A New ATP-Sensitive Potassium Channel Opener Protects the Kidney from Hypertensive Damage in Spontaneously Hypertensive Rats

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ABSTRACT

The effects of iptakalim, a new ATP-sensitive potassium channel opener, were studied in spontaneously hypertensive rats (SHR). Treatment of 12-week-old male SHR (six animals in each group) with iptakalim by gastric lavage at doses of 1, 3, or 9 mg/kg/day for 12 weeks resulted in a lowering of blood pressure. Iptakalim provided significant renoprotection to SHR rats as measured by decreased proteinuria and improved renal function. Histological evidence demonstrated that iptakalim could reverse renal vascular remodeling (of afferent arterioles, arcuate arteries, or interlobular arteries), and improve pathological changes of glomerular, renal interstitial, and glomerular filtration membranes. These effects were accompanied by the decreased circulation and intrarenal concentrations of endothelin 1 and transforming growth factor β1 (TGF-β1), and downregulated overexpression of genes for ET-1, endothelin-converting enzyme 1, TGF-β1, and the subunits of ATP-sensitive potassium channels (KATP), Kir1.1 and Kir6.1, in the kidney during hypertension. Abnormal expression of matrix components [collagen IV, fibronectin, matrix metalloproteinase 9 (MMP-9) and MMP tissue inhibitor 1 (TIMP-1)] was also significantly reversed by iptakalim. Our results demonstrate that chronic treatment with iptakalim not only reduces blood pressure but also preserves renal structure and function in SHR. In addition to reducing blood pressure, the renoprotective of iptakalim may be involved in inhibiting the circulation and intrarenal concentrations of endothelin 1 and TGF-β1, regulating the expression of KATP genes and correcting MMP-9/TIMP-1 imbalance in renal tissue, which may result in reducing the accumulation of extracellular matrix molecules.

The kidney is a critical target organ of hypertension-related damage. Although much progress has been made in providing more effective treatment for hypertension, the incidence of hypertension related to end-stage renal disease (ESRD) has increased in recent decades. Available data indicate that hypertension is second only to diabetic nephropathy as a primary etiology for ESRD and accounts for 30% of patients with ESRD (USRDS, 1998). These findings imply that optimal strategies for the treatment of hypertension to prevent renal damage have not been sufficient. So renal protection by antihypertensive agents, in addition to their efficacy for hypertension, has become an increasingly important target for medical therapy.

Iptakalim has been established as a newly selective ATP-sensitive potassium channel opener (KATP opener) by substantial pharmacological, biochemical, and electrophysiological studies as well as a receptor-binding test (Wang, 2003). Iptakalim selectively relaxes small arteries (Jia et al., 2004), and it has a selective antihypertensive action, which is more potent in the hypertensive state (Long et al., 2003c). As well as effectively decreasing blood pressure, iptakalim can simultaneously reverse hypertensive vascular remodeling (Long et al., 2003b; Xie et al., 2003, Wang et al., 2005) and cardiac remodeling (Long et al., 2003a; Wang et al., 2005). It has been suggested that the selective antihypertensive efficacy and reversal of hypertensive cardiovascular remodeling by
iptakalim may be related to regulation of cardiovascular K<sub>ATP</sub> mRNA expression.

Physiological and molecular studies have shown that renal K<sub>ATP</sub> channels are located on the tubular system, afferent arteriolar, and glomerular membranes. Pinacidil, a potassium channel opener, evokes afferent arteriolar diameter dilatation in kidneys from both rats with insulin-dependent diabetes mellitus (IDDM) (induced by streptozotocin injection) and sham-operated rats, but the dilator responses to pinacidil are more significant in kidneys from IDDM rats than from sham-operated rats. Glibenclamide does not alter afferent arteriolar diameter in sham-operated rats, but it reduces afferent arteriolar diameter in IDDM rats. This finding suggests that these vessels are endowed with K<sub>ATP</sub> channels that are normally quiescent. The function and activation of renal K<sub>ATP</sub> channels are changed under pathological conditions, which may be related to renal function (Ikenaga et al., 2000). However, the relationship between the change in conditions, which may be related to renal function (Ikenaga et al., 2000). However, the relationship between the change in K<sub>ATP</sub> channel function and renal function remains largely unknown. Therefore, we investigated the experimental therapeutic effects and molecular mechanism of iptakalim on hypertensive renal damage with a SHR model and compared its effect with that of benazepril (an angiotensin II receptor blocker) and pinacidil.

SHR is a genetic model of hypertension in which there is a definitive evidence for hypertension-dependent abnormalities in renal function and preglomerular vessel hypertrophy, and it has been used for evaluating the nephroprotective effects of different antihypertensive agents (Skov et al., 1996; Sabbatini et al., 2000). Therefore, we investigated the experimental therapeutic effects and molecular mechanism of iptakalim on hypertensive renal damage with a SHR model and compared its effect with that of benazepril (an angiotensin-converting enzyme inhibitor whose renoprotective action has been originally demonstrated by a large-scale clinical trial; Maschio et al., 1996) to further explore its antihypertensive mechanism and to provide theoretical basis for its use in clinical management.

