

Visualization of Renal Microcirculation in Isolated Munich-Wistar Rat Kidneys: Effects of Endothelin-1 on Renal Hemodynamic Activity

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ABSTRACT—The aim of the present study was to visualize the superficial glomeruli of the Munich-Wistar (MW) rat and to characterize the responses of the renal microvasculature to endothelin-1 (ET-1). We first examined the distribution of superficial glomeruli of the MW rat compared to that in a control strain (Wistar rat). Secondly, we examined the effects of ET-1 on the renal microcirculation of the MW rat. The right kidney was perfused with a Krebs-Ringer solution containing fluorescein isothiocyanate dextran (FITC-dextran) and was visualized under an epi-illuminated fluorescence microscope system. Changes in perfusion pressure and diameter of the microvessels accompanying the administration of ET-1 (10 fmole–300 pmole) were measured. The number of superficial glomeruli was greater in the MW rat than in the Wistar rat. ET-1 had long-lasting and dose-dependent pressor effects. Perfusion pressure showed a 3.5-fold increase compared with the control, and the afferent arterioles showed greater dose-dependent vasoconstriction than the efferent arterioles. These findings suggest that the MW rat is a useful animal model for the study of renal microcirculation and that the renal microcirculation is extremely sensitive to ET-1.

Keywords: Intravital microscope system, Renal microcirculation, Fluorescence microangiography, Endothelin-1, Munich-Wistar rat

The homeostatic regulation of the hemodynamics and the metabolic functions of the kidney is mediated by multiple control mechanisms. It has recently been suggested that endothelin-1 (ET-1), a potent long-lasting vasoconstrictor, may play an essential role in the regulation of blood flow in the arteries and veins of almost all organs (1–5). For example, the inappropriate release of ET-1 could conceivably contribute to the pathogenesis of a vast number of cardiovascular diseases, including systemic hypertension, vascular spasm, myocardial ischemia and congestive heart failure (4). Some studies have demonstrated that ET-1 stimulated the generation of inositol triphosphate (IP₃) in vascular smooth muscles, which then mobilized calcium ions from intracellular stores (6). However, the mechanisms by which endothelin mediates vascular tonus in the cardiovascular system have not yet been completely elucidated.

In the kidney, ET-1 has been shown to produce marked and sustained reductions in both renal blood flow and glomerular filtration rate (7). Therefore, it is suggested that ET-1 may be an important factor in the pathogene-

sis of hemodynamically-mediated renal failure. In fact, high affinity binding sites for ET-1 have recently been identified both in the glomeruli and in the inner medulla (8–10). Autoradiographic studies in the rat kidney have also revealed the localization of ET-1 binding in various blood vessels and tissues such as the renal artery and vein, glomerulus, arcuate artery, interlobular artery, vascular bundle and renal papilla (11). Nevertheless, the direct actions of ET-1 on the renal microcirculation have not yet been determined in detail, and evidence that ET-1 is produced within the renal microvasculature is currently lacking. To shed some light on this area, the effects of endothelins on renal microcirculation should be investigated under direct intravital microscopic observation, if a proper animal model is available.

The glomerulus is generally surrounded by convolutions of the proximal tubules, and thus is not directly accessible from the surface. In the Munich strain of Wistar rats (Munich-Wistar; MW), however, some glomeruli are located near the surface of the kidney (12, 13). This unique characteristic of MW rat kidney has permitted the

direct measurement of hydrostatic pressure in glomerular capillaries and Bowman's spaces, and the evaluation of various factors that may determine the glomerular filtration rate (14). Such characteristics of the MW rat have particular advantages over those of other species in visualizing the renal microcirculation by intravital microscopy. Furthermore, since some intrarenal mechanisms that indirectly affect renal hemodynamics are lacking in isolated perfused kidney preparations, an *in vivo* model may allow the additional effects of ET-1 to be investigated by microscopy.

The present study was performed to visualize the microcirculation in the superficial glomeruli using the isolated perfused MW rat kidney and to directly characterize the actions of ET-1 on the afferent and efferent arterioles. Our findings indicate that ET-1 is a potent renal vasoconstrictor that exerts its vasoconstrictive effects predominantly in the arterioles, and in particular in the afferent arterioles.

