Original Article

Effects of Efonidipine Hydrochloride on Renal Arteriolar Diameters in Spontaneously Hypertensive Rats

Masuhisa NAKAMURA, Mitsuru NOTOYA *, Yuka KOHDA, Junji YAMASHITA, Yuko TAKASHITA, and Munekazu GEMBA

Efonidipine, a calcium antagonist, has been reported to dilate not only afferent but also efferent arterioles, thereby reducing glomerular hydrostatic pressure. We investigated the effect of chronic treatment with efonidipine or lisinopril on the afferent and efferent arteriolar diameters by the vascular cast technique. Four-week-old spontaneously hypertensive rats (SHR) were divided into three groups: untreated, efonidipine (25 mg/kg/day)-treated, and lisinopril (3 mg/kg/day)-treated. At 22 weeks of age, the renal vasculatures were fixed at the maximally dilated condition. The morphometrical measurements showed that the treatments with efonidipine and lisinopril caused structural alteration of the vasculature, resulting in significantly greater efferent arteriolar diameters than in untreated SHR. In addition, lisinopril-treated rats had wider afferent lumina. The renoprotective effect of efonidipine and lisinopril might be partly due to the structurally larger efferent arteriolar lumen. (Hypertens Res 2002; 25: 751–755)

Key Words: efonidipine, efferent arteriole, vascular cast

Introduction

Blood pressure control is the most important component in the management of patients with hypertension and renal disease. Hypertension is a significant and independent predictor of end-stage renal diseases. In addition, attention has been focused on the importance of therapeutic interventions, which may be renoprotective (1, 2). Clinical studies have established that angiotensin-converting enzyme (ACE) inhibitors have beneficial effects on slowing the progression of renal damage. The results of experimental studies have indicated that ACE inhibitors exert their protective effect on the kidney through a decrease in efferent arteriolar resistance, and hence, through a reduction in glomerular hydrostatic pressure (3).

Calcium antagonists also are highly effective antihypertensive agents, although their renoprotective effects have not been studied as intensively as those of ACE inhibitors. Most of the calcium antagonists preferentially dilate afferent arterioles, which could elevate the glomerular pressure, and could be detrimental to renal function in the long term (4). Recent studies on renal hemodynamics have shown that the calcium antagonists efonidipine, nilvadipine, manidipine and aranidipine dilate efferent as well as afferent arterioles (5–11), and thereby reduce glomerular hydrostatic pressure. Therefore, these calcium antagonists may have a more pronounced renoprotective effect than others. And indeed, some of these antagonists have been shown to retard the progression of chronic renal failure in experimental settings (12–16).

The aim of the present study was to investigate the chronic effects of efonidipine on afferent and efferent arteriolar diameters. In vivo arteriolar diameter is determined by the constriction/relaxation state of the vessel and by chronic alteration of the structure through vascular remodeling. Because accumulating evidence has shown that a structurally altered diameter is important in renal hemodynamics (17–19), we chose to investigate the structurally defined arteriolar diameters.
ter specifically. To accomplish this, we fixed the renal vasculature at a maximally relaxed state, and then measured the arteriolar diameters.

Methods

Animals

Male spontaneously hypertensive rats (SHR) were obtained at 3 weeks of age from Charles River Breeding Laboratories (Hino, Shiga, Japan). The rats were maintained on normal rat chow (CA-1; CLEA Japan, Inc., Tokyo, Japan) and given water ad libitum.

Rats were divided into three groups: an SHR (control) group given vehicle only (n = 5); an SHR + LIS group given lisinopril (3 mg/kg/day, n = 5); and an SHR + EFO group given efonidipine hydrochloride (25 mg/kg/day, n = 5). The drugs or vehicle (0.5% methylcellulose solution, 2.0 ml/kg) was given orally once a day through a gavage at 6:00 PM, from age 4 weeks to 22 weeks. The dosages of lisinopril and efonidipine were chosen according to our preliminary studies and the literature (12), respectively, so that an increment of systolic blood pressure in SHR could be prevented. The body weight of the rats was measured biweekly. Systolic blood pressure was measured by the indirect tail-cuff compression method in conscious animals. All procedures were in accordance with our institutional guidelines.

