Effects of Indapamide, a Thiazide-Like Diuretic, on Structure of Cerebral Arterioles in Hypertensive Rats

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Abstract—We examined the effects of indapamide, a thiazide-like diuretic, on cerebral arterioles in spontaneously hypertensive rats (SHR). The structure and mechanics of cerebral arterioles were examined in untreated Wistar Kyoto rats (WKY) and SHR that were untreated or treated for 3 months with a low (1 mg/kg per day) or a high (10 mg/kg per day) dose of indapamide. We measured pressure, diameter, and cross-sectional area of the vessel wall (CSA) in maximally-dilated (EDTA) cerebral arterioles. Treatment of SHR with the high dose of indapamide normalized cerebral arteriolar mean pressure (62±4 [mean±SEM] versus 59±3 mm Hg in WKY and 88±6 mm Hg in untreated SHR; P<0.05), pulse pressure (13±1 versus 10±1 mm Hg in WKY and 20±1 mm Hg in untreated SHR; P<0.05), and CSA (1080±91 versus 1100±48 μm² in WKY and 1439±40 μm² in untreated SHR; P<0.05). In contrast, treatment of SHR with the low dose of indapamide did not normalize arteriolar mean (72±3) and pulse pressure (20±1 mm Hg), but did normalize CSA (1091±52 μm²). Treatment with either dose of indapamide failed to increase external diameter in cerebral arterioles of SHR (89±4 and 92±4 μm, respectively, versus 103±6 μm in WKY and 87±4 μm in untreated SHR). Finally, treatment with indapamide attenuated the rightward shift of the stress-strain curve in SHR, suggesting that treatment with indapamide attenuated increases in distensibility of cerebral arterioles in SHR. These findings suggest that, whereas thiazide-like diuretics may not attenuate eutrophic inward remodeling of cerebral arterioles in SHR, they may attenuate hypertrophic inward remodeling via a mechanism unrelated to their pressor effects. (Hypertension. 2004;43:1092-1097.)

Key Words: hypertension ■ diuretic ■ hypertrophy ■ remodeling

Chronic hypertension alters structure and mechanics of cerebral arterioles. Cerebral arterioles in spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP) undergo both eutrophic inward remodeling (an increase in the wall thickness-to-lumen ratio that cannot be attributed to a change in the amount of wall material or wall stiffness) and hypertrophic inward remodeling (an increase in the wall thickness-to-lumen ratio that can be attributed directly to an increase in wall material).1,2 Despite hypertrophy of the vessel wall, cerebral arterioles undergo a paradoxical increase in passive distensibility during chronic hypertension in SHR and SHRSP.2,3 Because the increase in wall thickness-to-lumen ratio of cerebral arterioles likely plays an important role in impaired cerebral vascular function during chronic hypertension, it is important to understand the effects of antihypertensive treatment on cerebral vascular structure and mechanics.

We found previously that treatment with angiotensin-converting enzyme (ACE) inhibitors (perindopril and cilazapril) prevents both eutrophic and hypertrophic inward remodeling of cerebral arterioles in SHRSP.4,5 Furthermore, eutrophic, but not hypertrophic inward remodeling, was attenuated by a low dose of perindopril that had minimal effects on arterial pressure in SHRSP.4 In contrast, we have shown that whereas both a β-blocker (propranolol) and a vasodilator (hydralazine) prevent hypertrophic inward remodeling of cerebral arterioles in SHRSP, probably by their antihypertensive effects,4,5 neither treatment prevents eutrophic inward remodeling.

Assuming that alterations in cerebral vascular structure contribute to the increased incidence of lacunar or ischemic stroke associated with hypertension,6 the above findings suggest that ACE inhibitors might be more effective than other forms of antihypertensive treatment in preventing strokes during treatment of hypertension. This supposition, however, appears to be contradicted by the finding that the thiazide-like diuretic indapamide prevents cerebral infarcts and hemorrhages in SHRSP fed a high-sodium diet as effectively as the ACE inhibitor delapril.7 The goal of this study, therefore, was to examine effects of treatment with indapamide on structure and mechanics of cerebral arterioles in SHR. To determine whether effects of indapamide on cerebral arterioles are pressure-dependent, we used a high dose to normalize arterial pressure in SHR relative to normo-
tensive Wistar-Kyoto rats (WKY), and a low dose to minimize reductions in arterial pressure in SHR.