**Materials and Methods**

**Animals.** Male SHR and normotensive Wistar-Kyoto (WKY) rats 12 weeks of age were obtained from the Institute of Cardiovascular Diseases, Chinese Academy of Medical Sciences (Beijing, China). The rats were housed in groups of five, under a 12-h light/dark cycle at a temperature of 24 ± 1°C and relative humidity of 56 ± 10%, with free access to water and normal diet (22% protein). All animal procedures were performed in accordance with the Declaration of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication 85-23, revised 1985).

**Chemicals.** Iptakalim was synthesized by the Beijing Institute of Pharmacology and Toxicology (Beijing, China). Benazepril was a gift from Novartis (Beijing, China). All other chemicals and materials were obtained from local commercial sources.

**Pharmacological Treatment.** SHR were randomly assigned to five groups (n = 6 rats/group) and treated with iptakalim (1, 3, and 9 mg/kg/day), benazepril (3 mg/kg/day), or vehicle. Six age-matched WKY rats treated with vehicle were also used as a control group. The compound or vehicle was orally administered once a day in 2 ml/kg for 12 weeks.

Body weight, systolic pressure, and heart rate were determined every week throughout the study. The systolic blood pressure (SBP) and heart rate were measured indirectly using a standard tail-cuff method (BP recorder, RBP-1; China-Japan Friendship Hospital, Beijing, China) in conscious rats after prewarming at 38°C for 10 min.

**Tissue Preparation.** After 12 weeks treatment, rats were anesthetized with an intraperitoneal injection of sodium pentobarbitone (60 mg/kg) and placed on a thermostatically controlled operating table. After thoracolaparotomy, venous blood was obtained from the right atrium; serum was isolated and stored at −20°C until assayed for biochemicals and TGF-β1.

Blood samples for determination of ET-1 and angiotensin II were collected on ice in tubes containing EDTA and centrifuged at 3000g for 15 min at 4°C to isolate plasma. Plasma samples were then frozen at −20°C until the assay was performed.

Then, the kidneys were rapidly removed without oclucing the blood supply, to prevent capillary collapse. The right kidney was weighed and homogenized at 4°C in 2 ml of saline, and the homogenate was centrifuged at 3000g for 15 min at 4°C. The supernatant was aliquoted and immediately stored at −70°C until assayed for endothelin 1, angiotensin II, and TGF-β1 protein. The left kidney was hemisected and rinsed in ice-cold saline to remove blood. Half of the left kidney was fixed in 10% buffered formalin and stored at −70°C until RNA extraction.

**Renal Function Determinations.** On the 12th week of experiment, animals were put in metabolic cages and 24-h urine samples were collected. Urinary protein excretion was determined using the Coomassie Light Blue assay. Serum creatinine, blood urea nitrogen, plasma glucose, and serum cholesterol and triglyceride levels were determined with an automated biochemical analyzer (7600-DDP-ISE; Hitachi Software Engineering, Yokohama, Japan).

**Histological Analysis.** The kidney tissue fixed in 10% phosphate-buffered formalin was embedded in paraffin, cut into 4-μm sections, and stained with hematoxylin and eosin. The morphological changes in the renal tissue were observed with light microscopy. The kidney tissue fixed in 2.5% glutaraldehyde solution was embedded in resin, sliced using an LKB slicer, and double stained with uranyl acetate and lead citrate. Changes in the glomerular basement membrane were observed using transmission electron microscopy (JEM1230; Japan Electron Optics Laboratory, Tokyo, Japan).

For vascular morphometry, five consecutive sections stained with hematoxylin and eosin were viewed under a microscope. The media thickness (M) and lumen diameter (L) of afferent arterioles, arcuate arteries, or interlobular arteries were measured using a computerized morphometric system, and the ratio of M to L (M/L) was calculated. On each section, five corresponding vessels were examined. The interlobular artery was identified as a single muscular artery associated with an interlobular artery, if necessary demonstrated in serial sections. Arcuate arteries were identified along the corticomedullary junction and surrounded by tubules. We included only vessels that were situated in the renal cortex, that had an inner lumen diameter less than 35 μm, and fulfilled at least one of the following criteria for being afferent arterioles: 1) presence of a microsphere in the lumen or in a glomerulus, providing that it was possible in serial sections to show that the microsphere had passed through the vessel to that glomerulus; 2) presence of an internal elastic lamina, because this lamina is absent in efferent arterioles even where many smooth muscle cells are seen in the vascular wall; and 3) a close relationship to an interlobular artery, if necessary demonstrated in serial sections.

**Endothelin 1, Angiotensin II, and TGF-β1 Measurement.** The concentrations of ET-1 and angiotensin II in the plasma and renal tissue were measured using a commercial radioimmunoassay kit (Eastern Asia Radioimmunity Research Institute, Beijing, China) according to the instructions provided by the manufacturer. TGF-β1 concentration in the serum and renal tissue was measured by enzyme-linked immunosorbent assay (Shenzhen Jingmei Biotechnological Company, Beijing, China).
Immunohistochemistry. Rabbit polyclonal antibodies against rat collagen IV, fibronectin, MMP-9, TIMP-1, or TGF-β1 (Boster Biological Technology, Wuhan, China) were applied. Specimens were stained using the avidin-biotin technique using an ABC kit (Huamei Biotechnology, Beijing, China). As negative controls, slides were incubated with phosphate-buffered saline instead of primary antibodies. Five sections were investigated for each parameter. The results were quantified with the aid of image analysis software (CMIAS analysis system, Image Center of the University of Aviation and Spaceflight, Beijing, China) and are expressed as average optical densities.

