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The intrarenal blood flow distribution and role of nitric oxide in diabetic rats

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Abstract

A few attempts have so far been made to determine the regional renal blood flow distribution in experimental diabetic rats. In the present experiment, 3 weeks after successful streptozotocin injection in diabetic rats (n = 8), the blood flows in the renal superficial and deep cortexes and outer medulla with implanted fibers were measured by laser-Doppler techniques. Renal blood flow was measured by an ultrasonic flow probe placed around the renal artery. Studies were performed at the baseline condition, during the administration of nonselective nitric oxide synthesis inhibitor, nitro-L-arginine methyl-ester (L-NAME), and during the postinfusion period.

The results showed that superficial cortical blood flow and deep cortical blood flow were significantly greater ($P < .05$) in diabetic rats compared with control rats (n = 8) (superficial cortical blood flow, 2.18 ± 0.22 vs 1.55 ± 0.21 V; deep cortical blood flow, 1.32 ± 0.13 vs 0.99 ± 0.14 V) with the significant increase in renal blood flow (18.1 ± 3.3 vs 14.5 ± 2.7 mL/min). Furthermore, it was shown that in diabetic rats the intravenous infusion of a low dose of L-NAME, which did not alter medullary blood flow, decreased cortical blood flow (CBF) ($P < .05$), whereas in control rats L-NAME did not affect CBF but a high dose of L-NAME decreased medullary blood flow ($P < .05$). We conclude that in early diabetic nephropathy the blood flow is increased in both the superficial and deep cortexes, and nitric oxide plays an important role in regulating the CBF during the development of diabetic nephropathy.

1. Introduction

Diabetic nephropathy is the single largest cause of end-stage renal disease. In the early phase of diabetic nephropathy, increases in renal perfusion and glomerular filtration rate, and histological glomerular hypertrophy and inappropriate dilation of afferent arterioles are seen in experimental diabetes [1,2]. However, few attempts have so far been made to determine the regional renal blood flow (RBF) distribution in experimental diabetic rats [3]. Clarification of the RBF distribution is very important to understand the pathogenesis of diabetic nephropathy.

Recently, a number of studies have demonstrated that nitric oxide (NO) plays an important role in the development of diabetic nephropathy, whereas some studies have found that NO action is decreased or unchanged in diabetic rats, and the issue of NO in renal pathophysiology is often controversial [4-6]. Several experiments have shown that in the early phase of diabetic nephropathy an increase in the synthase and action of NO could induce the elevation of glomerular filtration [2,7]. In a further study, enhanced NO synthesis (NOS) by ecNOS in afferent arterioles and glomerular endothelial cells caused the dilatation of afferent arterioles resulting in glomerular enlargement and hyperfiltration [8]. This increase in NO could be one of the pathogenic mechanisms in the development of diabetic nephropathy.

We also previously reported that the chronic systemic administration of nitro-L-arginine methyl-ester (L-NAME) decreased the renal medullary blood flow (MBF), which may result in the retention of sodium and water, and the development of hypertension in rats [9]. Furthermore, Mattson and Higgins [10] reported that the renal medulla contains more NOS than the renal cortex, by Western blotting technique. These data indicate that the endogenous renal medullary NO system plays an important role in the renal medullary circulation. Nitric oxide could be an important paracrine regulator of renal medullary function.

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Therefore, the first objective of the present experiment was to estimate the regional RBF determined by laser-Doppler techniques in streptozotocin (STZ)-induced diabetic rats. Changes in the surface and deep cortical blood flow (DCBF) and the outer MBF were measured in the early phase of diabetic nephropathy. Second, whether or not the nonselective NOS inhibitor, L-NAME, affected the regional RBF was studied.

