Angiogenic protection from focal ischemia with angiotensin II type 1 receptor blockade in the rat

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Forder, Joan P., Diane H. Munzenmaier, and Andrew S. Greene. Angiogenic protection from focal ischemia with angiotensin II type 1 receptor blockade in the rat. Am J Physiol Heart Circ Physiol 288: H1989–H1996, 2005. First published October 21, 2004; doi: 10.1152/ajpheart.00839.2004.—Angiogenesis within an ischemic region of the brain may increase tissue viability and act to limit the extent of an infarct. The ANG II pathway can both stimulate and inhibit angiogenesis depending on the tissue and the activated receptors. Previous work showed that 2-wk losartan administration (ANG II type 1 receptor blockade) initiates a significant cerebral angiogenic response. We hypothesized that administration of losartan in the drinking water of rats for 2 wk before initiation of focal ischemia would decrease the extent of the resulting infarct. Adult male Sprague-Dawley rats were given losartan (50 mg/day) in drinking water for 2 wk before initiation of cerebral focal ischemia produced by cauterization of cortical surface vessels. Controls received normal drinking water. In control animals, three main vessels feeding the whisker barrel cortex were cauterized, resulting in cessation of blood flow. The same protocol was followed for losartan-treated animals but did not result in cessation of blood flow in the whisker barrel cortex. Another group of losartan-treated animals received between 8 and 14 cauterizations of surface vessels feeding the whisker barrel cortex, and cessation of blood flow was verified. Rats were killed 72 h after surgery. Morphological examination revealed angiogenesis, maintained vascular delivery, and significantly decreased infarct size in losartan-treated animals compared with controls. These results demonstrate that pretreatment with losartan reduces infarct size after cerebral focal ischemia and support the hypothesis that cerebral angiogenesis may be one of the mechanisms responsible.

MATERIALS AND METHODS

All animal procedures were conducted in accordance with the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society. Male Sprague-Dawley rats (12 wk of age; n = 6 in each group) weighing 350–400 g were used for this study. All animals had free access to standard laboratory chow and were fasted overnight before surgery. Animals in the experimental groups were provided with losartan (50 mg/day; generously donated by Merck) in the drinking water for 2 wk before initiation of focal ischemia. Rats were housed individually and had access to its own drinking bottle. The daily intake of drug was closely monitored and adjusted as follows. A stock solution of losartan was created at a concentration of 1 mg/ml drinking water. Each rat was housed individually and had access to its own drinking bottle. The amount of water consumed was measured daily, and the concentration of the drug was adjusted according to the previous day’s measurement. Records were kept on each individual rat and then averaged. The average losartan consumption in rats was 50.07 ± 10.220.33.6 on October 1, 2017 http://ajpheart.physiology.org/ Downloaded from http://ajpheart.physiology.org/ by 10.220.33.6 on October 1, 2017
ment in a stereotaxic apparatus, a 6- to 8-mm cranial window was created over the right somatosensory cortex (2 mm caudal and 5 mm lateral to bregma). Small boluses (10–20 μl) of the vital dye Patent Blue Violet (10 mmol/l; Sigma) were injected into the right ECA to demonstrate transits through surface cortical vessels. A video recording was obtained at this time. For all rats, three critical arteriolar branches of the MCA around the barrel cortex were selected and electrically cauterized (Promhovet). The bolus injections of dye were repeated, and another video recording was made. For the untreated group, dye transits displayed complete lack of flow into surface vessels. For the three-vessel losartan (3V) group, surgery was halted after three arteriolar branches were cauterized. However, for the 3V group, dye transits indicated that retrograde flow occurred into the three cauterized vessels. For the remaining rats (all-vessel losartan (AV) group), cauterization continued until dye transits displayed complete lack of flow into surface vessels. A video recording was obtained at this time point for this group of rats. The window was replaced, and the scalp was sutured. The catheter was removed, and the anterior neck was sutured. All rats were allowed to recover and resume normal activities for 72 h after surgery. No evidence of gross neurological deficits was seen in any animal. To visualize the extent of hypoxia after focal ischemia surgery, six rats in each of three groups (untreated, 1 wk losartan treated, and 2 wk losartan treated) were infused with 0.50 ml of Hypoxyprobe-1 (30 mg/ml iv; Chemicon International) over a 30-s time period through the left femoral vein 1 h before cauterization of surface vessels. At 72 h after surgery, all rats were anesthetized with 1 ml of pentobarbital sodium (50 mg/ml ip) and the brain was quickly dissected and placed at −20°C for 20 min. Each brain was coronally sliced (2 mm) and incubated for 15 min in 2% triphenyltetrazolium chloride (TTC) to visualize mitochondrial dehydrogenase activity. Each 2-mm slice was photographed at ×0.63 magnification. Both hemispheric regions and the infarct region from each photographed image were traced and recorded (Metamorph version 4.6; Universal Imaging, Downingtown, PA) (Fig. 1A). From the 2-mm slices, the area was outlined from each region (infarct region, right and left hemispheres) and the percent volume of infarct was calculated.