Gene Expression. Total RNA was extracted from the renal tissue of rats using TRIzol reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA). RNA was quantified by determination of ultraviolet absorbance at 260 nm, measuring the optical density ratio at 260 and 280 nm to assess its purity. The total RNA of rats using TRIzol reagent according to the manufacturer’s instructions was reverse transcribed using MMLV reverse transcriptase (Promega, Madison, WI) reverse transcriptase reaction mixture in a total volume of 25 μl contained 5 μg of total RNA, 0.5 μg of Oligo(dT) primer, 200 μM mixed dNTPs, 200 units Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI) reverse transcriptase, 25 units of rRNasin ribonuclease inhibitor, 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl2, and 10 mM dithirotreitol. The reverse transcription reaction was incubated at 42°C for 60 min. Then, the final reaction mixture was directly used for PCR amplification. The PCR mixture contained 2 μl of cDNA, 10 mM KCl, 10 mM Tris-HCl, pH 8.0, 8 mM (NH4)2SO4, 25 mM MgCl2, 200 μM mixed dNTP, 2.5 units of TaqDNA polymerase (Promega), and 1 pM each primer. The PCR solution was initially denatured for 5 min at 94°C and then thermal cycling started with denaturation at 95°C for 30 s, annealing at 55–59°C for 30 s, and extension at 72°C for 1 min for 30 cycles. The final cycle included extension at 72°C for 10 min. The PCR reaction products were obtained by electrophoresis on 2% agarose gels and visualized using ethidium bromide. The fluorescence intensity of each band was quantified using Labworks 4.0 software. The specific primers for KATP, isofoms [sulfonylurea receptor 2 (SUR2), Kir6.1, Kir1.1], ET, renin-angiotensin system, and TGF-β1 component genes and their oligonucleotide sequences are presented in the Table 1 designed with Primer 5.0 software.

The primer sequences for rat endothelin 1 were sense, 5′-GCT CCT GCT CCT CCT TGA TG-3′; and antisense, 5′-CTC GCT CTA TGT AAG TCG TGG-3′. The primer sequences for rat ECE-1 were sense, 5′-GCT AGC GAT AGT CTT AGC AC-3′; and antisense, 5′-GTG CCA CCA CAA AAC TAC AG-3′. The primer sequences for rat ACE were sense, 5′-CAG CCT CAT CAT CCA GTT CC-3′; and antisense, 5′-CTA GGA AGA GCA GCA CCC AC-3′. The primer sequences for rat AGT were sense, 5′-TTC AGG CCA AGA CCT CCC-3′; and antisense, 5′-CCA GCC GCG AGG TGC AGT-3′. The primer sequences for rat TGF-β1 were sense, 5′-CAA CAT CAC ACA CAG TA-3′; and antisense, 5′-GTT GGT GAG CCG TTT CCT CCA G-3′. The primer sequences for rat GAPDH were sense, 5′-TCC CTC AAG ATT GTC AGC AA-3′; and antisense, 5′-AGA TCC ACA ACG GAT CAT AA-3′. The primer sequences for rat MPP-9 were sense, 5′-CTC CAG TAG ACG ATC CTT GC-3′; and antisense, 5′-AGC CCA GAG AAC TGA TTA T-3′. The primer sequences for rat TIMP-1 were sense, 5′-CGG GAC CAC CTT ATG CCT CA-3′; and antisense, 5′-GAG AGC ACA GCT CCA G-3′. The primer sequences for rat collagen IV were sense, 5′-TCT CAC TCA CTC CCA CAT C-3′; and antisense, 5′-GTA GCG CCA CTT CAA GCA TA-3′. The primer sequences for rat SUR2 were sense, 5′-ACC ACC TGG ACA ACT ACG AG-3′; and antisense, 5′-ATG GCA AGG AGG AAG AGA GAC GA-3′. The primer sequences for rat Kir1.1 were sense, 5′-CGA GAA ACC TTC CAT CTT G-3′; and antisense, 5′-CAC CAG AAC TCA GCA AAC-3′. The primer sequences for rat Kir1.1 were sense, 5′-GGT TAC GGA TTC TTT GT-3′; and antisense, 5′-AGG GCT GTT GTG CTC AAC-3′.

Statistical Analysis. The results are expressed as mean ± S.E. Statistical differences among the groups were evaluated using one-way analysis of variance followed by Newman-Keuls multiple range tests. Statistical significance was accepted at p < 0.05.

Results

During the 12-week treatment period, the SBP and heart rate of normotensive WKY rats did not change significantly. Under the same conditions, the SBP and heart rate of the control SHR increased gradually (Fig. 1). Iptakalim at 1, 3, and 9 mg/kg/day and benazepril at 3 mg/kg/day significantly decreased SBP, respectively, to around 37, 23, 23, or 21 mm Hg after 2-, 1-, 1-, or 1-week treatment (p < 0.01). Furthermore, the SBP of the four treatment groups was stabilized at a similar level from the fourth week (Fig. 1A). After 2 weeks of treatment, iptakalim at 3 and 9 mg/kg/day decreased heart rate, respectively, to around 37 and 45 bpm, whereas iptakalim at 1 mg/kg/day and benazepril at 3 mg/kg/day had no effect on heart rate (Fig. 1B). Body weight in the five SHR groups was lower than that in age-matched WKY rats at the beginning of experiment. During the 12-week treatment period, body weight in the five SHR groups and the normotensive control WKY rats showed a tendency to increase gradually. There was no statistical difference between the five SHR groups [body weights were 275 ± 22 g (no treatment), 277 ± 20 g (iptakalim at 1 mg/kg), 274 ± 20 g (iptakalim at 3 mg/kg), 278 ± 28 g (iptakalim at 9 mg/kg), and 277 ± 20 g (benazepril at 3 mg/kg)]. Kidney weight values were similar in WKY rats and in either control or pharmacologically treated SHR (data not shown).

