In vivo visualization of characteristics of renal microcirculation in hypertensive and diabetic rats

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Yamamoto, Tokunori, Yuichi Tomura, Hiroyoshi Tanaka, and Fumihiko Kajiya. In vivo visualization of characteristics of renal microcirculation in normal [Wistar-Kyoto (WKY)], spontaneously hypertensive (SHR), and streptoyotocin-induced diabetic rats (STZ). Am J Physiol Renal Physiol 281: F571–F577, 2001.—We developed a videomicroscope system with a charge-coupled device camera and evaluated it in the investigation of the glomerular microcirculation in normal [Wistar-Kyoto (WKY)], spontaneously hypertensive (SHR), and streptoyotocin-induced diabetic rats (STZ). In WKY, the diameter of the afferent arterioles (Af) was 11.9 ± 0.7 μm and that of the efferent arterioles (Ef) was 8.9 ± 0.7 μm. Af and Ef in each glomerulus could be visualized simultaneously with continuous recording of blood pressure and renal blood flow. In SHR, Af diameter was constricted to ~60% of that in WKY. A dose-dependent dilation of Af and Ef was observed after administration of barnidipine (1–10 μg/kg iv), a calcium channel antagonist, in all three groups. No change was seen in the Af-to-Ef diameter ratio (Af/Ef ratio) in WKY. In SHR, the Af/Ef ratio increased significantly because of the marked dilation of Af after barnidipine administration. In contrast, barnidipine diluted Ef in STZ, causing a significant reduction in the Af/Ef ratio. This system can analyze in vivo glomerular microcirculation and systemic macrocirculation simultaneously, allowing more direct investigation of the characteristics of and acute changes in glomerular microcirculation in pathological animals.

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The newly developed cone-shaped lens allows a tip diameter of 1 mm, magnification of \( \times 520 \), and spatial resolution of 0.86 \( \mu \text{m} \), permitting each erythrocyte in the glomerulus to be identified.

Using this system, we directly observed systemic hemodynamics and in vivo glomerular microcirculation in anesthetized rats. The characteristics of the glomerular microcirculation in SHR and STZ were demonstrated, and acute changes in glomerular microcirculation were studied after intravenous administration of \([3']\)-benzyl-3-pyrrolidinyl methyl[4S]-2,6-dimethyl-4-[m-nitrophenyl]-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride (barnidipine) (11), a calcium channel antagonist developed by Yamanouchi Pharmaceutical (Tokyo, Japan).

**MATERIALS AND METHODS**

Pencil-probe videomicroscope with a CCD camera. The experimental system consists of a special-ordered pencil-probe videomicroscope with a CCD camera (Nihon Kohden, Tokyo, Japan), micromanipulator, light source (LA-60Me, Hayashi, Tokyo, Japan), monitor (PVM-146J, Sony, Tokyo, Japan), videocassette recorder (VCR; WV-ST1, Sony), and a computer for image analysis (Power Macintosh G3, Apple Computer, Cupertino, CA) (Fig. 1). The system was modified for renal use from our previously reported needle-probe CCD videomicroscope system (9, 24). By employing a corn-shaped lens (optical magnification = \( \times 13.5 \), \( F \) number = 16.15) without an intervening relay lens, we were able to reduce the diameter of the tip to 1 mm. Ample light volume was secured from the xenon light source by distributing 8 optical fibers around the tip of the lens. The lens was fitted with a 12.7-mm grayscale CCD image sensor (IK-C41MF, Toshiba, Tokyo, Japan) at the focal length (200 mm) of the lens. A green filter to complement red was placed in front of the objective lens. A green filter (Toshiba, Tokyo, Japan) was secured from the xenon light source by distributing 8 optical fibers around the tip of the lens. The lens was fitted with a 12.7-mm grayscale CCD image sensor (IK-C41MF, Toshiba, Tokyo, Japan) at the focal length (200 mm) of the lens. A green filter to complement red was placed in front of the objective lens. A green filter (Toshiba, Tokyo, Japan) was secured from the xenon light source by distributing 8 optical fibers around the tip of the lens. The lens was fitted with a 12.7-mm grayscale CCD image sensor (IK-C41MF, Toshiba, Tokyo, Japan) at the focal length (200 mm) of the lens. A green filter to complement red was placed in front of the objective lens. A green filter (Toshiba, Tokyo, Japan) was secured from the xenon light source by distributing 8 optical fibers around the tip of the lens. 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Diabetes was induced in the STZ group by injection of 55 mg/kg STZ (Sigma, St. Louis, MO) in the tail vein of 12-wk-old WKY, 2 wk before the experiment.