The 2-mm slices were embedded in optimal cutting temperature compound (OCT; Tissue Tek) and frozen in 2-methylbutane maintained at −70°C by the addition of dry ice. Each frozen section was then used to produce 4-μm slices. These slices were immunohistochemically stained for either CD31 (platelet endothelial cell adhesion molecule 1) to visualize vessels or Hypoxyprobe-1 to visualize the extent of hypoxia. For both vessel visualization and visualization of hypoxia, slides were fixed in cold acetone at −20°C for 10 min, with endogenous peroxidase removed with 1:1 hydrogen peroxide-methanol for 10 min, and then blocked in 1% horse serum at room temperature for 30 min. Slides were incubated for 1 h in either monoclonal primary anti-rat CD31 antibody (BD Pharmingen, San Diego, CA) diluted in 3% horse serum (1:100) for visualization of vessels or monoclonal primary anti-rat Hypoxyprobe antibody (Chemicon International) diluted in 3% horse serum (1:500) for visualization of hypoxia. Slides were rinsed with Tris-buffered saline (TBS) and incubated for 30 min in biotinylated anti-mouse IgG, rat absorbed secondary antibody (Alexa Fluor 488, Molecular Probes, Eugene, OR) diluted in 3% horse serum (1:100). Slides were rinsed in TBS, incubated in the Vectastain Elite ABC system (Vector Laboratories, Burlingame, CA) for 30 min, and then quickly rinsed in distilled water before being exposed to diaminobenzidine (Vector) for 4 min. Slides were then rinsed in distilled water, air-dried, and coverslipped with the water-soluble mountant gelvatol. For vessel visualization, each 4-μm slice was photographed at ×1 magnification and previously outlined regions from the 2-mm pictures were overlaid onto the ×1 pictures to visualize the infarct region (Fig. 1B).

For vessel quantification, this overlaid image was used as a guide and ×20 magnification pictures were obtained from within the infarct, penumbra, and control regions (Fig. 1C). The control regions consisted of regions within the right hemisphere but far removed from the infarct and identical (symmetrical) regions from the left (control) hemisphere. With Metamorph software, each ×20 picture was ana-

Fig. 1. Immunohistochemical flowchart outlining visualization of cerebral microvessels. A: triphenyltetrazolium chloride (TTC)-stained 2-mm coronal section of the rat brain to visualize infarct area (white area). Right hemisphere (yellow outline) and infarct area (green outline) have been outlined and quantitated for area measurements. White bar, 2 mm. B: TTC-stained sections were cryosectioned at 4 μm and then stained immunohistochemically for CD31 (platelet endothelial cell adhesion molecule 1), a known vessel endothelial cell marker. Regions outlined in A were transposed onto ×1 pictures of immunohistochemically stained sections for localization of infarct. This “map” was then used to determine infarct, penumbra, and control regions. Quantification of vessel density (no. of vessels/0.26 mm²), average vessel size (μm²), and total vessel area (vessel density × average vessel size) were obtained from these pictures as described in MATERIALS AND METHODS. Black bar, 0.2 μm.

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lyzed for vessel density [the number of vessels in the field of view (FOV)], average vessel size of all vessels in the FOV, and total vessel area (product of vessel size × vessel density).