Effects on Hypertensive Functional Disorder. Urinary protein excretion rate for 24 h, serum creatinine, and blood area nitrogen in control SHR were significantly increased compared with that in WKY rats. Iptakalim, at doses of 1, 3, or 9 mg/kg/day, produced a dose-dependent decrease in urinary protein excretion rate for 24 h in SHR. The levels of serum creatinine and blood area nitrogen decreased to normal in the iptakalim-treated groups. Under the same experimental conditions, benazepril also effectively decreased urinary protein excretion rate for 24 h, serum creatinine, and blood area nitrogen. However, neither of the pharmacological treatments had any effect on blood glucose, cholesterol, and triglyceride (Table 1).

Effects on Hypertensive Histopathological Damage. Analysis of the morphology of renal glomeruli in control SHR revealed the occurrence of slight segmental sclerosis of glomerular capillaries compared with age-matched normoten- sive WKY rats. Treatment with iptakalim or benazepril reversed the damage (Fig. 2A). In the SHR, the afferent arterioles, arcuate arteries, and interlobular arteries revealed luminal narrowing, accompanied by an increase in media thickness compared with those of WKY rats. Treatment with iptakalim or benazepril decreased the media thickness (M), increased the lumen diameter (L), and decreased the ratio M/liter (Fig. 2, A–C; Table 2).

Electron micrographs showed that, in WKY rats, the threelayer structure of glomerular filtration membranes was normal, as characterized by the regular morphology of podocyte, the clear fenestra in endothelial cells, and absence of glomer-
ular basement membrane thickening (Fig. 3A). However, in control SHR, the glomerular basement membrane was irregularly thickened, and podocytes were mixed together locally (Fig. 3B). Treatment with iptakalim and benazepril for 12 weeks almost normalized the glomerular filtration membranes apart from local podocyte mixing (Fig. 3, C–F).

Effects on the Extracellular Matrix Component in Intrarenal Arterial Walls. We further investigated the effects of iptakalim on the expression of collagen IV and fibronectin protein in the arterial wall by immunohistochemistry. In SHR, collagen IV and fibronectin proteins revealed higher expression in arcuate arteries (2.0- or 1.9-fold; \( p < 0.01 \)) and interlobular arteries (2.6- or 3.1-fold; \( p < 0.01 \)) compared with WKY rats (Fig. 4, A and B). Iptakalim or benazepril treatment significantly suppressed the increase in collagen IV and fibronectin protein expression compared with SHR (\( p < 0.01 \); Fig. 4, A and B).

Effects on the Extracellular Matrix Degradation System. First, MMP-9, TIMP-1, collagen IV, and TGF-β1 protein expression were investigated by immunohistocytology. In SHR, collagen IV and fibronectin proteins revealed higher expression in arcuate arteries (2.0- or 1.9-fold; \( p < 0.01 \)) and interlobular arteries (2.6- or 3.1-fold; \( p < 0.01 \)) compared with WKY rats (Fig. 4, A and B). Iptakalim or benazepril treatment significantly suppressed the increase in collagen IV and fibronectin protein expression compared with SHR (\( p < 0.01 \); Fig. 4, A and B).

<table>
<thead>
<tr>
<th>Group</th>
<th>Cr (µM)</th>
<th>BUN (mM)</th>
<th>GLU (mM)</th>
<th>TC (mM)</th>
<th>TG (mM)</th>
<th>UAE (mg/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>37.8 ± 1.3</td>
<td>6.19 ± 0.14</td>
<td>4.28 ± 0.20</td>
<td>4.20 ± 0.18</td>
<td>1.11 ± 0.04</td>
<td>4.65 ± 0.19</td>
</tr>
<tr>
<td>SHR</td>
<td>68.2 ± 2.0**</td>
<td>8.38 ± 0.19</td>
<td>5.35 ± 0.22</td>
<td>4.89 ± 0.19</td>
<td>1.15 ± 0.02</td>
<td>140.3 ± 5.42**</td>
</tr>
<tr>
<td>SHR-Ipt 1 mg/kg/day</td>
<td>45.4 ± 1.9**</td>
<td>6.99 ± 0.22*</td>
<td>4.65 ± 0.19</td>
<td>4.70 ± 0.18</td>
<td>1.11 ± 0.04</td>
<td>89.65 ± 3.56**</td>
</tr>
<tr>
<td>SHR-Ipt 3 mg/kg/day</td>
<td>42.6 ± 1.7**</td>
<td>6.60 ± 0.17*</td>
<td>4.64 ± 0.19</td>
<td>4.64 ± 0.18</td>
<td>1.14 ± 0.04</td>
<td>51.07 ± 1.22**</td>
</tr>
<tr>
<td>SHR-Ipt 9 mg/kg/day</td>
<td>43.1 ± 1.9**</td>
<td>6.71 ± 0.17*</td>
<td>4.54 ± 0.18</td>
<td>4.59 ± 0.18</td>
<td>1.10 ± 0.04</td>
<td>44.18 ± 1.04**</td>
</tr>
<tr>
<td>SHR-Ben 3 mg/kg/day</td>
<td>44.8 ± 1.82**</td>
<td>6.66 ± 0.20*</td>
<td>4.60 ± 0.19</td>
<td>4.60 ± 0.17</td>
<td>1.14 ± 0.47</td>
<td>49.58 ± 1.03**</td>
</tr>
</tbody>
</table>