MATERIALS AND METHODS

All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals established by the University of Tsukuba, and these guidelines are in agreement with those of the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Two series of experiments, i.e., morphological studies for observing the distribution of glomeruli and the intravital microscopic observation of renal microcirculation, were performed.

Morphological studies

In this series of experiments, the distribution of superficial glomeruli in the kidney of the MW rat (Charles River Laboratories, Atsugi) was compared with that in a control (Wistar) strain (Charles River Laboratories). Male MW rats ($n=5$) and Wistar rats ($n=5$) of the same age (7–8 weeks), weighing 200–230 g, were sacrificed by an intravenous overdose of pentobarbital sodium, and the right kidneys were immediately removed. The kidneys were fixed in 10% formaldehyde, cut into 10- μ m-thick sections and stained with hematoxylin-eosin.

The numbers of glomeruli in the MW and Wistar rats were counted on light photomicrographs. Each photomicrograph was divided into 5 area zones of the same width (200 μ m), parallel to the renal surface. For each zone, the total number of glomeruli was counted manually for both strains. Measurements were repeated three times on the same photomicrograph, and the measured values were averaged.

Intravital observation of the renal microcirculation

The MW rats ($n=10$) were anesthetized with intraperitoneal injection of pentobarbital sodium (50 mg/kg), and sodium heparin (200 units/kg) was injected via the femoral vein for anticoagulation.

The kidney was then exteriorized, and microscopic observation of the renal microcirculation was carried out while the perfusate was constantly circulated. In these experiments, the right kidneys were chosen for the perfusion experiments, because the superior mesenteric artery branches were at the same level as the right renal artery, and, consequently, a cannula to perfuse the kidney could be passed from side to side without causing blood loss and without interrupting the blood flow to the kidney during preparation (15). After laparotomy, loose ligatures were placed around the right renal and the superior mesenteric arteries. The right renal artery was then cannulated by introducing a polyethylene cannula (3-french) for the injection of ET-1 and for monitoring the perfusion pressure through the superior mesenteric artery. The right kidney was perfused with Krebs-Ringer solution by means of a roller pump (Masterflex PA-21; Cole-Parmer Instrument Co., Chicago, IL, USA) at a constant flow rate of 2–2.5 ml/min during the experiments. The vena cava was dissected, and the kidney was quickly removed, after which the surrounding connective tissue was then removed.

The composition of the perfusate was: 113 mM NaCl, 4.8 mM KCl, 2.2 mM CaCl_2 , 1.2 mM MgSO_4 , 25 mM NaHCO_3 , 5.5 mM, containing 5.5 mM glucose and 0.8 mg/ml bovine serum albumin (Fraction V; Sigma Chemical Co., St. Louis, MO, USA). The medium was maintained at 37°C and was aerated with 95% O_2 -5% CO_2 . Its pH was adjusted to approximately 7.4.

The microcirculatory perfusate blood flow in the isolated perfused kidney was then observed by an intravital television microscope system. The arrangement of the intravital microscope system and the perfusion circuit is shown schematically in Fig. 1. This system and the microvascular visualization technique were essentially the same as those that we used previously to visualize the myocardial microcirculation (16). In short, to obtain a clear visualization of the perfused flow in the microvessels, fluorescein isothiocyanate (FITC)-labeled dextran (FITC-dextran-150S, MW 150,000; Sigma Chemical Co.) was dissolved in the perfusate (1 mg/ml), and its fluorescence was visualized with a CCD video camera (model VC-D300; Sanyo Electric Co., Ltd., Osaka) under an epi-illuminated fluorescence microscope system (model VANOX; Olympus Optical Co., Ltd., Tokyo). Video images from the CCD camera were recorded with a video cassette recorder (model AG-2860; Matsushita Electric Industry Co., Ltd., Osaka).

