Mineralocorticoid Receptor Activation Causes Cerebral Vessel Remodeling and Exacerbates the Damage Caused by Cerebral Ischemia

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Abstract—Mineralocorticoid receptor antagonists protect against ischemic cerebrovascular disease; this appears to be caused by changes in cerebral vessel structure that would promote blood flow. Therefore, we hypothesized that mineralocorticoid receptor activation with deoxycorticosterone acetate would cause deleterious remodeling of the cerebral vasculature and exacerbate the damage caused by cerebral ischemia. Six-week-old male Wistar rats were treated with deoxycorticosterone acetate (200 mg/kg) for 6 weeks. At 12 weeks of age, the deoxycorticosterone acetate–treated rats had elevated systolic blood pressure compared with age-matched controls (157 ± 5.9 versus 124 ± 3.1 mm Hg deoxycorticosterone acetate versus control; \( P < 0.05 \)). The area of ischemic damage resulting from middle cerebral artery occlusion was greater in the deoxycorticosterone acetate–treated rats than control (63.5 ± 3.72 versus 46.6 ± 5.52% of the hemisphere infarcted, deoxycorticosterone acetate versus control; \( P < 0.05 \)). Middle cerebral artery structure was assessed using a pressurized arteriograph under calcium-free conditions. Over a range of intralumenal pressures, the lumen and ODs of the middle cerebral arteries were smaller in the deoxycorticosterone acetate–treated rats than the control rats (\( P < 0.05 \)). There was also an increase in the wall thickness and wall:lumen ratio in the vessels from deoxycorticosterone acetate–treated rats (\( P < 0.05 \)). The vessels from the deoxycorticosterone acetate–treated rats were stiffer than those from control rats as evidenced by a leftward shift in the stress/strain curve. These novel data suggest that mineralocorticoid receptor activation without salt loading and nephrectomy is sufficient to elicit deleterious effects on the cerebral vasculature that lead to inward hypertrophic remodeling and an increase in the ischemic damage in the event of a stroke. (Hypertension. 2006;47[part 2]:590-595.)

Key Words: mineralocorticoids | cerebral ischemia | remodeling | cerebral arteries | aldosterone

Stroke, in particular, ischemic stroke, is a leading cause of death and disability in the Western world. Yet, the factors that increase an individual’s risk of having a stroke or exacerbate the damage caused by a stroke are still not completely understood. Studies using stroke-prone spontaneously hypertensive rats (SHRSPs) suggest that cerebral vascular structure plays an important role in the pathogenesis of cerebral ischemia. SHRSPs have larger cerebral infarcts when ischemia is induced by middle cerebral artery (MCA) occlusion than their normotensive counterparts, the Wistar Kyoto rats.1,2 This difference appears to be because of remodeling of the cerebral vessels that results in the vessels having a smaller lumen diameter and an impaired ability to dilate in response to ischemia.3–5

It is becoming increasingly clear that aldosterone is involved in the pathophysiology of cardiovascular disease.6 We have shown previously that chronic treatment of SHRSPs with spironolactone, the aldosterone antagonist, reduces the ischemic cerebral infarct size after MCA occlusion.2 This protection from stroke appears to occur at the level of the vasculature, because spironolactone causes an increase in the lumen and ODs of the MCA from SHRSPs.7 This suggests that aldosterone or mineralocorticoid receptor (MR) activation is involved in the remodeling process in the cerebral vessels.

Studies of the systemic vasculature also suggest that MR activation affects vessel structure. The media/lumen ratio, a marker of vascular hypertrophy, is increased in coronary and mesenteric arteries from rats made hypertensive by the administration of aldosterone or deoxycorticosterone and salt.8,9 Similarly, the aorta undergoes hypertrophy in deoxycorticosterone–salt-hypertensive rats.10,11 In these studies, the animals were uninephrectomized and were given a high-salt diet. To the best of our knowledge, no studies have assessed the effects of MR activation in naive rats fed a normal-salt diet. We hypothesized that deoxycorticosterone acetate (DOCA) administration to Wistar rats would exacerbate the damage caused by cerebral ischemia and reduce the lumen diameter and increase the wall thickness of the MCA.