Vascular Cast Study

At 22 weeks of age, with rats under Nembutal anesthesia (50 mg/kg IP; Dainippon Pharmaceutical, Osaka, Japan), a polyethylene catheter (PE-90) was inserted in a retrograde manner into the abdominal aorta. The ligature around the aorta proximal to the origin of the right renal artery was tied. Then, both kidneys were perfused with a buffered solution of the following composition: 112 mmol/l NaCl, 5.0 mmol/l KCl, 1.0 mmol/l NaH_{2}PO_4, 1.2 mmol/l MgSO_4, 25 mmol/l NaHCO_3, 11.2 mmol/l glucose, 0.1 mmol/l sodium nitroprusside, 15 g/l dextran, and 1,000 U/l heparin. The vena cava was opened by a small incision to allow escape of blood and perfusate. The flow rate of the perfusate was set at 2.0 ml/min. Notoya et al. (18) have suggested that the renal vasculature is maximally dilated in this perfusion condition.

After 10 min of perfusion, the perfusate was switched to the fixative (2.0% (para)formaldehyde, 0.5% glutaraldehyde, 75 mmol/l phosphate buffer, pH 7.2). In our routine experiments, under light and electron microscopic observation, shrinkage or swelling of cells in the renal vasculatures was minimal. After 10 min of perfusion with the fixative, the left renal artery was ligated. Then, acryl resin (Mercox, Daininpon Ink and Chemicals, Tokyo, Japan) was infused to make a cast of the vascular system in the right kidney.

After the cast had cured sufficiently, the renal tissue of the right kidney was digested and removed in 20% sodium hydroxide at approximately 50˚C. Digestion was done several times until renal tissue was completely removed. The cast was then rinsed several times in distilled water, air-dried, and dissected and mounted on stubs. The sample was coated with gold palladium with an ion sputter coater (SC500A; Emscope, Ashford, England) and examined with a scanning electron microscope (S-800; Hitachi, Tokyo, Japan).

Arteriole diameters were measured on the photographic prints taken at ³ 350. In both afferent and efferent arterioles, diameters were measured at three points ³ 30, 40, and 50 µm from the glomerular vascular pole ³ and averaged. Only the vessels of glomeruli in the outer cortex were examined. Identification of each arteriole was based on the finding that the afferent arterioles branched from the interlobular artery, whereas the efferent arterioles branched to the peritubular capillary network.

Statistics

Results are expressed as the mean ³ SE. Statistical analysis was performed with one-way analysis of variance (ANOVA) and Dunnett’s multiple comparison test. Values of p ³ 0.05 were considered to indicate statistical significance.

Results

Body Weight and Systolic Blood Pressure

Throughout the experimental period, the body weights of the three groups were similar (Fig. 1). At 4 weeks of age, systolic blood pressure (SBP) was comparable among the three groups (Table 1). At the end of the study, the SHR + LIS and
SHR + EFO groups showed a significant reduction in SBP compared with untreated SHR. Heart rate was not significantly different among the three groups.

Renal Arteriolar Diameters

Figure 2 presents the vascular casts of glomeruli with the afferent and efferent arterioles from the outer cortex, and Fig. 3 shows the arteriolar diameters measured from the vascular casts. The diameter of afferent arterioles in the SHR + LIS group was markedly and significantly larger than that of the untreated SHR group (23.2 ± 1.1 µm in SHR + LIS vs. 19.3 ± 0.8 µm in SHR, p < 0.05). In the SHR + EFO group, the afferent arteriolar diameters (18.4 ± 0.7 µm) were similar to those in the untreated SHR group. The efferent arteriolar diameters were significantly larger in the SHR + LIS and...
SHR + EFO groups than in the untreated SHR group (12.5 ± 0.6 μm in SHR vs. 18.8 ± 1.1 μm in SHR + LIS, p < 0.01; 16.2 ± 0.5 μm in SHR + EFO, p < 0.05).