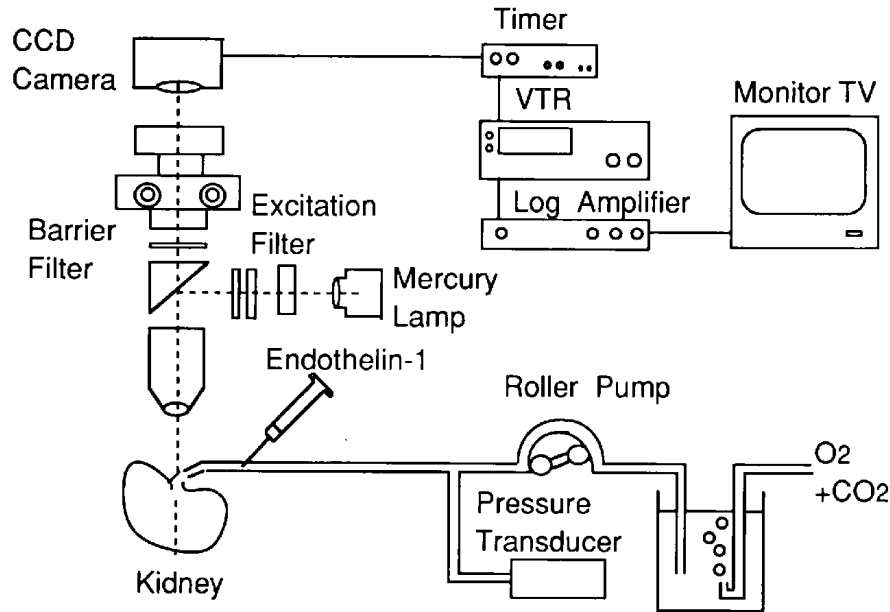


Fig. 1. Schematic diagram of the apparatus used to study microvessels in isolated perfused Munich-Wistar rat kidneys. The kidneys were perfused with Krebs-Ringer solution containing FITC-dextran and were observed under fluorescent microscopy. Renal flow was maintained constantly with a roller pump.

The observed microvessels were identified as arterioles or venules by observing the direction of movement of unstained red blood cells added to the perfusate solution (1 ml/150 ml). This perfusion technique enabled clear visualization of the perfused flow in the microvasculature, particularly in the afferent and efferent arterioles. Perfusion pressure was measured with a pressure transducer (Model MPU-0.5A; Nihon Kohden, Tokyo) through the cannula placed in the renal artery, and recorded on a polygraph system (RM-6000, Nihon Kohden).

Endothelin-1 (Peptide Institute, Osaka) was dissolved and stored in a 0.05% albumin solution to avoid deactivation. After ET-1 administration, changes in the afferent and efferent arteriolar diameters were measured in each experiment. The kidneys were allowed to equilibrate for at least 15 min before control measurements were taken. After control data were obtained, ET-1 (10 fmole–300 pmole) was administered cumulatively through a cannula placed in the perfusion circuit, and perfusion pressure and the vessel diameter were measured at 1 min after each administration of ET-1.

After each experiment, the microvascular diameter was measured directly on a printed photomicrograph of the replayed still video images of the microvasculature using a video printer (model UP-850; Sony Co., Ltd., Tokyo). Measurements of the vessel diameter were repeated three times in 5 points from the glomerulus to a distance of 30 μm on the same photograph, and the measured values were averaged.

Statistical analyses

All data are expressed as means \pm S.E.M. The contractile responses of the arterioles are expressed as percent decrease in diameter. Data were analyzed by one-way analysis of variance (ANOVA). If ANOVA indicated a significant difference, dose-dependent changes in contractile response within experimental groups were subjected to Fisher's analysis; differences between the two groups were assessed by the two-tailed unpaired Student's *t*-test (17). Probability values of $P < 0.05$ were considered statistically significant.

RESULTS

Morphological studies

Figure 2 shows a photomicrograph of a superficial glomerulus in the MW rat kidney. The photomicrographs allowed us to quantify the density of the glomeruli within an arbitrarily selected region of interest by directly counting the number of glomeruli. In this particular example, the diameter of the superficial glomerulus was estimated to be 120 μm . Our preliminary observations showed that MW rats at the age of 7–8 weeks had higher densities of superficial glomeruli than those at an age of 9–10 weeks.