Materials and Methods
Experiments were conducted on male WKY rats and SHR (Harlan Laboratories, Indianapolis, Ind). At 4 weeks of age, SHR were divided into 3 groups: an untreated group (n=13), a group treated with a low dose of indapamide (1 mg/kg per day; Inda-L; n=14), and a group treated with a high dose of indapamide (10 mg/kg; Inda-H; n=15). A group of WKY served as normotensive controls (n=14). The drug was administered in the food.

After about 3 months of treatment, we examined the mechanics of cerebral arterioles in 4-month old animals using a surgery similar to the one previously described. Procedures followed in this study were in accordance with the institutional guidelines as set forth by the University of Iowa.

Measurement of Cerebral Arteriolar Pressure and Diameter
Pressure and diameter were measured in first order arterioles on the surface of the cerebrum after making a craniotomy over the left parietal cortex as previously described. Pressure was measured continuously in cerebral arterioles with a micropipette connected to a servo-null pressure measuring device (model 5, Instrumentation for Physiology and Medicine, Inc, San Diego, Calif). Arterioles were monitored through a Leitz microscope (NPI objective) connected to a closed-circuit video system with a final magnification of ×356. Arteriolar diameter was measured from digitized images (video frame grabber, Quick Image 24, MASS Microsystems, Sunnyvale, Calif) by the use of image analysis software (NIH Image, National Institutes of Health, Research Services Branch, NIMH).

The precision of this system is 0.4 to 0.6 μm.

Experimental Protocol
After measurements under baseline conditions, pressure-diameter relationships were obtained in deactivated cerebral arterioles (EDTA, 67 mMol/L) using hemorrhage to reduce arterial pressure in decrements of 10 mm Hg between cerebral arteriolar pressures of 70 and 10 mm Hg. Maximally dilated arterioles were fixed at physiological pressure in vivo by perfusion of vessels with glutaraldehyde fixative (2.25% glutaraldehyde in 0.10 mol/L cacodylate buffer) while maintaining cerebral arteriolar pressure at baseline levels. The arteriolar segment used for pressure-diameter measurements was processed for electron microscopy and embedded in Spurr’s low viscosity resin while cross-sectional orientation was maintained. Cross-sectional area of the vessel wall (CSA) was determined histologically from 1-μm sections using a light microscope interfaced with the video image analyzing system described above.

Calculation of Mechanical Characteristics
Circumferential stress, circumferential strain, tangential elastic modulus, wall thickness, wall thickness-to-lumen ratio, and external diameter were calculated as described in detail previously. We also calculated the remodeling index and the growth index as previously defined.

Statistical Analysis
Analysis of variance was used to compare systemic mean pressure, arteriolar pressures, diameters, CSA, and slope of tangential elastic modulus versus stress. Probability values were calculated using a Student’s t test.

Results
Baseline Values
Systemic mean arterial pressure and cerebral arteriolar mean and pulse pressures were significantly greater in untreated SHR than in WKY (Table). The low dose of indapamide reduced systemic arterial mean pressure and cerebral arteriolar mean pressure in SHR to a level about halfway between untreated SHR and WKY. The low dose of indapamide had virtually no effect on cerebral arteriolar pulse pressure in SHR (Table). The high dose of indapamide reduced systemic arterial mean and cerebral arteriolar mean and pulse pressures in SHR to levels that were comparable to those in WKY (Table).

Before deactivation of cerebral arterioles with EDTA, the diameter of cerebral arterioles was significantly less in untreated SHR than in WKY (Table). Treatment of SHR with both doses of indapamide significantly increased cerebral arteriolar diameter (Table). Diameters remained substantially less, however, in both groups of SHR treated with indapamide than in untreated WKY (Table).