For hypoxia quantification, the ×1 photographed images were analyzed with Metamorph software to determine the extent of hypoxia as a percentage of the affected hemisphere. Sham-treated animals were also analyzed at each time point to ensure that visualized hypoxia was due to focal occlusion. More specifically, each image was thresholded to eliminate any background effects. Measurements of the area of visualized Hypoxyprobe antibody as well as the total hemispheric area were obtained, and a calculation of the percent area covered by the displayed Hypoxyprobe antibody was made. It is important to note that no attempt was made to determine the intensity of the antibody staining but merely to look at the percentage of the area of the hemisphere that was displayed as taking up the Hypoxyprobe antibody.

During surgery, observation of the surface vessels indicated a possible increase in the extent of vascular branching visible through the cranial window of losartan-treated animals. To quantitate this observation, a precauterization image was captured for each rat and each image was converted to gray scale (Microsoft Photoshop version 7.0). With Metamorph software, automated measurement of the percentage of cortex area within a predetermined rectangular area (3 mm × 2 mm) over the whisker barrel cortex-constituting vessels was obtained.

To quantify microvascular delivery of blood within the whisker barrel cortex, a dye transit event was captured from each of the pre- and post-three-vessel cauterization videos from all rats in all groups (untreated, 3V, and AV groups) as well as the post-all-vessels cauterization videos from the AV group. This method involved using Adobe Premier software, by which 5 frames/s were captured starting 1 s before visible identification of dye in the arterioles and lasting until all visible dye had left the venules. For each event, each frame was converted to gray scale (Microsoft Photoshop version 7.0) and each frame of each event was stacked in the computer’s frame buffer to allow for automated analysis of dye intensity changes over the event period. A standardized circular area of 0.5 mm² was positioned in an area that was determined to be devoid of large visible vessels (“avascular” region), and dye intensity data were collected for all frames in each event. The same avascular region was used in all videos obtained for each rat.

Data analysis. A two-way analysis of variance was performed for all measurements. All data are expressed as means ± SE. P < 0.05 was considered significant.

RESULTS

Infarct size. Cauterization of vessels feeding the whisker barrel cortex of control animals resulted in a reproducible focal lesion confined to the whisker barrel cortex (Fig. 2A, left). TTC staining of the 2-mm-thick slices demonstrated a sharp delineation of the infarct region that was confined to the cortex. Only one of six rats in the 3V group displayed any infarct (Fig. 2A, right). When a bolus injection of dye was repeated after the three main arterioles were cauterized, all rats in this losartan group displayed backfilling of dye into the major surface arterioles, whereas untreated animals did not. For the remaining six losartan-pretreated animals (AV group), cauterization was continued beyond the three original vessels until a bolus injection of dye revealed no backfilling into the major surface arterioles. This cauterization resulted in 8–14 occlusion points (Fig. 2A, middle). For the AV group, the average infarct size was significantly smaller (0.40% ± 0.16 of right hemisphere) than in untreated rats (2.91% ± 0.54 of right hemisphere) but was significantly larger than the average infarct size of the 3V group (0.031% ± 0.03 of right hemisphere; Fig. 2B).

Angiogenesis. Only one of the six 2-wk losartan-pretreated animals displayed any visual infarct. Therefore, in the animals that did not display any visible infarct, an area of the whisker barrel cortex equivalent to the infarct size and location of the animal that did show a visible infarct was used for analysis and was termed the “area at risk.” The decrease in vessel density seen in the infarct region of untreated animals in response to focal ischemia was attenuated in 1-wk losartan-pretreated animals and increased even further in 2-wk losartan-pretreated animals (Fig. 3A). The increase in vessel density seen in control regions of untreated animals in response to focal ischemia was not seen in 1-wk or 2-wk losartan-pretreated animals. The increase in vessel density seen in the penumbra region of untreated animals in response to focal ischemia was also present in 1-wk, but not in 2-wk, losartan-pretreated animals. Comparing sham-surgery animals, a significant increase in vessel density was seen in all cortical areas of 2-wk losartan-pretreated animals (102.5 vessels/0.26 mm²) compared with untreated animals (76.5 vessels/0.26 mm²) (P < 0.001). Analysis of all sham-surgery groups indicated no significant differences throughout the cortex as follows: untreated
Vessel density in 1-wk pretreated sham-surgery animals was not significantly different from untreated sham-surgery animals (Fig. 3A).