Figure 3 shows the density of glomeruli thus estimated for each zone in the MW rats (solid columns) and the Wistar rats (open columns). These morphological studies showed a greater density of superficial glomeruli in the MW rats. Comparison of the densities of the glomeruli showed a significant difference between the kidneys of the

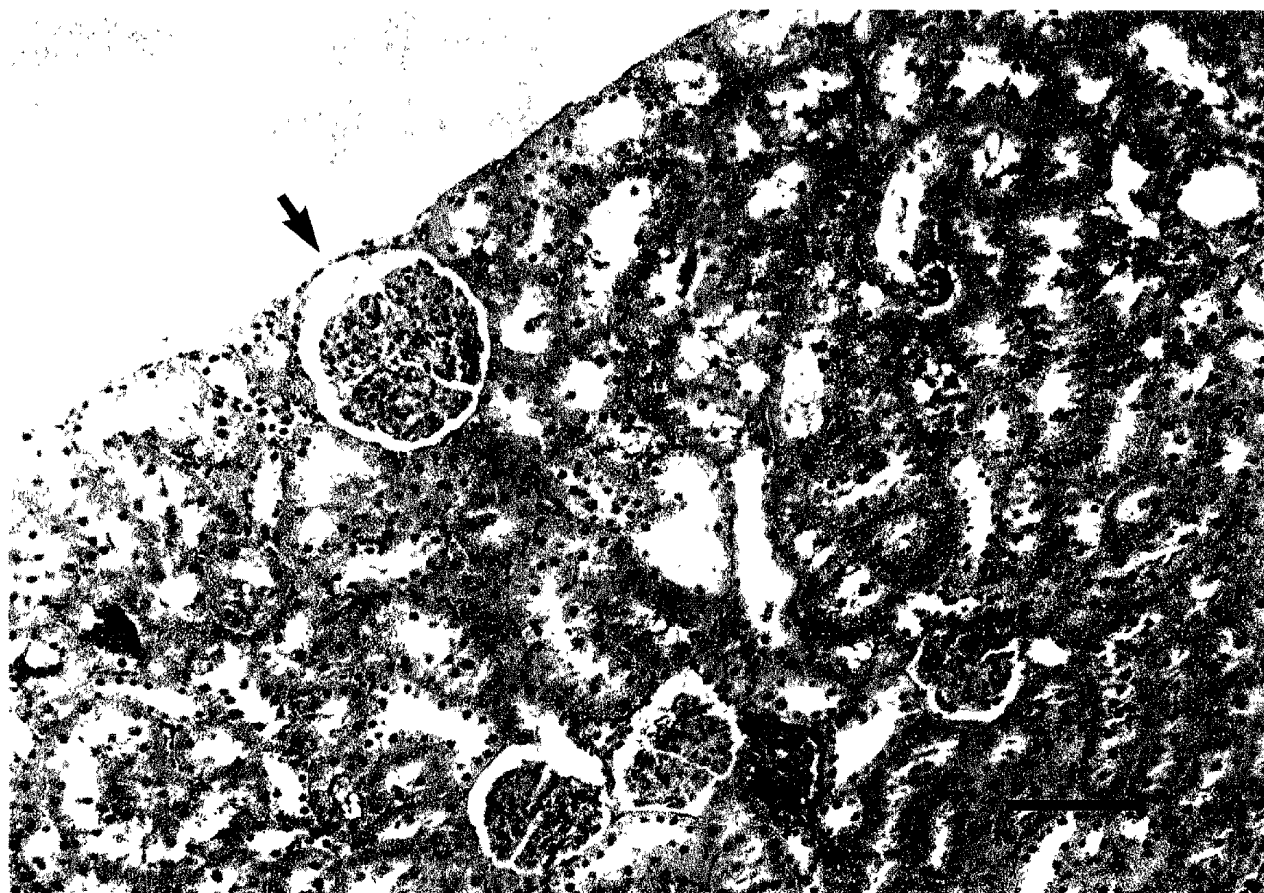


Fig. 2. Superficial glomerulus (arrow) of the Munich-Wistar rat kidney. Bar: 100 μm .

MW and the Wistar rats for depths of 0–200 μm ($P < 0.01$) and 200–400 μm ($P < 0.05$) beneath the surface