Ipt, iptakalim; Ben, benazepril; Cr, creatinine; BUN, blood urea nitrogen; GLU, glucose; TC, total cholesterol; TG, triglycerides; UAE, urinary albumin excretion rate.

\* \( p < 0.05 \).
\** \( p < 0.01 \) vs. WKY.
\# \( p < 0.05 \).
\## \( p < 0.01 \) vs. SHR.
chemistry. In SHR, the MMP-9 protein expression was lower than that in WKY rats \((p < 0.05; \text{Fig. } 5A)\), whereas the expression of TIMP-1, collagen-IV, and TGF-β1 were much higher than in WKY rats \((p < 0.01; \text{Fig. } 5A)\). Treatment with iptakalim or benazepril resulted in up-regulation of the decreased MMP-9 expression and down-regulation of the increased protein expression of TIMP-1, collagen-IV, and TGF-β1 compared with those in SHR \((p < 0.05–0.01; \text{Fig. } 5A)\).

Second, we examined the mRNA expression for these proteins in kidney using RT-PCR. This demonstrated that the level of MMP-9 mRNA was down-regulated, and TIMP-1, collagen-IV, and TGF-β1 mRNAs were up-regulated in SHR \((p < 0.05–0.01; \text{Fig. } 5B)\) compared with WKY rats. After long-term treatment with iptakalim or benazepril, MMP-9 mRNA expression was up-regulated, and TIMP-1, collagen-IV, and TGF-β1 mRNA expression was down-regulated compared with that in SHR \((p < 0.05–0.01; \text{Fig. } 5B)\).

Third, we measured the TGF-β1 in plasma and renal tissue. In SHR, the concentration of TGF-β1 was increased compared with that in WKY rats \((p < 0.01; \text{Table } 3)\). Twelve weeks of treatment with iptakalim or benazepril significantly }
suppressed the increase in TGF-β1 level (p < 0.05–0.01; Table 3).

**Effects on the Endothelin System.** In SHR, the concentrations of endothelin 1 (plasma and renal tissue) significantly increased compared with those in WKY rats (p < 0.01; Table 3). Twelve weeks of treatment with iptakalim or benazepril significantly suppressed the increase in endothelin 1 level (p < 0.05–0.01; Table 3). The renal expression of ET-1 or ECE-1 mRNA was significantly higher in SHR compared with that in WKY rats. Benazepril or iptakalim treatment suppressed the increase in mRNA expression of ET-1 and ECE-1 (p < 0.05–0.01; Fig. 6A) compared with SHR.

**Effects on the Renin-Angiotensin System.** In control SHR, the concentration of angiotensin II (plasma or renal tissue) significantly increased compared with that in WKY rats. Twelve weeks of treatment with benazepril significantly suppressed the increase in angiotensin II level. Under the same experimental conditions, iptakalim did not alter the concentration of angiotensin II (Table 3). Angiotensinogen and angiotensin-converting enzyme (ACE) are two critical

### TABLE 2
Morphological parameters of afferent arteriole, interlobular artery, and arcuate artery after 12 weeks of treatment

