesartan-treated rats; P<0.05).

**Relationship Between Decrease in CBF and Area of Ischemia**

In brain areas of severe ischemia and neuronal death, CBF was reduced to values below the 0.50 mL/g per minute threshold or even below the 0.25 mL/g per minute threshold (Figures 2B, 3, and 4A). Conversely, areas with substantial decreases in blood flow that did not reach the 0.50 mL/g per minute threshold (Figure 2B) did not reveal ischemia and neuronal death (Figures 3 and 4A).

In SHR pretreated with candesartan and submitted to MCA occlusion, there was a significant reduction of the area volume where CBF was <0.25 mL/g per minute and in the combined area volume where CBF was <0.50 mL/g per minute, and there was a significant increase of the area volume where CBF was >0.5 mL/g per minute compared with vehicle-treated rats (Figures 2C, 3, 4A, and 4B). The total area volume where CBF was <0.50 mL/g per minute correlated well with the total area of ischemia, and candesartan treatment reduced both figures by 30% (Figures 3, 4A, and 4B).

Conversely, pretreatment with 0.1 mg/kg per day nicardipine, which failed to protect against ischemia, did not produce any redistribution of CBF after ischemia (Figure 4B).

**Effect of Antihypertensive Treatments on External Diameter and Media Thickness of MCA**

AT₁ receptor blockade significantly increased the external MCA diameter and significantly decreased the media thickness of the vessel wall and the external diameter/media thickness ratio (Figure 5). Treatment with nicardipine or captopril did not significantly affect the external diameter, the media thickness of the MCA, or the external diameter/media thickness ratio (Figure 5).

**Discussion**

Improvement of local perfusion during ischemia is a rational approach to rescue brain tissue. We tested the hypothesis that improvement of the collateral circulation resulting in reduced CBF decrease was an important mechanism in the

![Figure 3. Angiotensin AT₁ receptor blockade decreases the area of CBF values under a 0.50 mL/g per minute threshold after MCA occlusion. Figures represent typical images of CBF obtained with the 4-[N-methyl-¹⁴C]antipyrine method (see Materials and Methods) at 4 levels in the anteroposterior plane in individual rats treated with vehicle (left) or candesartan 0.3 mg/kg per day (right). For each group, figures on the right represent actual film images of CBF with image scanning obtained with the use of a Macintosh-based image processing system, NIH Image 1.62. For each group, figures on the left show comparative images obtained with the use of the image processing system and computerized image analysis to reveal the area corresponding to a CBF below the 0.50 mL/g per minute threshold (shown in white). Scale bar is 1 cm.](http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/01.STR.93.12.2300/-/DC1/figure3.jpg)
AT<sub>1</sub> receptor blockade for 28 days offered protection from ischemia after permanent MCA occlusion similar to that obtained by 14 days of pretreatment before MCA occlusion with reperfusion<sup>12</sup>; however, a short (3 days) pretreatment was ineffective, in parallel with the normalization of the cerebrovascular regulation after AT<sub>1</sub> receptor blockade, which occurs only after 7 to 14 days of treatment.<sup>12</sup> This suggests that the length of pretreatment is crucial for the protective effect to occur. While inhibition of Ang II synthesis with captopril for 28 days protected equally against brain ischemia, pretreatment with nicardipine for 28 days did not, in agreement with reports that calcium channel blockers did not prevent cellular damage during ischemia.<sup>23</sup> This indicates that protection against ischemia is dependent on Ang II system inhibition<sup>6,7,12</sup> and not the consequence of differences in the progression of the antihypertensive effects because all compounds decreased blood pressure very early in the treatment. All prolonged antihypertensive treatments used here, including calcium channel blockade, protected from brain swelling, indicating that the mechanisms underlying brain swelling are not identical to those responsible for neuronal death and infarct size.