Rats were anesthetized with 100 mg/kg (ip) thiobutabarbital (Wako Chemicals, Osaka, Japan). A polyethylene catheter (PE-50) for measuring blood pressure was inserted in the right femoral artery. The right femoral vein was cannulated with a PE-50 catheter for administration of drugs. The left kidney was exposed via a flank incision and isolated from surrounding tissues. After the left renal artery was detached, a probe (inside diameter 1 mm) for measuring renal blood flow (RBF) was attached to it and connected to an ultrasonic flowmeter (Transonic Systems, Ithaca, NY). The arterial catheter was connected to a pressure transducer (TP-400, Nihon Kohden), and blood pressure was continuously recorded via a polygraph system (RM-6100, Nihon Kohden). Heart rate (HR) was measured by blood pressure pulse wave via a tachometer (AT-601G, Nihon Kohden). RBF was also continuously recorded via a polygraph system.

The capsule of the renal cortex was removed, and the renal surface was incised (diameter, 1 mm; depth \( \sim 0.5 \) mm) using a scalpel at an extrarenal (Zondek line) location with little vascular predominance as in nephrolithotomy. The tip of the pencil-probe CCD videomicroscope, which was fixed to a micromanipulator capable of adjusting the coordinates on the \( X \), \( Y \), and \( Z \)-axes, was then guided diagonally to the inside edge of the excision on the renal surface. Lateral glomeruli from the inside edge of the excision not affected by the excision were observed. Superficial glomeruli in which \( A_f \), \( E_f \), and the glomerular outer boundary could be confirmed and in which blood flow was not influenced by surgical insult were used in the experiment. One glomerulus per left kidney per rat was monitored in each protocol. The number of glomeruli and the number of animals is the same, and all parameters were measured in each rat. The direction of blood flow in \( A_f \)
RESULTS

Typical WKY, SHR, and STZ glomerular images acquired by this system are shown in Fig. 3. A, B, and C. It was possible to measure hemodynamic values (MBP, HR, RBF) simultaneously. After each parameter was measured, barnidipine (1 mg/kg, and each parameter was measured 10 min after each dose of barnidipine was increased to 3 mg/kg) was administered to the rats. Ten minutes later, hemodynamic parameters were measured before administration of drugs, after hemodynamic parameters were measured 10 min after each dose of barnidipine was increased to 3 mg/kg, and then to 10 mg/kg iv). Intravenous administration of barnidipine (3 and 10 mg/kg) was significantly (P < 0.01) larger than that of WKY (3 mg/kg) lowered MBP dose dependently in WKY (P = 0.01) (Table 1). The maximum decrease in MBP after administration of barnidipine was significantly (P < 0.01) larger than that of WKY. In the seven STZ, there were no significant increases in MBP, HR, and RBF after administration of barnidipine (10 mg/kg) also induced a significant (P < 0.01) increase in MBP (P < 0.01). Barnidipine did not affect the MBP, HR, and RBF simultaneously.

There was no difference in the effects of barnidipine on MBP and glomerular size. When the glomeruli were continuously recorded on a VCR, glomerular blood flow did not inhibit glomerular blood flow. The images of the glomeruli were visualized clearly, and it was acquired by this system are shown in Fig. 3. Af, Ef, and Af diameter were measured before administration of drugs, after hemodynamic parameters were measured before administration of drugs, after hemodynamic parameters were measured 10 min after each dose of barnidipine was increased to 3 mg/kg, and then to 10 mg/kg iv). Intravenous administration of barnidipine (3 and 10 mg/kg) was significantly (P < 0.01) larger than that of WKY. In the seven STZ, there were no significant increases in MBP, HR, and RBF after administration of barnidipine (10 mg/kg) also induced a significant (P < 0.01) increase in MBP (P < 0.01). Barnidipine did not affect the MBP, HR, and RBF simultaneously.