Data are expressed as mean ± S.E.M., n = 6 per group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WKY</th>
<th>SHR</th>
<th>SHR-Ipt 1</th>
<th>SHR-Ipt 3</th>
<th>SHR-Ipt 9</th>
<th>SHR-Ben3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Afferent arteriole</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media (μm)</td>
<td>6.09 ± 0.25</td>
<td>9.56 ± 0.36**</td>
<td>7.71 ± 0.29**</td>
<td>6.62 ± 0.34**</td>
<td>6.43 ± 0.26**</td>
<td>6.52 ± 0.26**</td>
</tr>
<tr>
<td>Lumen (μm)</td>
<td>7.21 ± 0.31</td>
<td>3.32 ± 0.22**</td>
<td>4.97 ± 0.24***</td>
<td>7.65 ± 0.29**</td>
<td>7.50 ± 0.16**</td>
<td>7.45 ± 0.40**</td>
</tr>
<tr>
<td>Media/lumen</td>
<td>0.85 ± 0.03</td>
<td>2.91 ± 0.13**</td>
<td>1.55 ± 0.07***</td>
<td>0.87 ± 0.08**</td>
<td>0.86 ± 0.04**</td>
<td>0.88 ± 0.03**</td>
</tr>
<tr>
<td><strong>Interlobular artery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media (μm)</td>
<td>10.74 ± 0.44</td>
<td>15.77 ± 0.62**</td>
<td>12.62 ± 0.45*</td>
<td>10.99 ± 1.02**</td>
<td>11.22 ± 0.47**</td>
<td>11.19 ± 0.44**</td>
</tr>
<tr>
<td>Lumen (μm)</td>
<td>25.54 ± 1.15</td>
<td>17.19 ± 0.67**</td>
<td>24.45 ± 0.96**</td>
<td>25.99 ± 2.52**</td>
<td>25.26 ± 0.91**</td>
<td>26.86 ± 1.09**</td>
</tr>
<tr>
<td>Media/lumen</td>
<td>0.41 ± 0.01</td>
<td>0.92 ± 0.03**</td>
<td>0.52 ± 0.02**</td>
<td>0.43 ± 0.05**</td>
<td>0.44 ± 0.01**</td>
<td>0.42 ± 0.02**</td>
</tr>
<tr>
<td><strong>Arcuate artery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media (μm)</td>
<td>15.01 ± 0.71</td>
<td>24.11 ± 1.00**</td>
<td>17.64 ± 0.81**</td>
<td>16.07 ± 0.74**</td>
<td>16.04 ± 0.76**</td>
<td>15.66 ± 0.73**</td>
</tr>
<tr>
<td>Lumen (μm)</td>
<td>45.73 ± 1.82</td>
<td>26.42 ± 1.04**</td>
<td>38.19 ± 1.59**</td>
<td>40.26 ± 1.84**</td>
<td>41.33 ± 1.38**</td>
<td>42.62 ± 1.63**</td>
</tr>
<tr>
<td>Media/lumen</td>
<td>0.34 ± 0.02</td>
<td>0.92 ± 0.02**</td>
<td>0.47 ± 0.02*</td>
<td>0.40 ± 0.02**</td>
<td>0.39 ± 0.02**</td>
<td>0.37 ± 0.02**</td>
</tr>
</tbody>
</table>

*p < 0.05.

**p < 0.01 vs. WKY.

#p < 0.05.

##p < 0.01 vs. SHR.

Fig. 3. Electron micrographs of the glomerular filtration membranes in a normotensive WKY rat (A), control SHR (B), SHR treated with iptakalim at 1, 3, or 9 mg/kg/day (C–E) and SHR treated with benazepril at 3 mg/kg/day (F). Normal three-layer structure of glomerular filtration membranes in WKY rats, including glomerular basement membrane (black arrow), podocytes (hollow arrow), and endothelial cells (arrowhead). Note in control SHR, irregular thickening of glomerular basement membranes ($) and podocytes mixed together (*). In pharmacologically treated SHR, glomerular filtration membranes were almost normal, apart from some local podocytes mixing. Cap, capillary. Scale bar, 1 μm.
components of the renin-angiotensin system. The renal expression of angiotensinogen and ACE mRNA was significantly higher in control SHR compared with that in WKY rats. Benazepril treatment suppressed the increase in mRNA expression of angiotensinogen and ACE, but iptakalim did not alter it (Fig. 6A).

**Effects on K<sub>ATP</sub> Channel Isoforms.** In rat renal tissue, the mRNA expression of K<sub>ATP</sub> subunits SUR2, Kir6.1, and Kir1.1 was studied using RT-PCR, with GAPDH included as an internal control. RT-PCR analysis showed that the mRNA expression levels of SUR2, Kir6.1, and Kir1.1 were increased in control SHR compared with those in WKY rats. Benazepril treatment suppressed the increase in mRNA expression of angiotensinogen and ACE, but iptakalim did not alter it (Fig. 6A).

**Discussion**

Iptakalim, which is a novel chemical type of potassium channel opener, possesses selective antihypertensive efficacy with a steady and lasting character, and a unique feature of antihypertensive action by protecting the organs involved in hypertension. Iptakalim can reverse hypertensive cardiovascular remodeling (Long et al., 2003a,b; Wang et al., 2005) and provides protection against hypertensive brain damage (Wang et al., 2004). This study further demonstrated that iptakalim has nephroprotective effects in SHR.

The effects of iptakalim on hypertensive renal damage were investigated in SHR. Preglomerular artery remodeling, especially afferent arteriolar remodeling, is one of the key pathological changes and is fundamental to hypertensive renal damage, because it directly affects glomerular perfusion and pressure, and leads to proteinuria, glomerulosclerosis, and interstitial renal fibrosis, as a result of renal dysfunction. Proteinuria is an early stage predictor of renal damage, which suggests glomerular hyperperfusion, hyperfiltration, and glomerular hypertension (Bidani and Griffin, 2002). These changes are important mechanisms in the development of glomerulosclerosis. Therefore, antihypertensive agents should not be limited to blood pressure reduction but should also correct pathophysiological mechanisms, to reverse hypertensive target organ damage. Antihypertensive agents that reduce BP, and at the same time reverse renal vascular remodeling, allow recovery of glomerular perfusion, and improve renal function, decrease proteinuria, and as a result decrease the incidence rate of ESRD induced by hypertension. Our findings suggest that iptakalim can decrease BP effectively, and simultaneously it can reverse pathological changes of glomerular filtration membranes, decrease proteinuria, reverse renal vascular remodeling, ameliorate pathological glomerular and renal interstitial changes, and improve renal function.

