Effects of Vasodilatory Antihypertensive Agents on Endothelial Dysfunction in Rats with Ischemic Acute Renal Failure

Masao KAKOKI, Yasunobu HIRATA, Hiroshi HAYAKAWA, Etsu SUZUKI, Daisuke NAGATA, Hiroaki NISHIMATSU, Kenjiro KIMURA, Atsuo GOTO, and Masao OMATA

Ischemic acute renal failure is associated with vascular endothelial dysfunction. We examined whether vasodilatory antihypertensive agents would improve endothelial function in rats with ischemia/reperfusion renal injury. Rat kidneys were isolated and perfused after clipping of the bilateral renal arteries for 45 min and reperfusion for 24 h, and renal perfusion pressure and nitric oxide concentration in the venous effluent (chemiluminescence assay) were monitored. Preischemic administration of celiprolol (a β-blocker; 100 mg/kg p.o.), benidipine (a calcium channel blocker; 1 mg/kg p.o.), or imidapril (an angiotensin converting-enzyme inhibitor; 3 mg/kg p.o.) restored endothelial function in rats subjected to acute renal ischemia (renal perfusion pressure [10−8 M acetylcholine]: sham −42±3%, ischemia −31±1%, ischemia + celiprolol −39±1% *, ischemia + benidipine −38±2% *, ischemia + imidapril −42±2% *; * p<0.05 vs. ischemia). Serum urea nitrogen and creatinine levels were also lower in the treated groups. Furthermore, ischemia-induced decreases in the response to acetylcholine and renal excretory function were smaller in SHR than in deoxycorticosterone-salt hypertensive rats, in which endothelial damage was marked. These results suggest that preischemic endothelial function may influence the degree of ischemic renal injury. Calcium channel blockers, converting-enzyme inhibitors, and endothelial NO synthase-activating β-blockers had beneficial effects on renovascular endothelial dysfunction due to ischemia.

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Key Words: β-blocker, calcium channel blocker, angiotensin converting enzyme inhibitor, nitric oxide

Introduction

Ischemic nephropathy is one of the cardiovascular complications of hypertension. However, the mechanisms by which ischemia induces renal injury remain undetermined. Ischemic acute renal failure (iARF), in particular, involves complex interrelated sequences of various mediators, including ATP depletion, increase in intracellular free Ca2+ and oxidant injury. More recently, it has been reported that nitric oxide (NO) also plays an important role in the pathogenesis of ischemia/reperfusion injury. However, it remains to be clarified whether NO plays a toxic or protective role in this context. Furthermore, it is also unclear if NO is increased or decreased in ischemic acute renal failure (1-3).

We previously found that renovascular endothelial dysfunction associated with ischemia/reperfusion injury was
due to reduced release of endothelium-derived NO. Inhibition of NO synthesis aggravates not only endothelial function but also renal excretory function. On the other hand, stimulation of NO synthesis by tetrahydrobiopterin improved both of these functions, in addition to ameliorating tubular damage (4). From this point of view, when ischemic insult occurs under condition of reduced action of NO, renal damage may be enhanced. Endothelial function, particularly the release of NO, decreases under most hypertensive conditions. Although many antihypertensive agents have been reported to be effective for ischemic renal injury, none has consistently proven beneficial (5). Thus, we here compared the susceptibility to ischemia in the kidneys of spontaneously hypertensive rats (SHR) and deoxycorticosterone acetate (DOCA)-salt hypertensive rats, in which endothelium-derived NO has been reported to be normal or elevated (6, 7) and markedly decreased (8), respectively. Furthermore, we examined the effects of 3 kinds of vasodilatory antihypertensive agents, i.e., celiprolol (a β-blocker), benidipine (a calcium [Ca] channel blocker) and imidapril (an angiotensin converting-enzyme [ACE] inhibitor), on endothelial and excretory dysfunction in rat kidneys subjected to acute ischemia/reperfusion.

Methods

Animals

All animal studies were performed in concordance with the University of Tokyo guidelines for animal experiments.