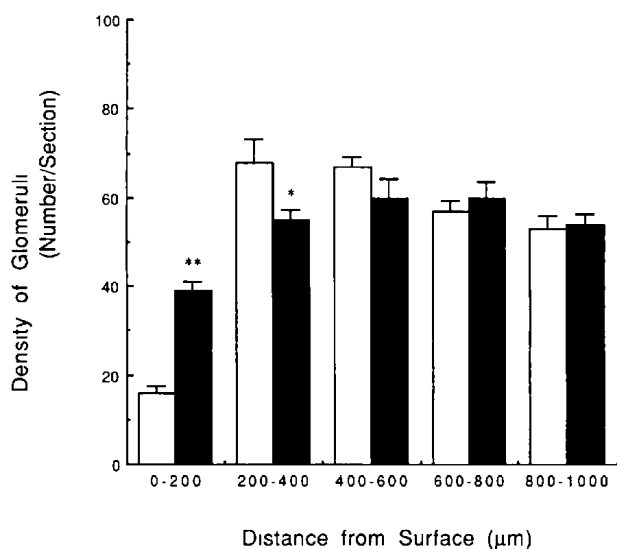


Fig. 3. Density of glomeruli in the Munich-Wistar rat (closed columns) and the Wistar rat (open columns) in each of 5 zones in the cortex. Values are expressed as means \pm S.E.M., $n=5$. * $P < 0.05$, ** $P < 0.01$ vs. Wistar rats.

of the kidney. However, the total number of glomeruli per section was not significantly different in the MW (267 ± 16) and Wistar rats (260 ± 16). All these findings suggested that the MW rat was a particularly suitable model for observing the renal microcirculation under the intravital microscope.

Intravital observation of the renal microcirculation

The isolated perfused kidneys of MW ($n=5$) and Wistar rats ($n=5$) were observed by intravital microscopy. Figure 4, taken from still playback images, shows a typical image of an efferent arteriole (diameter, 18 μm) under the fluorescent microscope. The afferent and/or efferent arterioles within the glomerulus were identified by observing the direction of movement of unstained red blood cells added to the perfusate solution.

Figure 5, in which dose-response curves for the increase in perfusion pressure following cumulative application of ET-1 are plotted, shows the pressor effects of ET-1 on the microvessels of the isolated MW rat kidney. ET-1 showed a long-lasting and dose-dependent pressor effect. The mean perfusion pressure increased significantly from 61 ± 3 mmHg at a dose of 300 fmole of ET-1 to 216 ± 15

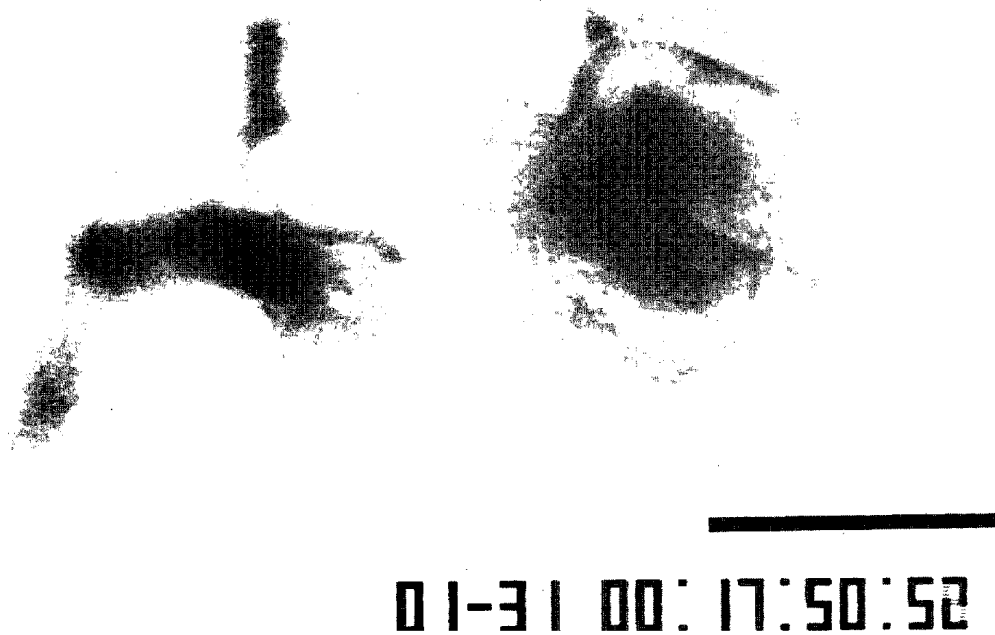


Fig. 4. Typical images of efferent arteriole in isolated perfused Munich-Wistar rat kidney under fluorescent microscopy. Bar: 50 μ m.