Received September 20, 2005; first decision October 12, 2005; revision accepted November 1, 2005.
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© 2006 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org DOI: 10.1161/01.HYP.0000196945.73586.0d

590
Methods

Animals
Male Wistar rats were obtained from Harlan (Indianapolis, IN). Rats were maintained on a 12-hour light/dark cycle, housed 2 per cage, and allowed access to normal chow and water ad libitum. At 6 weeks of age, rats were anesthetized with ketamine/xylazine (100/20 mg/kg IM), and a silastic pellet containing DOCA (200 mg/kg) was implanted subcutaneously in the nape of the neck. The pellet was replaced at 9 weeks of age to allow for the dose of DOCA to be adjusted for the increase in body weight. DOCA-treated rats were compared with age-matched control rats. Eighteen rats were treated with DOCA (9 for the induction of ischemia and 9 for the analysis of vessel structure), and 18 control rats were used. These studies were approved by the Institutional Animal Care and Use committee and were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals.

Measurement of Blood Pressure
Blood pressure was measured by tail cuff using a RTBP1001 rat-tail blood pressure system (Kent Scientific).

MCA Occlusion
Rats were anesthetized with isoflurane in oxygen and were allowed to ventilate spontaneously; body temperature was maintained at 37°C during the anesthesia. The MCA occlusion was carried out according to the technique of Longa et al.12 The common carotid artery was exposed by a midline incision, and the branches of the external carotid artery were cauterized. A 3-0 monofilament thread with a rounded tip was introduced into the carotid artery and advanced cranially into the internal carotid artery over a distance of 19 mm, measured from the bifurcation of the common carotid artery. The thread was left in place, and the rats were allowed to recover. Blood flow to the region surrounding the MCA was measured using a laser Doppler flow probe to confirm MCA occlusion. Postocclusion (24 hours), the rats were anesthetized and decapitated and the brains carefully removed. The area of the infarction was quantified using 2,3,5-triphenyltetrazolium staining as described previously.2 Two of the DOCA-treated rats died during the surgery to induce ischemia; thus, there were 9 control rats and 7 DOCA-treated rats.

MCA Structure
MCAs were isolated and placed in cold physiological salt solution (PSS; in mM: 141.9 NaCl, 4.7 KCl, 1.7 MgSO4, 0.5 EDTA, 2.8 CaCl2, 10.0 HEPES, 1.2 KH2PO4, and 5.0 glucose). The first branch-free segment of the MCA most proximal to the Circle of Willis was mounted on 2 glass micropipettes in a small vessel arteriograph (Living Systems Instrumentation). Vessels were bathed with warm oxygenated PSS, and the intraluminal pressure was set at 75 mm Hg before the vessels were allowed to equilibrate for 30 minutes. Vessels that did not hold pressure were discarded (vessels from 3 control rats were discarded for this reason). For the analysis of vessel structure, the vessels were bathed in calcium-free PSS containing 2 mmol/L EGTA, and the intralumenal pressure was increased from 0 to 180 mm Hg in 20-mm Hg increments. Videomicroscopy was used to measure lumen diameter, external diameter, and wall thickness at each pressure after a 5-minute equilibration. The wall/lumen ratio, circumferential wall stress, and wall strain were calculated using the method of Baumbach and Hajdu.13 The elastic modulus (β-coefficient) was calculated from the stress/strain curves for the individual vessels, and these curves were fitted to an exponential model (y = ae^βx) where β is the slope of the curve: the higher the β-coefficient the stiffer the vessel. The remodeling and growth indices were calculated for the vessels at an intraluminal pressure of 100 mm Hg using the formulas described by Heagerty et al.14 Blood was obtained by cardiac puncture at the time of euthanasia for the analysis of plasma potassium and sodium using the Synchron EL-ISE electrolyte system (Beckman Coulter).

Statistics
All of the results are represented as a mean ± SE. Cerebral vascular structure data were analyzed by 2-way repeated measures ANOVA with a Bonferroni post-test. Blood pressure, cerebral infarct size, plasma electrolytes, body weights, and β-coefficients were compared using the Student t test. A P value ≤0.05 was considered significant.

Chemicals and Supplies
All of the chemicals and supplies were purchased from Sigma Chemical Company (St. Louis, MO). Silastic was purchased from Dow Corning Corp (Midland, MI).

Results

Body Weights and Plasma Electrolytes
At 12 weeks of age, the DOCA-treated rats weighed significantly less than the control rats; however, there was no difference in the brain weight between the 2 groups. The heart weight and heart/body weight ratios were increased in the DOCA-treated rats compared with the control. There was no significant difference in the plasma sodium levels between the DOCA-treated and control rats. There was, however, a 20% reduction in the plasma potassium levels in the DOCA-treated group (Table). DOCA treatment caused a significant increase in the systolic blood pressure after 6 weeks of treatment (Figure 1; n = 8 for the DOCA-treated and 6 for the control; P < 0.05).