Discussion

The vascular casting technique is a useful method for the measurement of renal arteriolar diameters. Using this technique, identification of afferent and efferent arterioles can be easily achieved under a scanning electron microscope. Gattone et al. (20) and Kimura et al. (21) used this method to conduct morphometric studies on the renal arterioles in SHR. They attempted to fix the arterioles at the functional constriction/relaxation state, by maintaining a perfusion pressure equal to the blood pressure of the animals. Their vessel diameter values may therefore have reflected both functional and structural factors. In this study, on the other hand, we fixed the vasculature at the maximally relaxed condition by perfusing Ca²⁺-free buffer with 0.1 mmol/l nitroprusside at low perfusion pressure. It was thus possible to eliminate the influence of the functional state when determining the vascular diameters (18).

The major finding in this study is that long-term treatment with lisinopril or efonidipine caused structurally larger efferent arteriolar lumina. Because a wider efferent arteriolar diameter leads to a reduction in glomerular pressure, this effect may be a favorable one in regard to renoprotection. And because vessel diameter is determined by structural and functional factors, structural widening of efferent arterioles might lead to a persistent effect on the renal hemodynamics. Previous studies have shown that ACE inhibitors affect the structure of renal vasculatures by decreasing renal vascular resistance (18, 19), and that they have a persistent effect on blood pressure after treatment withdrawal (19, 22). Therefore, it would be of interest to study the relationship between the effect of efonidipine on renal hemodynamics and arteriolar structure after treatment withdrawal.

Functionally, it has been demonstrated that efonidipine inhibits both afferent and efferent vasoconstriction induced by angiotensin II (5–7). However, the mechanism by which efonidipine inhibits efferent vasoconstriction remains undetermined. Because the vasodilatory effect on the efferent arterioles varies among the calcium antagonists (e.g., efonidipine, manidipine > nifedipine, nicardipine)(5), this effect is likely mediated by a mechanism other than L-type voltage-dependent calcium channel (L-VDCC) blockade. Furthermore, previous studies have suggested that angiotensin II-induced efferent constriction may not be dependent on L-VDCC activation (23, 24). In addition to vasoconstriction, angiotensin II activates several nuclear transcription factors, e.g., AP-1, STAT and NF-κB, thereby inducing cytokine expression that may play important roles in the structural vascular remodeling (25). Of interest, a recent report showed that efonidipine inhibited PMA-induced activation of NF-κB in mesangial cells, while the same concentration of vera-

damil or nifedipine did not (26). We could speculate that efonidipine might have widened the efferent arterioles structurally through its inhibition of NF-κB, but this hypothesis will require further investigation.

In the present study, treatment with lisinopril caused widening of the afferent arteriolar lumina, consistent with earlier reports (18) and with the general finding that ACE inhibitor treatment results in decreased renal vascular resistance (22). The observation that the lumen diameter was similar between efonidipine-treated and untreated SHR kidneys was unexpected, because efonidipine, as well as other calcium antagonists, has been shown to dilate afferent arterioles functionally. The reason for this apparent discrepancy is not clear. A possible explanation may be that efonidipine-treatment dilates afferent arterioles functionally, but does not cause structural alteration. However, in view of a report that cilazapril increases the diameter of afferent arterioles, even under a relaxed condition induced by papaverine perfusion (19), this explanation might be unlikely. We cannot exclude the possibility that the afferent arteriolar diameter was underestimated in this experiment.

In conclusion, our data showed that long-term treatment with efonidipine or lisinopril affected the structurally-defined efferent arteriolar diameter in SHR. This raises the possibility that the structural alterations might be beneficial in maintaining the hemodynamic effects of the drugs, which will be the subject of our future study.

References