Twelve-week-old male Wistar rats were used in the present study. The in vivo model of iARF was prepared as described elsewhere (4). In brief, after anesthesia with pentobarbital sodium (40 mg/kg i.p.), a midline abdominal incision was made and the bilateral renal arteries were clamped with silver clips for 45 min after the injection of heparin (10 U/kg, i.m.); thereafter, the clamps were removed and the incision was closed. Celiprolol (100 mg/kg), benidipine (1 mg/kg), imidapril (3 mg/kg), or vehicle (0.5% methylcellulose 400 cps) were given by gavage just before the induction of ischemia. Twenty-four hours after the start of reperfusion, 0.1 ml arterial blood was drawn to determine the serum levels of urea nitrogen and creatinine, and the right kidney was isolated and perfused. The in vivo model of renal ischemia was also prepared in 12-week-old DOCA-salt hypertensive rats and SHR. SHR were purchased from the Disease Model Cooperative Research Association (Kyoto, Japan). DOCA-salt rats were prepared by left heminephrectomy and subcutaneous implantation of silicone pellets containing 200 mg/kg DOCA in 6-week-old male Wistar rats. Saline (0.9%) was then given as drinking water for 6 weeks. Blood pressure was determined by the tail cuff method.

Measurement of Perfusion Pressure and Amount of NO Release in the Isolated Perfused Kidney

Rats were anesthetized with pentobarbital sodium (40 mg/kg i.p.). The kidneys were isolated and perfused as described previously (4, 7, 8). In brief, after an abdominal incision the right kidney was exposed and the renal artery was cannulated with a needle via the superior mesenteric artery. The kidneys were perfused with Krebs-Henseleit buffer maintained at 37°C, saturated with 95%O₂-5%CO₂, and containing 10⁻⁵ mol/l indomethacin, at a constant flow of 5 ml/min/g kidney. Renal perfusion pressure (RPP) was maintained by 10⁻⁶ mol/l phenylephrine. RPP was continuously monitored using a Statham pressure transducer (Gould, Cleveland, OH), and recorded on a polygraph recorder (MR-7000, Nihon-Kohden Co., Ltd., Tokyo).

The effluent perfusate from the renal vein was obtained continuously (2 ml/min), mixed with a chemiluminescence probe (0.5 ml/min; 2 mmol/l H₂O₂, 18 μmol/l luminol, 2 mmol/l potassium carbonate and 150 mmol/l desferrioxamine) and then forwarded into a chemiluminescence analyzer (825-CL, Jasco Corp., Tokyo) as previously described (9) to measure NO output. After a 60-min equilibration period, we assayed the effects of vehicle, 10⁻⁹, 10⁻⁸, and 10⁻⁷ mol/l of acetylcholine (ACh), and 10⁻⁴ mol/l N²-nitro-L-arginine on RPP and NO release in a cumulative manner at 10-min intervals.

Drugs and Chemicals

Celiprolol, benidipine, and imidapril were kindly donated by Nippon Shinyaku Co. (Kyoto, Japan), Kyowa Hakko Co. (Tokyo), and Tanabe Seiyaku Co. (Osaka, Japan), respectively. Laboratory reagents and chemicals used for the preparation of Krebs-Henseleit solution and H₂O₂ were purchased from Wako Pure Chemical (Osaka, Japan). All other chemicals were from Sigma-Aldrich Japan (Tokyo).

Statistical Analysis

Data are expressed as mean±SEM. Statistical comparisons were made using analysis of variance followed by the Student-Neumann-Keuls test. P values less than 0.05 were considered to indicate statistical significance.

Results

Table 1 lists the body weight, kidney weight and baseline RPP of all groups of rats studied.

Figure 1 shows the changes in systolic blood pressure before and after the induction of ischemia/reperfusion renal injury. Baseline systolic blood pressure was around 120 mmHg in normotensive Wistar rats treated with anti-
hypertensive agents. Systolic blood pressure was significantly decreased in rats with iARF. However, the rats administered antihypertensive agents showed a further decrease in blood pressure. There were no differences in blood pressure among the three groups administered antihypertensive agents.

In the sham-operated isolated kidneys, ACh markedly decreased RPP in a dose-dependent manner. This dilatory effect of ACh was associated with substantial increases in NO. Addition of NG-nitro-L-arginine to ACh elevated RPP and diminished NO release. However, in the kidneys isolated from rats with iARF, ACh-induced vasodilation and NO release were attenuated. Figure 2 demonstrates the effects of pretreatment with vasodilatory antihypertensive agents on the impairment of ACh-induced relaxation of renal vessels and NO release in rats with acute renal ischemia. The decreases in endothelium-dependent vasorelaxation and NO release due to ischemia were significantly reversed by celiprolol, benidipine, and imidapril.

Figure 3 shows the effects of pretreatment with vasodilatory antihypertensive agents on serum urea nitrogen
and creatinine levels. Each of the 3 antihypertensive agents significantly lowered both serum urea nitrogen and creatinine in rats with iARF. However, both levels were lower in the imidapril group than in the other groups.