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Intravenous administration of barnidipine (1, 3, and 10 μg/kg) caused a dose-dependent reduction in MBP in SHR (Table 1). Barnidipine (1 and 3 μg/kg iv) resulted in a dose-dependent increase in RBF (P < 0.01), but barnidipine (10 μg/kg iv) decreased RBF (P < 0.01). Af diameter after administration of barnidipine (3 and 10 μg/kg) increased significantly (P < 0.01) in a dose-dependent manner compared with the basal measurement. Similarly, barnidipine (3 and 10 μg/kg iv) also resulted in a significant (P < 0.05, P < 0.01, respectively) dose-dependent increase in Ef diameter in SHR. Barnidipine (3 and 10 μg/kg iv) caused an increase in the Af/Ef ratio (Fig. 4). A typical image of the acute effects of barnidipine on glomerular microcirculation in SHR is shown in Fig. 5.

Barnidipine (1, 3, and 10 μg/kg iv) lowered the MBP dose dependently in STZ (Table 1). After administration of the maximum dose (10 μg/kg), RBF decreased and Af were dilated in STZ (P < 0.01). On the other hand, Ef diameter was also increased in STZ after administration of barnidipine (3 μg/kg), and a greater increase was observed in Ef diameter after administration of 10 μg/kg (P < 0.01). The maximum dose of barnidipine (10 μg/kg) resulted in a significant reduction in the Af/Ef ratio (P < 0.01) (Fig. 4).

Table 1. Systemic and glomerular hemodynamics before and after barnidipine administration in all experimental groups

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>MBP, mmHg</th>
<th>RBF, ml/100 g BW</th>
<th>Af Diameter, μm</th>
<th>Ef Diameter, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n = 6)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Basal</td>
<td>79 ± 4</td>
<td>1.60 ± 0.17</td>
<td>11.9 ± 0.8</td>
<td>8.8 ± 0.7</td>
</tr>
<tr>
<td>Barnidipine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 μg/kg iv</td>
<td>79 ± 4</td>
<td>1.58 ± 0.15</td>
<td>12.5 ± 0.8</td>
<td>8.8 ± 0.8</td>
</tr>
<tr>
<td>3 μg/kg iv</td>
<td>74 ± 3§</td>
<td>1.46 ± 0.15</td>
<td>13.1 ± 0.8</td>
<td>10.3 ± 0.7§</td>
</tr>
<tr>
<td>10 μg/kg iv</td>
<td>58 ± 2§</td>
<td>0.99 ± 0.10</td>
<td>13.7 ± 0.9§</td>
<td>11.0 ± 0.8§</td>
</tr>
<tr>
<td>SHR (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>147 ± 6†</td>
<td>1.48 ± 0.13</td>
<td>6.8 ± 1.1§</td>
<td>10.0 ± 0.6</td>
</tr>
<tr>
<td>Barnidipine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 μg/kg iv</td>
<td>135 ± 0.6§</td>
<td>1.59 ± 0.13§</td>
<td>7.8 ± 1.1</td>
<td>10.6 ± 0.3</td>
</tr>
<tr>
<td>3 μg/kg iv</td>
<td>117 ± 4§</td>
<td>1.75 ± 0.13§</td>
<td>9.0 ± 0.9§</td>
<td>11.2 ± 0.4§</td>
</tr>
<tr>
<td>10 μg/kg iv</td>
<td>82 ± 2§</td>
<td>1.10 ± 0.13§</td>
<td>11.6 ± 1.7§</td>
<td>12.5 ± 0.8§</td>
</tr>
<tr>
<td>STZ (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>81 ± 5</td>
<td>1.84 ± 0.16</td>
<td>14.0 ± 1.9</td>
<td>9.4 ± 0.7</td>
</tr>
<tr>
<td>Barnidipine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 μg/kg iv</td>
<td>77 ± 5‡</td>
<td>1.81 ± 0.17</td>
<td>14.5 ± 2.1</td>
<td>10.3 ± 0.8</td>
</tr>
<tr>
<td>3 μg/kg iv</td>
<td>72 ± 6§</td>
<td>1.67 ± 0.18</td>
<td>15.3 ± 1.8</td>
<td>11.5 ± 0.7‡</td>
</tr>
<tr>
<td>10 μg/kg iv</td>
<td>63 ± 5§</td>
<td>1.35 ± 0.17</td>
<td>15.7 ± 1.8§</td>
<td>13.9 ± 1.3§</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. MBP, mean blood pressure; RBF, renal blood flow; Af, afferent arteriole; Ef, efferent arteriole; BW, body weight; WKY, Wistar-Kyoto; SHR, hypertensive rats; STZ, streptozotocin-induced diabetic rats. *P < 0.05, †P < 0.01 vs. basal values in WKY. ‡P < 0.05, §P < 0.01 vs. basal values in each experimental group.
The percent change from basal values in MBP is shown in Fig. 6. The depressor effect induced by barnidipine administration was significantly \( P < 0.01 \) larger in SHR compared with WKY, but no difference was seen between STZ and WKY (Fig. 6). Barnidipine administration did not produce a change in glomerular size in WKY. Additionally, there was no difference in the percent change in glomerular size between SHR and WKY, although it decreased in STZ compared with WKY \( P < 0.05 \) (Fig. 6).