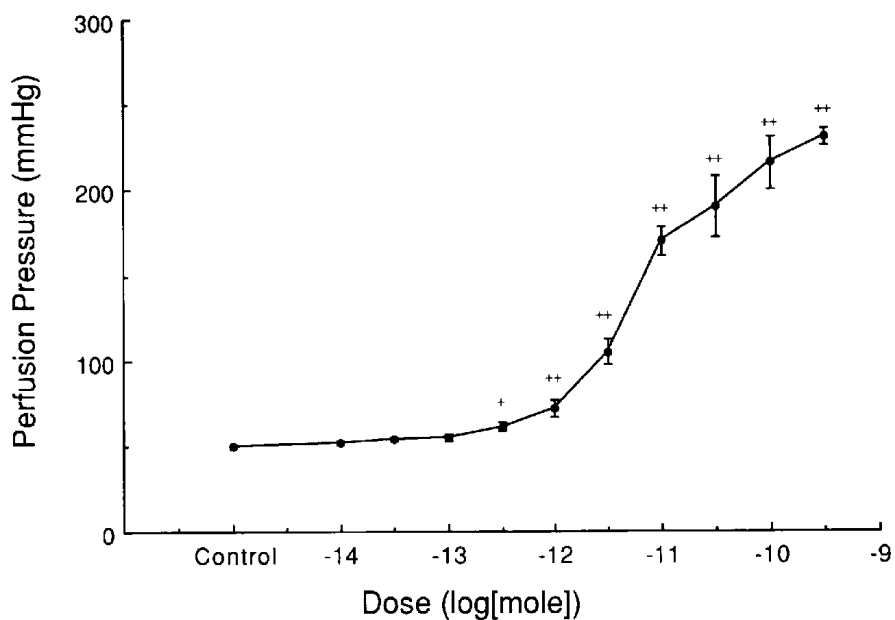


Fig. 5. Dose-dependent changes in perfusion pressure after cumulative injection of ET-1 in isolated perfused Munich-Wistar rat kidneys. Values are expressed as means \pm S.E.M., $n=10$. * $P<0.05$, ** $P<0.01$ vs. control.

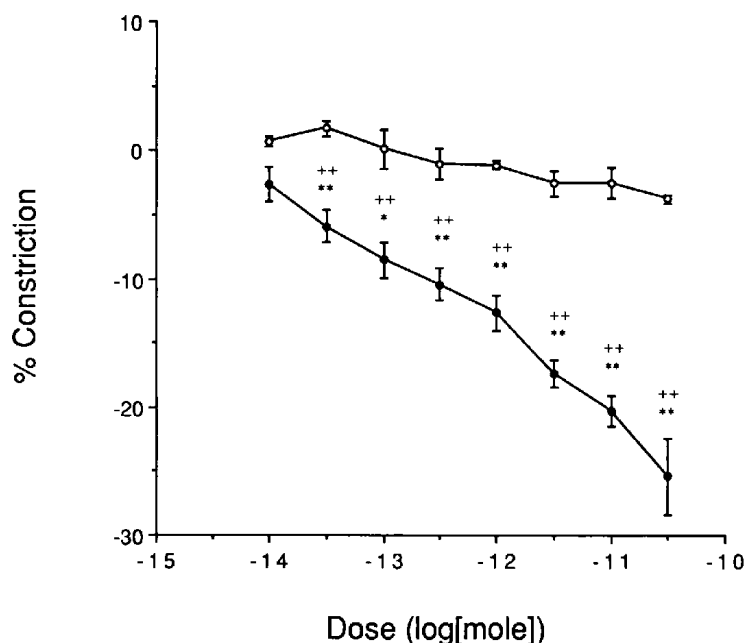


Fig. 6. Vasoconstrictive effects of ET-1 on afferent (closed circles) and efferent (open circles) arteriolar diameter in isolated perfused Munich-Wistar rat kidneys. Values are expressed as means \pm S.E.M., $n=5$. * $P<0.05$, ** $P<0.01$ vs. efferent arteriole. +++ $P<0.01$ vs. control.

mmHg at 100 pmole; i.e., a 4.3-fold increase compared with the control values (50 ± 1 mmHg). Such pressor effects of ET-1 showed significant differences from the control at doses of more than 300 fmole ($P<0.05$).