The average vessel size in control and penumbra regions of untreated focal ischemia animals was significantly increased from that of sham-surgery animals (Fig. 3B) and remained increased in both 1-wk and 2-wk losartan-pretreated animals. Although the average vessel size within the area at risk of the untreated animals was not significantly different from sham-surgery animals, it was significantly increased in both 1-wk and 2-wk losartan-pretreated animals (P < 0.001). Comparing sham-surgery animals, no significant difference in average vessel size was seen between treatment groups. Furthermore, no significant difference was seen in all cortical regions within each sham-surgery group (control, area at risk, or penumbra). Therefore, all three cortical regions were combined for each treatment group.

Total vessel area is a product of vessel density and average vessel size. A significant decrease was seen in the total vessel area in the infarct region in untreated animals in response to focal ischemia (3,765.8 μm²/0.26 mm²) compared with total vessel area in a comparable region in untreated sham-surgery animals (4,810.6 μm²/0.26 mm²) (P < 0.01; Fig. 3C). The total vessel area in the infarct area of 1-wk losartan-pretreated animals was significantly increased (6,894.9 μm²/0.26 mm²) compared with a comparable region in 1-wk losartan-pretreated sham-surgery animals (4,319.2 μm²/0.26 mm²) (P < 0.001). The total vessel area was significantly increased in the area at risk in 2-wk losartan-pretreated animals (10,674.3 μm²/0.26 mm²) compared with 2-wk losartan-pretreated sham-surgery animals (7,859.5 μm²/0.26 mm²) (P < 0.001; Fig. 3C). The increase in total vessel area seen in control regions of untreated animals in response to focal ischemia was also seen in 1-wk losartan-pretreated animals but was not seen in 2-wk losartan-pretreated animals (Fig. 3C). The increase in total vessel area seen in the penumbra region of untreated animals in response to focal ischemia was also present in 1-wk and 2-wk losartan-pretreated animals (Fig. 3C). Comparing sham-surgery animals, no significant difference was seen in all cortical regions within each sham-surgery group (control, area at risk, or penumbra). Therefore, all three cortical regions were combined for each treatment group (Fig. 3C). A significant increase in total vessel area was seen in all cortical areas of 2-wk losartan-pretreated sham-surgery animals (7,859.5 μm²/0.26 mm²) compared with untreated sham-surgery animals (4,810.6 μm²/0.26 mm²) (P < 0.001). A trend toward significance was seen in 1-wk losartan-pretreated sham-surgery animals compared with untreated sham-surgery animals. Therefore, the changes seen within each region appear to increase in a time-dependent manner.

Extent of hypoxia. Immunohistochemistry of the hypoxic region with Hypoxyprobe-1 antibody revealed a significantly smaller visible hypoxic region within the hemisphere that received focal occlusion in 2-wk losartan-pretreated animals (3.9%) compared with untreated animals (13.8%) (P < 0.001; Fig. 4). The extent of the hypoxic area was not significantly different between untreated and 1-wk losartan-pretreated animals.

Surface vascularization. Quantification of the percent area covered by surface vessels revealed that 2-wk losartan-pretreated animals displayed a significantly higher surface vascul-
larization (31.8% ± 0.92 of surface area) compared with animals given normal drinking water (untreated animals; 16.7% ± 0.84 of surface area) (Fig. 5). Surface vascularization was not significantly different between untreated and 1-wk losartan-pretreated animals.

**Vascular delivery.** Small bolus injections of dye were made before and after cauterization for each rat. Precauterization dye intensity changes in the avascular area of the cranial window for all animals indicated a rapid initial increase in intensity with a slower emptying (Fig. 6). Cauterization of control animals resulted in cessation of vascular delivery into the area. However, for losartan-pretreated animals, the same cauterization resulted in only a delayed vascular delivery. Cauterization of an additional 8–14 points in the losartan-pretreated animals resulted in complete cessation of vascular delivery into the area. One-week losartan-pretreated animals displayed an attenuated, but still present, vascular delivery after the standard three-vessel occlusion of surface vessels (data not shown).