We compared the size of the infarct with the absolute values of CBF in the affected areas with and without treatment. During brain ischemia, candesartan pretreatment decreased the proportion of CBF reduction in cortical areas at the periphery of the infarct, as determined with the Doppler technique.<sup>12</sup> However, the Doppler method offers only relative measurements of CBF in a few areas, whereas the [14C]iodoantipyrine technique used here allows for the detailed study of actual CBF values in all regions of interest. In vehicle-treated SHR, we found that the area of reduction of CBF below a crucial threshold of 0.50 mL/g per minute clearly matched the area of infarct and neuronal death.

After pretreatment with the AT<sub>1</sub> antagonist for 28 days, both the area of CBF below the 0.50 mL/g per minute threshold and the area of infarct were significantly and equivalently reduced and closely matched, suggesting that preservation of CBF above a crucial threshold is a critical factor in the protection from ischemia after AT<sub>1</sub> blockade.<sup>12</sup> Protection of CBF during ischemia and normalization of cerebrovascular autoregulation might be based on the inhibition of the growth-promoting effects of Ang II in cerebral arteries,<sup>24</sup> resulting in increased arterial compliance and increased capacity of collateral brain arteries to dilate during ischemia, a mechanism similar to that of the protective effects of ACE inhibitors.<sup>5,6,8,11,13</sup> Pretreatment with the AT<sub>1</sub> receptor antagonist for 28 days significantly increased the external diameter and reduced the media thickness of the MCA wall, while nicardipine was ineffective. These findings support the hypothesis that Ang II plays an important role as a growth-promoting agent in the increase in brain arterial wall thickness in hypertension<sup>24</sup> and that blockade of the growth-promoting effects of Ang II in cerebral arteries by AT<sub>1</sub> receptor inhibition is an important mechanism underlying the increased arterial compliance and the relative CBF protection during ischemia. Decreased arterial blood pressure, as produced by calcium channel blockade, is not sufficient to
reverse the structural changes in mid-caliber cerebral arteries such as the MCA.

Pretreatment with an ACE inhibitor protected against ischemia but failed to modify MCA morphology. There are several explanations for this discrepancy, including effects of ACE inhibitors in smaller-caliber vessels and AT1 receptor stimulation due to only partial prevention of Ang II synthesis. AT1 receptor blockade elevates plasma levels of Ang II, increasing Ang II AT1 receptor stimulation, which inhibits vascular growth and promotes vasodilatation. AT1 antagonists increase formation of the vasodilator bradykinin, upregulating nitric oxide formation through AT2 receptor stimulation, but the half-life of the peptide is short. ACE inhibitors inhibit bradykinin degradation, making the potentiation of its effects more durable. Unopposed AT1 receptor stimulation after AT1 receptor blockade could be responsible for the morphological changes observed in the MCA, while prolonged stimulation of bradykinin effects by ACE inhibitors may contribute to the protection from ischemia predominantly through effects localized to smaller-caliber arteries.

In conclusion, AT1 receptor blockade, as a consequence of inhibition of hypertension-induced arterial remodeling, significantly protects against brain ischemia through increased arterial compliance, normalization of cerebrovascular autoregulation, increased capacity to dilate when confronted with decreased CBF, and enhancement of collateral circulation during hypoperfusion. The degree of protection against neuronal injury parallels the degree of protection against hypoperfusion. Selection of antihypertensive medications that inhibit the cerebrovascular and brain Ang II systems may result in significant end organ protection during brain ischemia.

Acknowledgments

This study was supported in part by a grant from ASTRA GmbH Germany (Dr Terrón). We thank Dr Berend Friedrich, ASTRA GmbH Germany, for his generous supply of candesartan, and Dr Gustavo Baiardi for help in preparing the figures.

Dr Terrón was on leave from the Department of Pharmacology, CINVESTAV-IPN, Mexico City, Mexico.

References


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<th>Media thickness (µm)</th>
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Figure 5. Morphometry of MCA after antihypertensive treatment. Photographs are of representative histological cross sections of rat MCA stained with hematoxylin and eosin, selected from horizontal planes corresponding to H 4.2 (plate 84). Bar is 50 µm. Values are mean ± SEM. *P<0.05, candesartan-treated compared with other groups.
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Stroke. 2002;33:2297-2303
doi: 10.1161/01.STR.0000027274.03779.F3

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