After deactivation of cerebral arterioles with EDTA, internal and external diameters of cerebral arterioles were significantly smaller in untreated SHR than in WKY (Table). Values of the remodeling index suggest that about 80% of the reduction in internal diameter in untreated SHR was because of inward eutrophic remodeling (Table). Internal and external diameters were not significantly greater in SHR treated with either the low or high dose of indapamide than in untreated SHR (Table). These findings suggest that indapamide does not significantly attenuate reductions in internal diameter in SHR. Furthermore, values of remodeling index suggest that the reductions in internal diameter in treated SHR were due entirely to eutrophic inward remodeling.

CSA, wall thickness, and wall-thickness-to-lumen ratio were significantly greater in cerebral arterioles in untreated SHR than in WKY (Table). The growth index (defined as the percentage change in wall CSA) indicates that wall CSA increased by about 30% in SHR compared with WKY (Table). This finding suggests that the 20% of reduction in internal diameter not accounted for by eutrophic remodeling was the result of inward hypertrophic remodeling. Both the high and low doses of indapamide significantly reduced CSA, wall thickness, and wall thickness-to-lumen ratio in SHR (Table). Effects of treatment on the growth index indicate that the low, as well as the high, doses of indapamide prevented hypertrophy of cerebral arterioles in SHR (Table).

Vascular Mechanics
After maximal dilatation of cerebral arterioles with EDTA, internal and external diameters were significantly less in SHR than in WKY at all levels of cerebral arteriolar pressure between 70 and 10 mm Hg (Figure 1). Neither the low or high doses of indapamide significantly increased internal or external diameters of cerebral arterioles in SHR at any level of arteriolar pressure (Figure 1).

The stress-strain curve in cerebral arterioles of untreated SHR was shifted to the right of the curve in WKY (Figure 2), and the slope of tangential elastic modulus versus stress was less in untreated SHR than in untreated WKY (Table). Thus, passive distensibility was increased in cerebral arterioles of SHR, despite hypertrophy of the vessel wall. Treatment of SHR with both the low and high dose of indapamide attenuated the rightward shift of the stress-strain curve (Figure 2) and the decrease in the slope of tangential elastic modulus.
modulus versus stress (Table). These findings suggest that indapamide may attenuate increases in distensibility of cerebral arterioles in SHR.

**Discussion**

There were two major findings in this study. First, whereas both the high and low doses of indapamide prevented hypertrophic inward remodeling of cerebral arterioles in SHR, only the high dose of indapamide normalized cerebral arteriolar mean and pulse pressures relative to normotensive WKY. These findings suggest that effects of treatment with indapamide on cerebral arteriolar hypertrophic inward remodeling occur, at least in part, independently of the pressor effects of indapamide. Second, neither the high dose nor the low dose of indapamide attenuated eutrophic inward remodeling of cerebral arterioles in SHR, even though the high dose completely normalized cerebral arteriolar mean and pulse pressures. These findings suggest that indapamide does not attenuate cerebral arteriolar eutrophic inward remodeling in SHR. The findings also confirm previous observations\(^2\)\(^-\)\(^4\) that increases in arterial pressure are not an important determinant of cerebral arteriolar eutrophic inward remodeling during chronic hypertension. On the basis of previous observations that indapamide influences microvascular density in the coronary circulation in hypertensive rats,\(^1\) one might predict that indapamide alters cerebral microvascular density in SHR. We cannot rule out this possibility because we did not evaluate vascular density in this study.