**DISCUSSION**

This study provides the first quantitative description of a vascular angiogenic protective role for the AT1-specific antagonist losartan against cerebral ischemia. This protection was characterized by decreased infarct size, increased microvascu-
lar measures of vessel density, average vessel size, and total vessel area, reduced resultant hypoxia, increased surface vascularization, and sustained vascular delivery in losartan-pretreated animals in a time-dependent manner. These findings provide strong evidence for increased collateralization in the cerebral vascular network resulting in little or no visible infarct. The increase in average vessel size after focal ischemia provides additional evidence for a remodeling response in support of previously postulated theory (28). Remodeling provides greater blood flow, whereas the increase in collateralization would provide alternate routes for blood flow into tissue that has been subjected to ischemic stroke. This combination could provide adequate tissue perfusion and assist in maintaining the viability of the tissue. The increased vessel density seen in the penumbra of infarcted rats that had not been given losartan implies a compensatory adaptation 72 h after focal ischemia. Even when all surface vessels were cauterized in losartan-pretreated animals, the infarct size was still significantly smaller than in untreated animals, suggesting possible cerebral collateralization from deeper vessels and remodeling of the existing vascular bed. As indicated in the 1-wk losartan-pretreated animals, in which remodeling but not increased vessel density was seen, this remodeling may precede the growth of new vessels and provide a degree of vascular protection.

Previous studies demonstrated a decrease in infarct size in various animal models after chronic losartan pretreatment (5, 19). However, the length of pretreatment, the mode of administration, and the animal used varies from study to study. For example, a single bolus pretreatment dose of losartan (10 mg/kg) significantly increased the survival rate of gerbils subjected to gradual occlusion of the carotid artery while increasing blood flow in the ipsilateral hemisphere (5). Such an acute effect of losartan may involve immediate opening of ipsilateral circulation, which would be crucial in the gerbil as this species has an incomplete circle of Willis and therefore cannot obtain increased blood flow from the contralateral hemisphere to the same extent as other species.

In a recent study using spontaneously hypertensive rats (SHR), the ANG I-converting enzyme inhibitor captopril, the Ca²⁺ channel blocker nicardipine, or the AT₁ receptor blocker candesartan was given to rats for 3 (acute) or 28 (chronic) days (19). The size of infarct and cerebral blood flow (CBF) were measured after permanent MCA occlusion (19). Acute administration did not affect either infarct size or CBF, which is in contrast to the effect seen in gerbil studies and therefore does not support the vasodilation theory of protection from infarct. Rats, having a complete circle of Willis, would be more capable of collateral recruitment than gerbils. Thus, as outlined by Ito et al. (19), if increased blood flow due to vasodilation of ipsilateral vessels was the mechanism responsible for the decreased infarct size in gerbils, then rats should also see an increased blood flow and decreased infarct size with acute administration of the AT¹ blocker. However, this was not the case (19). We did not measure the effect of acute losartan pretreatment on blood flow in our normotensive rat strain in the current study but plan to do so in the future.

The Ito et al. (19) study found that, in contrast to acute pretreatment, chronic pretreatment of either captopril or candesartan, but not nicardipine, reduced infarct size. The reduced infarct size cannot be accounted for by an increased CBF because nicardipine and captopril both significantly decreased CBF in the peripheral area of ischemia whereas chronic pretreatment with candesartan in SHR resulted in only a slight decrease in CBF (19). Chronic pretreatment with candesartan did result in a concurrent increased MCA external diameter and a reduced media thickness of the MCA (19). Because all three drugs decreased blood pressure equally but only captopril and candesartan reduced infarct size, Ito et al. (19) concluded that the protection against ischemia is dependent on ANG II system inhibition. The present study used a normotensive rat strain and indicates an angiogenic component of ischemic protection that supports the findings of Ito et al. (19). Although we have found a strong effect with losartan, recent studies indicate that other AT₁ receptor antagonists may cross the blood-brain barrier more effectively (12, 13).

It is important to note that although the MCA is a resistance vessel, the downstream arterioles also play a major role in local blood flow regulation and must be examined for possible increased compliance and sensitivity to ischemic injury. Furthermore, AT₁ blockade has been shown to increase plasma ANG II levels (15), thus providing for an increased stimulation of other ANG II receptor subtypes. Either of these possibilities could provide additional evidence for a remodeling response in the protection against ischemia is dependent on ANG II system inhibition.