Accumulation of extracellular matrix molecules in the kidney is one of the main pathological features of hypertensive renal damage. Iptakalim was shown to decrease the overex-
In addition, TGF-β1, a cytokine on renal damage (Bottinger et al., 1997). Forces of this cytokine on renal damage, indicating a direct cause a progressive loss of renal function, suggesting a direct effect of this cytokine on renal damage and fibronectin, in the wall of arcuate arteries and interlobular arteries under hypertensive conditions. This effect primarily contributes to its ability to produce vascular structural improvement.

Accumulating data indicate that TGF-β1 is involved in the pathophysiological process of hypertensive renal damage. Data from TGF-β1 transgenic mice have shown that high levels of circulating TGF-β1 can mediate renal fibrosis and cause a progressive loss of renal function, indicating a direct effect of this cytokine on renal damage (Bottinger et al., 1997). In addition, TGF-β1 can directly stimulate the synthesis of extracellular matrix and can inhibit the degradation of extracellular matrix by increasing the synthesis of TIMPs and reduce the production and secretion of MMPs, with the consequence of glomerulosclerosis and renal interstitial fibrosis (Lijnen et al., 2003). Our findings showed that antihypertensive therapy with iptakalim and benazepril ameliorates hypertensive renal damage, consistent with decreasing intrarenal TGF-β1 concentration and mRNA expression in SHR. This suggests that iptakalim protects the kidney from hypertensive damage in hypertension partly through inhibiting TGF-β1 expression and regulating MMP-9 or TIMP-1 expression in the renal tissue. The data showed that renoprotection by iptakalim may be related to the reduction of circulating and intrarenal TGF-β1, leading to a correction of extracellular matrix synthesis and degradation imbalance, by up-regulating MMP-9 expression and down-regulating that of TIMP-1.

Fig. 6. Effects of iptakalim and benazepril on endothelin 1 (ET-1), ECE-1, ACE, and angiotensinogen (AGT) (A) and SUR2, Kir1.1, and Kir6.1 (B) mRNA expression in the whole kidney of SHR after 12 weeks of treatment. Each mRNA expression level was quantified by the ratio of the intensity of the internal control band of GAPDH to target band. Data are means ± S.E.M., n = 6 per group. *p < 0.05, **p < 0.01 vs. WKY. #p < 0.05, ##p < 0.01 versus SHR.

TABLE 3
Changes in plasma and intrarenal ET-1, TGF-β1, and Ang II concentration after 12 weeks of treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum</th>
<th>Renal Tissue</th>
<th>Serum</th>
<th>Renal Tissue</th>
<th>Serum</th>
<th>Renal Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pg/ml</td>
<td>pg/mg</td>
<td>pg/ml</td>
<td>pg/mg</td>
<td>pg/ml</td>
<td>pg/mg</td>
</tr>
<tr>
<td>WKY</td>
<td>76.0 ± 2.9</td>
<td>2.55 ± 0.10</td>
<td>20.1 ± 0.7</td>
<td>1.40 ± 0.04</td>
<td>46.9 ± 2.2</td>
<td>4.5 ± 0.19</td>
</tr>
<tr>
<td>SHR</td>
<td>110.2 ± 3.9**</td>
<td>4.43 ± 0.17**</td>
<td>37.3 ± 1.1**</td>
<td>2.45 ± 0.08**</td>
<td>101.5 ± 3.7**</td>
<td>8.6 ± 0.31**</td>
</tr>
<tr>
<td>SHR-Ipt 1 mg/kg/day</td>
<td>91.1 ± 3.6**</td>
<td>3.36 ± 0.12**</td>
<td>28.1 ± 0.9**</td>
<td>1.98 ± 0.07**</td>
<td>109.9 ± 3.9**</td>
<td>8.0 ± 0.37**</td>
</tr>
<tr>
<td>SHR-Ipt 3 mg/kg/day</td>
<td>90.5 ± 2.9**</td>
<td>2.96 ± 0.09**</td>
<td>24.1 ± 0.9**</td>
<td>1.56 ± 0.05**</td>
<td>96.1 ± 3.9**</td>
<td>8.3 ± 0.21**</td>
</tr>
<tr>
<td>SHR-Ipt 9 mg/kg/day</td>
<td>88.9 ± 3.4**</td>
<td>2.87 ± 0.10**</td>
<td>23.0 ± 0.8**</td>
<td>1.46 ± 0.06**</td>
<td>98.3 ± 4.0**</td>
<td>8.3 ± 0.27**</td>
</tr>
<tr>
<td>SHR-Ben 3 mg/kg/day</td>
<td>88.0 ± 3.5**</td>
<td>2.99 ± 0.12**</td>
<td>23.8 ± 0.8**</td>
<td>1.44 ± 0.06**</td>
<td>55.5 ± 2.5**</td>
<td>5.6 ± 0.22**</td>
</tr>
</tbody>
</table>

Ang II, angiotensin II.
* p < 0.05.
** p < 0.01 vs. WKY.
# p < 0.05,
## p < 0.01 versus SHR.

Expression of two extracellular matrix components, collagen IV and fibronectin, in the wall of arcuate arteries and interlobular arteries under hypertensive conditions. This effect primarily contributes to its ability to produce vascular structural improvement.