**Hypertrophic Inward Remodeling**

Cerebral arterioles undergo hypertrophic inward remodeling in several models of experimental hypertension including SHR, SHRSP, renal hypertension, and \(N^\text{-nitro-L-arginine methylester (L-NAME)-induced hypertension. In contrast, hypertension in SHR apparently is not associated with hypertrophy in mesenteric small resistance arteries.\(^1\) One possible explanation for this apparent discrepancy is that mesenteric arterioles may not respond to the factors that contribute to development of cerebral vascular hypertrophy during chronic hypertension. Determinants that may contribute to vascular hypertrophy during chronic hypertension include increases in pressure,\(^1\)
Eutrophic Inward Remodeling

During chronic hypertension in SHRSP and SHR, cerebral arterioles undergo eutrophic inward remodeling of the vessel wall with a reduction in external diameter. 1,2 Determinants of vascular eutrophic inward remodeling of cerebral arterioles in SHR and SHRSP may include genetic factors 2,20 and the renin-angiotensin system 4,5 but not increases in pressure. Results obtained in the present experiment confirm this observation as both the high and low dose of indapamide had no effect on vascular eutrophic inward remodeling, despite the fact that the high dose of indapamide normalized blood pressure in SHR compared with WKY. However, treatment with indapamide, especially in higher doses, has been re-

Figure 2. Stress-strain relationships in arterioles on the cerebrum in untreated WKY (filled circle, n = 14) and SHR that were untreated (filled square, n = 13) or treated with the high dose (Inda-H, open triangle, n = 14) or low dose (Inda-L, open square, n = 15) of indapamide during maximal dilatation with EDTA. Values are mean ± SEM. DI indicates cerebral arteriolar diameter; DIo, original cerebral arteriolar diameter.

neurohumoral factors, 13-16 genetic factors, 17 and endothelial factors such as endothelin 18,19 and nitric oxide (NO). 20 Of these various determinants, we 21 and others 22 have suggested previously that arterial pulse pressure may be an important determinant of vascular hypertrophic inward remodeling. We have to remain cautious, however, as a previous report suggests that pulse pressure is not an important determinant of small artery structure in human essential hypertension. 23

In support of an important role for pulse pressure in the development of hypertrophic inward remodeling, we found previously that treatment of SHRSP with the vasodilator, hydralazine, in a dose that normalized cerebral arteriolar pressure, prevented hypertrophic inward remodeling of cerebral arterioles, even though arteriolar mean pressure remained significantly elevated. 5 Thus, we were surprised to find in this study that both the low and the high dose of indapamide prevented cerebral arteriolar hypertrophic inward remodeling in SHR, despite the failure of the low dose to normalize arteriolar pulse pressure. On the other hand, it has been reported that even a moderate decrease in arterial pressure with indapamide is more effective than the thiazide diuretic, hydrochlorothiazide, in reducing myocyte hypertrophy in SHRSP. 24 Furthermore, it has also been shown that indapamide is more effective than the ACE inhibitor, enalapril, in reducing left ventricular hypertrophy in hypertensive patients. 25 These findings, therefore, suggest that indapamide may be more effective than vasodilators, ACE inhibitors, and other thiazide diuretics in preventing vascular hypertrophic inward remodeling. In addition, our findings in this study suggest that the effectiveness of indapamide in preventing arteriolar hypertrophic inward remodeling may depend on other determinants of hypertrophy than increases in arterial pressure, and in particular, pulse pressure. It should also be noted, however, that the ability of indapamide to prevent hypertrophic inward remodeling may vary from one vascular bed to another. For example, indapamide apparently does not prevent medial hypertrophy of small coronary arteries in SHRSP. 24 Furthermore, a study performed on subcutaneous arteries of hypertensive patients treated with a thiazide diuretic or a calcium channel blocker showed that the thiazide diuretic, in contrast to the calcium channel blocker, did not have a significant effect on peripheral resistance artery. 26

Prevention of cerebral arteriolar hypertrophy in SHR by indapamide may be mediated by NO. This possibility is based on the following observations. First, indapamide has been show to improve endothelium-dependent relaxation, an effect that may be mediated by an increase in NO production. 27 Second, it has been shown previously that NO inhibits the proliferation of rat vascular smooth muscle cells in vitro. 28 Finally, we have reported that reductions in available NO induced by nitric oxide synthase inhibition results in hypertrophy of cerebral arterioles independently of increases in cerebral arterioal pressure. 20 Thus, indapamide may prevent hypertrophy of cerebral arterioles in SHR by increasing availability of NO, thereby inhibiting growth of smooth muscle in cerebral arterioles.