Twelve-week-old DOCA-salt hypertensive rats and SHR had a similarly high systolic blood pressure (DOCA: 225 ± 18; SHR: 201 ± 9 mmHg). However, the ACh-induced vasorelaxation and NO release were lower in DOCA-salt hypertensive rats than in 12-week-old SHR (Fig. 4). In the ischemic groups, ACh-induced vasorelaxation and NO release were markedly attenuated in DOCA-salt hypertensive rats compared with sham-operated DOCA-salt rats. In SHR, the renal response to ACh tended to decrease under ischemic conditions, but the de-
gree of the impaired response to ACh was significantly smaller than that observed in DOCA-salt hypertensive rats. Serum urea nitrogen and creatinine levels also increased in both types of hypertensive rats subjected to acute renal ischemia. The increases in urea nitrogen and creatinine were also greater in DOCA-salt rats (Fig. 5).

Discussion

It is well established that ischemia/reperfusion injury is accompanied by vascular endothelial damage. It is possible that the degree of tissue damage induced by ischemia is influenced by the state of endothelial function before ischemia, because in the present study ischemia-induced injury of the endothelium in SHR, in which endothelium-derived NO release was preserved, was mild, whereas in DOCA-salt hypertensive rats, in which NO release was markedly decreased, ischemia-induced injury was severe. Our results were in agreement with those reported by Sabbatini et al. (10), who demonstrated that the kidneys of aged rats, in which NO production was attenuated, were more vulnerable to hypoxia. Moreover, Janssens et al. (11) have reported that under hypoxic conditions constriction of pulmonary vessels in rats with adenovirus-mediated overexpression of endothelial NO synthase was less than in controls. It was also demonstrated that 2-week treatment with 17β-estradiol, which may increase NO release, improved canine coronary endothelial function after ischemia (12). These studies, along with our present results, strongly suggest the importance of preischemic NO release in preventing renal damage by ischemia. This mechanism may also influence the effects of the antihypertensive agents used in this study.

It has been suggested that the Ca-channel blockers have beneficial effects on ischemic injury in the heart, kidneys, brain and liver, probably by preventing intracellular Ca2+ overload under ischemic conditions (13-17). The beneficial effects of Ca-channel blockers are unlikely to be due to the improvement of posts ischemic hemodynamics, because Ca-channel blockers also have protective effects in cultured cardiomyocytes. The increase in the intracellular level of calcium in ischemia/reperfusion has been ascribed to a dysfunction of the Na+-Ca2+ exchanger due to a dysfunction of 2Na+-K+ ATPase, or the deactivation of Ca2+-ATPase (18). It has been reported that benidipine increases NO release in the heart via unknown mechanisms (19, 20). However, the reported antioxidant activities of benidipine may explain its effect on NO release (21, 22).

It is well established that ACE inhibitors prevent not only chronic cardiovascular remodeling after ischemia but also acute tissue injury caused by ischemia/reperfusion in the heart, lung, liver and kidneys (23-27). Interestingly, it has been demonstrated that the beneficial effects of ACE inhibitors in the acute phase are mainly due to the activation of bradykinin-NO or of the bradykinin-prostacyclin cascade, rather than to the suppression of angiotensin II, because angiotensin type 1 receptor antagonists have been clearly shown to be less beneficial than ACE inhibitors in previous studies (28, 29), and because addition of bradykinin type 2 receptor antagonists has been shown to abolish the effects of ACE inhibitors (28-30).

A striking finding of the present study was that celiprolol, a β-blocker which has no antioxidant properties, exerted significantly beneficial effects on endothelial function after ischemia/reperfusion injury. Recent studies suggest that endothelium-derived NO is involved in vasodilatation induced by several β-blockers. We have previously proposed that celiprolol strongly activates NO synthase through its effects on endothelial 5-HT1A receptors (31). It has also been shown that nebivolol, which is another NO synthase-activating β-blocker, reduces ischemia-induced myocardial damage (32). Therefore, it is possible that several β-blockers that activate NO synthase have beneficial effects on endothelial function after ischemia/reperfusion injury.

In conclusion, the severity of ischemia/reperfusion renal injury appeared to depend on the preexisting endothelial function. Ca-channel blockers, ACE inhibitors, and endothelial NO synthase-activating β-blockers lessened renovascular endothelial dysfunction due to ischemia, suggesting that these drugs can be expected to provide organ protection when administered as baseline antihypertensive agents.
agents.

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References