The data from TGF-β1 transgenic mice show that high levels of circulating TGF-β1 can be pathogenically involved in the progression of hypertensive renal damage. Data from TGF-β1 transgenic mice show that high levels of circulating TGF-β1 can mediate renal fibrosis and cause a progressive loss of renal function, indicating a direct effect of this cytokine on renal damage (Bottinger et al., 1997). In addition, TGF-β1 can directly stimulate the synthesis of extracellular matrix and can inhibit the degradation of extracellular matrix by increasing the synthesis of TIMPs and reduce the production and secretion of MMPs, with the consequence of glomerulosclerosis and renal interstitial fibrosis (Lijnen et al., 2003). Our findings showed that antihypertensive therapy with iptakalim and benazepril ameliorates hypertensive renal damage, consistent with decreasing intrarenal TGF-β1 concentration and mRNA expression in SHR. This suggests that iptakalim protects the kidney from hypertensive damage in hypertension partly through inhibiting TGF-β1 expression and regulating MMP-9 or TIMP-1 expression in the renal tissue. The data showed that renoprotection by iptakalim may be related to the reduction of circulating and intrarenal TGF-β1, leading to a correction of extracellular matrix synthesis and degradation imbalance, by up-regulating MMP-9 expression and down-regulating that of TIMP-1.

So far, endothelin 1 is one of the strongest vasoconstrictor substances known in vivo, and it is regarded as an important pathological factor promoting glomerulosclerosis. Endothelin 1 can directly constrict glomerular afferents and efferent arterioles and mesangial cells to increase renal vascular resistance and decrease renal blood flow and glomerular filtration rate. In pathological conditions, endothelin 1 can stimulate the growth of inherent renal cells and the secretion of the extracellular matrix (Lariviere and Lebel, 2003). Furthermore, renal tissue endothelin 1 concentration increases with increased proteinuria and poor renal function prognosis. Our study showed that plasma and renal tissue endothelin 1 concentration and renal tissue endothelin 1 concentration in SHR is directly involved in progressive renal damage by stimulating hypertrophy and hyperplasia of vascular endothelial cells, promoting the deposition of extracellular matrix and inhibiting the degradation of extracellular matrix (Klahr and Morrissey, 2000). Moreover, intrarenal angiotensin II preferentially constricts efferent arterioles, leading to glomerular hypertension (Palmer, 2001), and contributes to proteinuria, either by inducing glomerular hypertension and hypertrophy or by causing contraction of mesangial cells and therefore
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altering intraglomerular circulation and glomerular filtration membranes perme selectivity. In this study, we observed that plasma and intrarenal angiotensin II, angiotensinogen, a substrate of synthesis angiotensin II, and mRNA expression of the specific converting angiotensin II enzyme ACE increased. Antihypertensive therapy with benazepril can ameliorate hypertensive renal damage, consistent with decreased overproduction of angiotensin II and overexpression of ACE and angiotensinogen. However, iptakalim can also decrease BP and ameliorate hypertensive renal damage, but it has no effect on the renin-angiotensin system. This finding suggests that renoprotection of iptakalim is different from that of benazepril, which did affect the renin-angiotensin system.

The effects of iptakalim on K\textsubscript{ATP} channels were investigated in SHR. Physiological and molecular studies have shown that renal K\textsubscript{ATP} channels play an important role in renal potassium homeostasis and renal microvascular tone under physiological conditions (Carmines and Fujiwara, 2002). The structure and function of renal K\textsubscript{ATP} channels may be changed under pathological conditions (Sgard et al., 2003). Renal ischemia followed by reperfusion provokes differential regulation of K\textsubscript{ATP} subunits. In hypertension, K\textsubscript{ATP} channel activity in renal microvasculature was attenuated in SHR, which may be related to elevated preglomerular vascular resistance (Mimuro et al., 1998). Furthermore, the attenuated K\textsubscript{ATP} channel activity can be reversed by long-term antihypertensive treatment (Ohya et al., 1996). This indicates that such alternation in channel activity might contribute, at least in part, to the elevated preglomerular vascular tone in hypertension. Our study further demonstrated that the renal mRNA expression of K\textsubscript{ATP} subunits SUR2, Kir6.1, and Kir1.1 was up-regulated under hypertensive conditions. Hypotensive doses of iptakalim can down-regulate the overexpression of Kir6.1 and Kir1.1 with no effect on SUR2. Molecular and pharmacological studies have shown that the SUR subunit is regarded as a binding site for drugs, whereas Kir acts only a pore-forming protein to regulate potassium channels. However, recent studies have shown that Kir also plays an important role in effect of drugs on K\textsubscript{ATP} channels (Ashcroft and Gribble, 2000). Our study demonstrates that Kir6.1 or Kir1.1 may be a potential target for the hypertensive renal protection of iptakalim. However, benazepril can decrease BP and ameliorate hypertensive renal damage equally, but it has no effect on K\textsubscript{ATP} channels. This finding suggests that the molecular mechanism the renoprotection by iptakalim antihypertensive therapy is different from that of benazepril, which may be related to down-regulating the overexpression of Kir6.1 and Kir1.1.

In summary, our study indicated that, in the SHR model, iptakalim could not only reduce blood pressure effectively but also reverse hypertensive renal damage. Furthermore, long-term antihypertensive therapy with iptakalim could decrease circulation and intrarenal concentration endothelin 1 and TGF-β1 and correct the elevated renal expression endothelin 1, ECE-1, TGF-β1, and the K\textsubscript{ATP} channels subunits Kir6.1 and Kir1.1.

References


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