Cerebral Infarct Size
To investigate the effect of DOCA treatment on the outcome of cerebral ischemia, the MCA was occluded, and the infarct size was measured after 24 hours. Treatment of Wistar rats with DOCA caused a marked increase in the percentage of the hemisphere damaged by ischemia (Figure 2; n = 7 for the DOCA-treated and 9 for the control group; P < 0.05). The infarct was limited to the cortex and basal ganglia. Blood flow to the area supplied by the MCA was assessed by laser Doppler to ensure that the MCA was similarly occluded in both groups of rats. The percentage drop in blood flow at the time of occlusion was the same in both groups (52.4 ± 4.58% for the DOCA-treated and 51.9 ± 3.35% for the control group).

MCA Structure
Vascular structure was analyzed using a small vessel arteriograph under zero-calcium and zero-flow conditions. The MCAs from the DOCA-treated rats showed a marked inward remodeling as evidenced by a reduction in both the IDs and ODs of the vessel over a range of intraluminal pressures from...
40 to 180 mm Hg (Figure 3; n=9 for the DOCA-treated and 6 for the control; * indicates a significant difference from control). This was confirmed by calculating the remodeling index for the DOCA-treated rats. At an intraluminal pressure of 100 mm Hg, the remodeling index was 90.9%. The vessel wall thickness and wall/lumen ratio were both increased in the DOCA-treated rats compared with control (Figure 4; n=9 for the DOCA-treated and 6 for the control; * indicates a significant difference from control). Similarly, the growth index for the DOCA-treated rats at an intraluminal pressure of 100 mm Hg was 8.03% indicating hypertrophy of the vessel wall.

To assess the compliance of the MCA circumferencial wall, stress and strain were calculated and plotted against each other. There was a leftward shift in the stress/strain curve for the DOCA-treated rats, indicating a reduction in vessel compliance (Figure 5A; n=9 for the DOCA-treated and 6 for the control; * indicates a significant difference from control). The $\beta$-coefficient, a marker of vessel stiffness, was increased in the DOCA-treated rats compared with control (Figure 5B; n=9 for the DOCA-treated and 6 for the control; * indicates a significant difference from control).

**Discussion**

There were 2 major findings of this study. First, MR activation caused an increase in the amount of damage caused by cerebral ischemia in Wistar rats. Second, MR activation in the absence of dietary sodium supplementation was sufficient to cause remodeling of the cerebral vasculature; this appears to be a hypertrophic inward remodeling, which also results in increased vessel stiffness.

Most previous studies of MR-dependent hypertension have used a model that combines a high-salt diet with a unilateral nephrectomy and mineralocorticoid treatment. This treatment regime results in a malignant form of hypertension. In this study, we observed an $\sim$40-mm Hg increase in blood pressure in the Wistar rats treated with DOCA, whereas an $\sim$60-mm Hg rise in pressure can be expected in Wistar rats that have undergone a unilateral nephrectomy and have been administered both DOCA and salt.15

DOCA treatment increased the damage caused by permanent focal cerebral ischemia. Laser Doppler blood flow measurements were used to confirm that a complete occlusion of the MCA had taken place. The similarity in the percentage drop in blood flow between the control and treated groups suggests that the difference in the cerebral infarct size cannot be accounted for by a difference in the effectiveness of the MCA occlusion. Therefore, the exacerbation of the ischemic damage in the DOCA-treated rats must relate to a change in the ability of the rats to respond to an ischemic insult. Whereas our results suggest that a change in vessel structure is responsible for the increase in the cerebral infarct size, it should be noted that other variables, such as PO2 and PCO2, can also affect the outcome of ischemia, although these variables were not measured here. We have shown previously that the ischemic cerebral infarct size in SHRSPs is reduced by spironolactone, suggesting that MR activation is involved in the pathogenesis of cerebral ischemia. There are also several studies in humans showing a link between elevated plasma aldosterone and the risk of stroke.16,17 Interestingly, a comparison of patients with essential hypertension and primary hyperaldosteronism suggests that the increased stroke
risk in patients with primary hyperaldosteronism is blood pressure independent. The blood pressure dependency of the response observed here remains unclear, and additional studies need to be carried out to evaluate this.