In Wistar rats, chronic pretreatment with candesartan resulted in significant improvement in neurological outcome and reduced infarct size and edema of the hemisphere with focal ischemia (15). Although both that study and the present study used normotensive rats and obtained similar findings concerning reduced infarct size, some significant differences are found. We administered losartan orally for 14 days as opposed to subcutaneously for 5 days. Previous studies have found that 7–14 days of pretreatment are necessary with AT₁ receptor blockade before normalization of the cerebrovascular regulation can occur (23). Our focal ischemic model was restricted to the somatosensory whisker barrel cortex of the rat, whereas the Groth et al. study (15) involved a more extensive complete MCA occlusion, thus allowing for more extensive neurological damage. Groth et al. (15) measured neurological behavioral outcome of the occluded rats, whereas we used quantitative vessel area and vascular delivery measures to assess the protective effect. With the use of a 14-day pretreatment period, a focal occlusion model, and quantitative measures, the present study has defined a possible angiogenic component to ischemic protection after stroke.

The decrease in infarct size following chronic administration of an AT₁ receptor antagonist such as losartan could be due to a number of different mechanisms: NO-dependent or bradykinin-dependent vasodilation of existing vessels, neuroprotection, or angiogenesis. Although strong evidence has been provided for the role of ANG II in limiting the decrease in blood flow after ischemia through the rapid recruitment of preexisting collateral circulation in noncerebral tissues (2, 23), direct evidence of this effect after cerebral focal ischemia has been lacking. Such a mechanism has been postulated in SHR (19) without direct experimental evidence. However, in a direct comparison between SHR and Wistar-Kyoto rats, only SHR displayed NO-dependent vasodilation after AT₂ stimulation (24). Other studies have reported a vasodilatory protective role of losartan initiated through a kinin-dependent mechanism in...
the aorta of stroke-prone SHR (11). However, this response could not be repeated in normotensive animals (11). Other studies have indicated that in SHR it is not the increase in CBF that is responsible for decreases in focal ischemic infarct size (30). Together, these studies indicate that vasodilation, through an NO- or kinin-dependent mechanism, cannot fully account for the protective role of losartan in reducing infarct size in normotensive animals after focal ischemia.

A neuroprotective action of AT1 antagonist pretreatment has been reported in the rat brain (4). Such a protective action has been shown to significantly improve the neurological outcome of focal cerebral ischemia with markedly reduced expression of c-Fos and c-Jun proteins in the cortex in the ipsilateral hemisphere (4). However, the presence of these proteins indicates cellular stimulation in response to stressors such as ischemia (16) but is not necessarily indicative of increased cellular death. Thus there may not be a direct neuroprotective role of AT1 antagonist pretreatment but instead a decreased ischemic stressor, as indicated by the reduced expression of these proteins. It has also been suggested that ischemia induces an overexpression of AT2 receptors in neuronal cell cultures, thus providing a cellular adaptation to hypoxia (26). Chronic administration of AT1 antagonist has been shown to remove the negative feedback to renin release and thus results in an increase in circulating plasma ANG II. This increased ANG II is believed to increase AT2 receptor stimulation without the opposing actions of AT1 receptor activation. However, direct evidence for this has not been reported to date. It is also possible that increased ANG II levels may result in increased metabolites such as ANG III or ANG IV and that the specific receptors to these metabolites may be involved in this phenomenon.

The remaining possible mechanism involves an angiogenic role of losartan. The angiogenic role of ANG II has been well documented in noncerebral organs such as skeletal muscle (14, 21) kidney (16, 17), rabbit cornea (6, 9), and various cell lines (25). These studies indicate systemically that it is the AT1 receptor that mediates angiogenesis and vasoconstriction, whereas the AT2 receptor opposes these actions. There have been recent indications that the roles of these two receptors may differ in the brain (22), with AT1 receptor blockade increasing cerebral microvessel density in rats. Thus pretreatment with losartan would block the angiogenic inhibition and favor microvessel proliferation. This increased angiogenesis would provide a more dense vascular bed. When a vessel is occluded, as is the case in stroke, the increased angiogenesis could allow for blood flow to the area from alternative routes. Evidence for this correlation between increased angiogenesis and more positive outcomes after stroke in humans showed that increased angiogenesis correlated strongly with longer survival rates after stroke when measured postmortem (20).

The present study provides direct evidence of increased angiogenesis, increased surface vascularization, and maintained microvascular delivery after chronic administration of losartan followed by permanent focal ischemia to small surface branches of the MCA over the whisker barrel cortex of normotensive rats. Because losartan is clinically administered to combat hypertension, it may also provide an inherent vascular protective role against cerebral ischemia.

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GRANTS

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