The vasoactive effects of ET-1 were easily quantified from the fluorescently visualized images of the microvasculature. Figure 6 shows the vasoconstrictive effects of ET-1 on the afferent and efferent arterioles of the MW rat. The mean diameter of the arterioles observed under the control conditions was $21.3 \pm 1.2 \mu\text{m}$ ($n=5$) and $19.8 \pm 1.4 \mu\text{m}$ ($n=5$) for the afferent and efferent arterioles, respectively. The afferent arterioles showed dose-dependent vasoconstriction after ET-1 administration, the degree of constriction of these vessels being much greater than in the efferent arterioles. The percent constriction of afferent arterioles was significantly different from that of the efferent arterioles at ET-1 doses of more than 30 fmole ($P<0.05$). Moreover, the contractile response of the afferent arterioles to ET-1 occurred evenly along the entire length of the vessel and showed a significant difference at doses of more than 30 fmole compared with the control. At higher doses of ET-1 (3–10 pmole), the blood flow in the afferent arterioles was markedly reduced. However, the contractile response of the efferent arterioles to ET-1 were not significantly different from the control values. In these experiments, unusual phenomena such as backward flow, stasis or shunt flow between the afferent and efferent arterioles were frequently observed. These findings seemed to provide evidence of drastic

microcirculatory disturbance due to administration of exogenous ET-1.

DISCUSSION

Endothelin-1 (ET-1), a peptide produced by vascular endothelial cells, is now recognized as the most potent mammalian vasoconstrictor peptide thus far described (1, 4, 18). Although the action of ET-1 as a paracrine or circulating mediator is not yet well understood, there is considerable evidence that endothelins play a substantial role in the regulation of cardiovascular and renal function, and there are clear indications of diseases in which they may be implicated (4).

Since, as yet, little is known about the mechanisms by which ET-1 controls the vascular tonus of the kidney, the role played by ET-1 in physiological and pathophysiological states in this organ is difficult to determine at present. With respect to the hemodynamic effects of ET-1 on the renal circulation, Harris et al. (19) reported that renal hemodynamic and tubular transport responses to low-dose infusions of ET-1 (1 and 10 ng/kg/min) in anesthetized rats were reduced towards their predicted physiological level, and the actions of ET-1 were predominantly depressor, vasodilator and natriuresis. Stacy et al. (20) examined the direct effects of continuous intrarenal infusions of ET-1 without inducing systemic effects and confirmed that ET-1 had profound direct actions in reducing renal hemodynamics and electrolyte excretion. These

previous studies showed that the changes in renal hemodynamics and tubular transport functions induced by ET-1 occurred at lower plasma levels than those inducing any systemic change. Furthermore, Kon et al. (21), who used anti-ET antibodies in an ischemic kidney model intending to deactivate endogenous ET, suggested that this peptide was involved in the onset of acute renal ischemic injury. Plasma levels of ET-1 have been shown to be elevated in patients undergoing hemodialysis treatment and in patients with acute or chronic renal failure (4, 22–24). Thus, although these studies show that ET-1 plays an important role in renal function and disease, we still have little information derived from the direct observation of physiological and pathophysiological states related to ET-1 in the kidney.

In the kidney, the afferent and efferent arterioles are the major sites exhibiting vascular resistance, and therefore their behavior is the most important determinant of renal blood flow and the glomerular filtration rate. Recent studies have addressed the effects of ET-1 on renal microcirculation. Badr et al. (7) reported that micropuncture pressure measurements with MW rats showed a marked increase in the afferent and efferent arteriolar resistance accompanying the intravenous administration of ET-1. Using a single isolated perfused arteriole from rabbit kidney, Edwards et al. (25) also reported that ET-1 induced potent vasoconstriction of both the afferent and efferent arterioles. In micropuncture studies using MW rats given continuous intravenous infusions of ET-1 (0.63 pmol/min), King et al. (26) reported that ET-1 caused a proportionately greater elevation of the vascular resistance of efferent rather than afferent arterioles, and they also found that a marked elevation of glomerular capillary hydraulic pressure and a lower glomerular capillary ultrafiltration coefficient were induced by ET-1. These indirect observations suggested that the renal vascular effects of ET-1 were localized to the afferent or the efferent arterioles. Recent studies utilizing video-microscopic observation of the microvascular blood flow in Wistar rats, as used in the present study, have shown that ET-1 reduced the diameter of these vessels. For example, Loutzenhiser et al. (27) reported that, in the isolated perfused hydronephrotic kidney, ET-1 was a potent vasoconstrictor of the afferent arterioles with a threshold vasoconstriction response at a dose of 0.01 nM, whereas it had a lesser effect in reducing the efferent arteriolar diameter. Fretschner et al. (28) used a split hydronephrotic rat kidney model and found that intravenous ET-1 (100 ng/kg/min) induced elevated systemic blood pressure, decreased glomerular blood flow and preferentially constricted larger preglomerular vessels such as the arcuate artery. However, to our knowledge, no direct demonstration of the involvement of the affer-