Fig. 4. Effects of barnidipine on the \( \text{Af/EF} \) in WKY, SHR, and STZ. Values are means \( \pm \) SE; \( n = \) number of rats. * \( P < 0.05 \), † \( P < 0.01 \) vs. basal values in each experimental group. \( \text{Af/EF} \), ratio of afferent arteriole diameters to efferent arteriole diameters.

Fig. 5. Typical glomeruli and hemodynamic status of SHR before and after barnidipine administration.

Fig. 6. Effects of barnidipine on mean blood pressure (MBP) and glomerular size in WKY, SHR, and STZ. Values are means \( \pm \) SE; \( n = \) number of rats. % change, Percent change from basal values in each group. * \( P < 0.05 \), † \( P < 0.01 \) vs. WKY.

Fig. 5. Typical glomeruli and hemodynamic status of SHR before and after barnidipine administration.
DISCUSSION

Using this system, in vivo Af and Ef diameter in vivo, Steinhausen et al. (22) reported that Af and Ef diameters were 7.9 ± 0.5 and 7.7 ± 0.5 μm, respectively, in hydronephrotic rats. Hirata et al. (10) reported Af and Ef diameters of 11.5 ± 0.3 and 10.8 ± 0.2 μm, respectively. Although it should be noted that these studies differed from ours in their use of hydronephrotic rats, the mean Af and Ef diameters found in this study (11.9 ± 0.8 and 8.8 ± 0.7 μm for Af and Ef, respectively) as calculated from still images of six WKY were nearly equivalent to the diameters reported in previous studies.

Basal Af diameter (6.8 ± 1.1 μm) in SHR with developed hypertension was demonstrated to be smaller than that in WKY (11.9 ± 0.8 μm). Enhancement of autoregulatory mechanisms has been reported in SHR, including a myogenic response (14) and a tubuloglomerular feedback response (3). However, there were controversial reports on basal Af tonicity in SHR. One report demonstrated that basal Af diameter in 20-wk-old SHR is significantly smaller than that in WKY of the same age as measured in a cast study (16). Another report using isolated, perfused glomeruli did not show any difference in basal Af diameters between WKY and SHR (13). It is possible that basal vascular tonicity was altered in those in vitro experiments.

This study is, to the authors’ knowledge, the first to demonstrate directly the in vivo diameters of Af and Ef near glomeruli in SHR. Af in the filtering glomeruli were constricted in SHR. Although it was previously reported using the in vitro blood-perfused juxtaglomerular nephron technique (20) or micropuncture method (27) that the Af diameter in STZ was dilated and the Af vascular resistance was decreased in STZ, this study is the first to provide direct in vivo visualization of Af near filtering glomeruli in diabetic rats. Basal Af diameter was 14.0 ± 1.9 μm in STZ rats. We succeeded in showing significant constriction of Af in SHR (P < 0.05), although Af dilation was not statistically significant in STZ. It is necessary to increase the number of measuring points, number of glomeruli, or number of animals to obtain higher statistical power for the comparison of basal Af and Ef diameters.