There are many factors that affect the outcome of cerebral ischemia, including vascular remodeling and an impairment of the ability of vessels to dilate in response to ischemia. In this study, a hypertrophic inward remodeling was observed, along with an increase in vessel wall stiffness in the DOCA-treated rats. This presents the possibility that there is both increased smooth muscle proliferation and extracellular matrix deposition in the cerebral vasculature of the DOCA-treated rats. There is evidence in the literature that vascular remodeling, in the form of hypertrophy, occurs in the peripheral vasculature in the DOCA–salt model of hypertension. There are few studies assessing the effects of DOCA–salt hypertension on the cerebral vasculature. However, by comparing the results obtained for peripheral resistance vessels with the results presented here, it appears that the extent of the changes in cerebral artery structure in our study are not as marked as those described previously in the DOCA–salt model. For instance, Li et al reported a 21% reduction in the lumen diameter of mesenteric arteries from DOCA–salt hypertensive rats compared with the 15% reduction we observed in the MCA. It is worth noting, however, that the DOCA–salt rats had a significantly higher blood pressure than the rats used in our study, and this may contribute to the differences in the results. It is also possible that the different vascular beds respond to DOCA administration in different ways.

It is possible that the hypertrophy of the MCA is associated with an increase in epidermal growth factor (EGF) signaling. EGF is a smooth-muscle mitogen that has been implicated previously in hypertensive vascular remodeling. We have shown that the expression of the EGF receptor (EGFR) mRNA is aldosterone sensitive, in vivo it is reduced by spironolactone, and in vitro it can be increased by aldoste-
Interestingly, studies suggest that mineralocorticoids stimulate vascular smooth muscle cell proliferation via rapid nongenomic actions. Aldosterone activates the EGFR in several cell types and acts synergistically with angiotensin II to increase vascular smooth muscle cell proliferation by activation of EGFR. Other tyrosine kinases have also been implicated in the rapid response to aldosterone including Big mitogen-activated protein kinase and c-Src and p-38 mitogen-activated protein kinase.

We also observed a reduction in the compliance of the MCAs from DOCA-treated rats; altered collagen turnover or fibrosis could be responsible for this. There is ample evidence in the literature to suggest that aldosterone can increase collagen deposition in the kidneys, heart, and aorta. Similarly, aldosterone antagonism reduces vascular collagen deposition in young hypertensive rats and in older normotensive rats. If aldosterone also increases collagen levels in the cerebral vasculature, then this would explain the reduction in the compliance of the vessels from the DOCA-treated rats.

In this study, DOCA treatment resulted in a reduction in the plasma potassium levels; whereas this was not an unexpected finding, it may have affected the results obtained. Many previous studies have suggested that dietary potassium supplementation is cardioprotective, and it reverses the remodeling of the aorta and mesenteric and carotid arteries from hypertensive rats. Thus, it is possible that the reduction in plasma potassium observed here had the opposite effect. Another limitation of this study is that we only measured vessel structure, and we did not assess whether DOCA treatment causes rarefaction of the cerebral vessels. The literature concerning the effects of DOCA–salt hypertension on vessel number is conflicting. One study suggests that there is no change in vessel number, whereas others have suggested that the third-, fourth-, and fifth-order pial arteries are less dense and smaller in DOCA–salt-treated rats. Rarefaction of the pial arteries could also explain the increase in the ischemic cerebral infract size in the DOCA-treated rats. It should also be noted that, in these studies, the DOCA treatment began before puberty; thus, it is not possible to rule out effects of DOCA on other hormones and vasomodulators during puberty. It also should be noted that, at this point, we are unable to determine the location of the MR responsible for the effects presented here. It is possible that the response is purely because of the elevation in blood pressure; it is also possible, however, that these are direct effects of MR activation at the level of the vascular smooth muscle.

Perspectives
Since the publication of the Randomized ALdactone Evaluation Study (RALES) and EPlerenone neuroHormonal Efficacy and SUrival Study (EPHESUS) trials, it has become increasingly evident that aldosterone or MR activation has deleterious extrarenal actions. It is clear from this study that MR activation exacerbates the damage caused by cerebral ischemia and causes remodeling of the cerebral vasculature in a manner that would impede cerebral blood flow. Until recently, aldosterone was thought to play only a minor role in human hypertension. It is now clear that ≥10% of hypertensive subjects have elevated aldosterone levels and that aldosterone levels at the high end of the normal range may predispose patients to hypertension. The current study suggests that these individuals are at an increased risk for ischemic stroke and highlights the potential therapeutic benefits for MR antagonism in patients at risk for stroke.

Acknowledgments
This work was supported by funds from the National Institutes of Health (HL077385).

References


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Hypertension. 2006;47:590-595; originally published online December 19, 2005;
doi: 10.1161/01.HYP.0000196945.73586.0d

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