Another mechanism by which indapamide may prevent cerebral vascular hypertrophy relates to its effects on sympathetic nerve activity. Indapamide has been shown to reduce sympathetic tone. 29 Activity of sympathetic nerves may be an important determinant of cerebral vascular growth. We have found, for example, that ablation of the superior cervical sympathetic ganglion prevents hypertrophy of cerebral arterioles of SHRSP and reduces CSA in cerebral arterioles of WKY. 13 These findings suggest that indapamide may prevent cerebral arteriolar hypertrophy in SHR by reducing activity of the sympathetic nervous system.

**Figure 1.** Pressure-internal diameter (left panel) and pressure-external diameter (right panel) relationships in arterioles on the cerebrum in untreated WKY (filled circle, n = 14) and SHR that were untreated (filled square, n = 13) or treated with the high dose (Inda-H, open triangle, n = 14) or low dose (Inda-L, open square, n = 15) of indapamide during maximal dilatation with EDTA. Values are mean ± SEM. *P < 0.05 vs WKY.
ported to activate the renin-angiotensin system. Thus, we cannot rule out the possibility that any attenuation of eutrophic inward remodeling of cerebral arterioles that might otherwise have occurred during treatment of SHR with indapamide could have been counteracted by activation of the renin-angiotensin system.

Finally, our findings confirm previous observations that eutrophic inward remodeling may play a larger role than hypertrophic inward remodeling in impairment of maximal dilator capacity of cerebral arterioles in SHR and SHRSP. This statement is based on the observation that the internal diameter of maximally-dilated cerebral arterioles was not significantly greater in indapamide-treated SHR than in untreated SHR, despite prevention of cerebral arteriolar hypertrophic inward remodeling in the treated group. If hypertrophic inward remodeling played a larger role than eutrophic inward remodeling in impaired maximal dilatation of cerebral arterioles, we would have expected a greater restoration of maximal dilator capacity in treated SHR.

Distensibility

Distensibility of fully relaxed cerebral arterioles is increased in SHRSP, SHR, and rats with 1-kidney and 1-clip renal hypertension, despite hypertrophy of the arteriolar wall. Furthermore, prevention of hypertrophy in cerebral arterioles of SHRSP by treatment with an ACE inhibitor or carotid clipping significantly attenuates increases in arteriolar distensibility. We were not surprised, therefore, by the finding in this study that, in association with prevention of hypertrophy, treatment with indapamide also attenuated increases in cerebral arteriolar distensibility.

We have previously proposed that increases in passive distensibility that accompany hypertrophy of cerebral arterioles may be because of a reduction in the proportion of stiff (collagen and basement membrane) to compliant (smooth muscle, elastin and endothelium) components of the arteriolar wall in cerebral arterioles. Therefore, a possible explanation for the finding in this study that treatment of SHR with indapamide attenuates cerebral arteriolar distensibility is that indapamide alters proportional composition of cerebral arterioles relative to untreated SHR. An alternative explanation is that indapamide improves distensibility of cerebral arterioles in SHR by reducing intracellular calcium and phosphate ions levels that may be involved in arterial rigidity.

Perspectives

A key finding in this study was that treatment with indapamide prevented hypertrophic inward remodeling of cerebral arterioles in SHR, even when administered in a dose that was minimally effective in reducing arterial pressure. This finding provides additional re-enforcement for the concept that effects of treatment on structure do not necessarily correlate with, and thus cannot be predicted by, the degree of reduction in arterial pressure. This concept is further buttressed by the previous findings that some forms of antihypertensive treatment that otherwise appear to normalize pressure, may not prevent, or reverse, arterial hypertrophic inward remodeling. Presumably, normalization of arterial structure is an important goal of antihypertensive treatment. Therefore, an implication that follows from the outcomes of this study and others is that establishing the efficacy of new antihypertensive agents with respect to their ability to normalize vascular structure will continue to be an important goal.

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