In the present experiment, intravenously administered barnidipine lowered MBP and dilated Af and Ef dose dependently in all three animal groups. In general, dihydropyridine-type calcium antagonists act on L-type calcium channels, which are thought to be silent in Ef. However, the vasodilator action of each dihydropyridine-type calcium antagonist on Ef is different (8).

Amlodipine dilated angiotensin II-constricted Ef slightly more than nifedipine, and efonidipine dilated both angiotensin II-constricted Af and Ef to the same degree. There were reports that amlodipine inhibited the N-type calcium current (6) and efonidipine inhibited the T-type calcium current (17), and therefore the N- or T-type calcium channel may regulate the tonicity of Ef. Barnidipine also inhibited the N-type calcium current (6). It has been reported that barnidipine dilates Af and Ef in hydronephrotic hypertensive rats (15). Although there is no evidence of the effect of barnidipine on T-type calcium channels, barnidipine possibly dilates Ef mediated by non-L-type voltage-dependent calcium channels. Our results showed that barnidipine dilates Af and Ef in filtering glomeruli.

Dose-dependent Af and Ef dilation occurred after administration of barnidipine (1 and 3 μg/kg iv) in SHR, and RBF increased even though MBP decreased. Af and Ef dilated to a greater degree after administration of barnidipine (10 μg/kg), but RBF decreased. The dilatation of the renal microvessels could no longer compensate for the reduction in MBP after administration of barnidipine (10 μg/kg). We examined these changes directly because our system allows simultaneous observation of MBP, RBF, and Af and Ef diameter.

This system can calculate the Af/Ef ratio in each glomerulus. The Af/Ef ratio is thought to be an important indicator of acute changes in glomerular microcirculation. When systemic blood pressure is constant, an increase in the Af/Ef ratio increases internal glomerular pressure, whereas a decrease lowers it. Barnidipine did not change the basal Af/Ef ratio in WKY at any of the doses used and therefore diluted both Af and Ef to the same degree. However, the Af/Ef ratio in SHR increased dose dependently after barnidipine administration, while in STZ it decreased. MBP was markedly reduced in SHR compared with the other two rat groups. It is possible that the pronounced dilation of Af resulted from a reduction in tonicity attributable to a myogenic response in SHR (21). In addition, the decrease in the Af/Ef ratio after barnidipine administration in STZ rats indicated attenuation of the Af dilatory function. This result is consistent with a report suggesting that Af dilatory dysfunction occurs in diabetic rats, because sodium nitroprusside-induced Af dilation is attenuated in STZ (20). It was possible to show the different characteristics of Af dilatory function in SHR and STZ using the present experimental system.

In this study, barnidipine induced a pronounced hypotensive effect in SHR compared with WKY, but internal glomerular pressure might not decrease, presumably because the Af/Ef ratio increased. It is possible that internal glomerular pressure decreased in STZ because both MBP and the Af/Ef ratio decreased. Several reports on ultrastructural studies (26) and isolated rat glomeruli (4, 5) suggest that glomerular volume and internal glomerular pressure are proportionally correlated. Barnidipine induced no change in glomerular size in WKY and SHR in this study, but it reduced the size of glomeruli in STZ. This system
cannot presently be used to measure internal glomerular pressure directly, but the acute, relative change in glomerular size was in agreement with the change in internal glomerular pressure inferred from the changes in MBP and the A/E ratio.

This newly developed CCD videomicroscope system allowed us to visualize directly the in vivo glomerular microcirculation in anesthetized rats, and acute changes in glomerular microcirculation and changes in systemic macrocirculation simultaneously. Moreover, it is possible to investigate the characteristics of glomerular microcirculation in pathological animals. Therefore, this system is useful for elucidating the pathophysiology in many pathological models and investigating the acute effects of drugs on the glomerular microcirculation.

REFERENCES