ent or efferent arterioles in normal kidneys has been made to date; the studies cited above were carried out in specialized kidneys, such as the hydronephrotic kidney. Similarly to previous reports (29), our present study of the isolated perfused MW rat kidney showed that the direct microvascular action of ET-1 was predominant in the afferent arterioles, the efferent arterioles being less affected. Moreover, our results showed a significant increase in perfusion pressure at concentrations from 300 fmole, and significant contraction of the arterioles from 30 fmole. Although we could not completely explain this discrepancy, it may be a result of differences in total renal vascular resistance and the sensitivity of afferent arterioles to ET-1.

The MW rat kidney was confirmed to be a valuable model for assessing the microvascular hemodynamic action of ET-1 in that glomeruli could be directly observed. The advantage of using this strain is that most glomeruli are located under the vicinal surface of the kidney, and with this glomerular localization, the glomerular vessels and fluid from the Bowman's capsule are directly accessible (12, 13). Therefore, extensive pharmacological and physiological studies of glomerular characteristics have been carried out with this strain (30–36). With respect to the localization of glomeruli, Hackbarth et al. (12) reported that an average of 3–4 superficial glomeruli was observed in each histological section in MW rats aged between 20 and 100 days, while in the control rat kidney, only 2 superficial glomeruli were found in all histological sections. They also reported that at ages of over 100 days, the number of superficial glomeruli diminished to about one per section. In the present study, we obtained similar results, in that the MW rats at ages of 7–8 weeks had a higher density of superficial glomeruli than those at 9–10 weeks of age. Therefore, we used the MW rats at 7–8 weeks age to observe the superficial glomeruli.

Although a few investigators have attempted to determine the effects of ET-1 on renal hemodynamics by micropuncture studies of the MW rat kidney, they were unable to determine the site of action of ET-1 in the renal microvasculature (7, 26, 36, 37). Thus, since a very limited number of studies have reported the effects of ET-1 in MW rat kidneys, we attempted in the present study to characterize the renal hemodynamic effects of ET-1 directly, in terms of morphological findings in the MW rat kidney. Moreover, since isolated perfused kidney models are free from systemic hemodynamic effects and metabolism by nonrenal tissues as well as the effects of accumulation of ET-1 in the perfusate, the present model enables the study of the effects of ET-1 *per se*. In the present study, under direct intravital microscopic observation, we demonstrated, for the first time, that the action of ET-1 was primarily characterized by a response of afferent ar-

terioles. These findings also supported the soundness of the use of the isolated perfused MW rat kidney in pharmacological and physiological studies of the renal microcirculation.

In this study, we used a fluorescently labeled tracer solution of high molecular weight (FITC-dextran, MW 150,000) to visualize or map the renal microvasculature. The use of this tracer was justified by the report of Steinhäuser et al. (38), who injected the tracer intravenously. They reported that the tubular passage of dextran was not marked and that no tubular passage was found even after increased amounts of dextran were injected. Pallone et al. (39) also reported in a micropuncture study using the MW rat that a buffer solution containing FITC-dextran (MW 2,000,000, 5 mg/ml) and albumin (MW 60,000, 0.1 g/dl) exerted only negligible intracapillary oncotic pressure (1.4 mmHg). Our preliminary study also showed that high molecular weight FITC-dextran (MW 150,000) did not alter renal hemodynamics. Therefore, we concluded that the present visualization technique was suited for experiments.

In conclusion, ET-1 alone was confirmed to be a potent vasoconstrictor with preferential activity in the renal microvasculature. The results of the present study provided evidence of the direct action of ET-1 on renal resistance vessels. Furthermore, the primary site of vasoconstriction induced by ET-1 was shown to be localized to the afferent arterioles. Under the present experimental conditions, no effects of ET-1 were observed in the efferent arterioles. Finally, the MW rat was confirmed to be a useful animal model for studying renal microcirculation.

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