2. Methods

Experiments were performed on 16 male Sprague-Dawley rats with 240 to 280 g body weights. The rats were housed in the Animal Resource Center at the Toho University of Medicine, with food and water provided ad libitum. Diabetes was induced by intravenous administration of STZ (Sigma, Japan) (50 mg/kg body weight) dissolved in isotonic saline. Only rats with plasma glucose level greater than 250 mg/dL and no signs of ketosis were included. Plasma glucose was measured by a standard glucose oxidase procedure. Moderate hyperglycemia was stable until the day of experiments (blood glucose level, 341 ± 43 mg/dL; on the day of experiments). In this experiment, malnourishment, catabolic state, weight loss, and hyperphagia were reduced because the 50 mg/kg STZ does not completely destroy all β-islet cells of the rat pancreas [11,12]. The results thus obtained in moderately hyperglycemic rats without insulin treatment may closely resemble the clinical condition. In control rats, a saline vehicle was infused instead of STZ. Diabetic and control rats were followed for 3 weeks. All rats received standard rat chow and tap water ad libitum. One day before the experiment, each rat was placed in a metabolic cage for urinary albumin excretion (UAE) for 24 hours. Urinary albumin excretion was measured using the developed radioimmunoassay system as previously described [13].

Three weeks after successful STZ injection in diabetic rats and saline vehicle infusion in control rats, all rats were anesthetized with Inactin (100 mg/kg IP) and placed on a heated surgical table to maintain a body temperature of 37 °C. Cannulas were placed in the femoral arteries for measurement of arterial blood pressure and collection of blood and in the jugular vein for infusion of solution. Surgical fluid losses were replaced by continuous intravenous infusion of 2% bovine serum albumin (Sigma, Japan) in a 0.9% sodium chloride solution at 1 mL/h per 100 g body weight through the experiment. The left kidney was exposed through a midline incision, isolated, and placed in a holder.

2.1. Laser-Doppler flowmetry techniques

Laser-Doppler flowmeters were used to study the changes in blood flow in 3 regions of the left kidney. The blood flow in the renal superficial cortex was measured in each rat with the external probe and implanted fiber. As previously described [14,15], the blood flows in the deep cortex and outer medulla were measured by laser-Doppler flowmetry with implanted fibers in each rat. The implanted fibers consisted of 0.5-mm-diameter fiber-optic strands connected to an external probe. The loss of light at the connection between the implanted optical fiber and the external probe was minimized by introduction of fused silica matching liquid into the connection.

The fibers were implanted in the renal cortex and medulla by inserting them directly into the kidney tissue through a small hole made in the renal capsule with a 26-gauge needle. The fiber tips were inserted 0.5 to 1 mm beneath the surface of the renal cortex to measure the net flux of red blood cells in the superficial renal cortex, and 3 to 4 mm deep and 6 to 7 mm deep to monitor changes in the deep cortex and inner medulla, respectively. The acute insertion of the fibers resulted in minimal bleeding, which stopped after several minutes. As the region from which blood flow was measured was the undamaged renal tissue beneath the tip of the fiber, we do not believe that implantation of the fiber significantly altered either the whole kidney or regional blood flow. The location of the implanted fibers was confirmed at the conclusion of each experiment by dissecting the kidney and viewing the regions surrounding the fiber tip. If the implanted fibers were incorrectly positioned or if excessive bleeding or tissue damage occurred because of accidental movement of the fibers during the experiment, the data from that rat were discarded.

Superficial cortical blood flow (SCBF), DCBF, and MBF were expressed as the respective laser-Doppler flow signals. An ultrasonic transit-time flow probe was placed around the left renal artery for measurement of RBF. The signals were transmitted to a transit-time flowmeter (PDV-20, Crystal Biotech, Northborough, Mass). This device measures absolute RBF in milliliters per minute.

The rats were studied in 2 groups: group 1, diabetic rats (n = 8); group 2, control rats (n = 8). The experiment consisted of a sequence of six 20-minute periods. Mean arterial pressure (MAP), RBF, SCBF, DCBF, and MBF were measured during two 20-minute control periods, two 20-minute periods with dose-dependent L-NAME (10 μg/kg per minute; 50 μg/kg minute was infused intravenously), and during two 20-minute postinfusion periods.

On the day of the experiment, each rat was surgically prepared as described above. Continuous measurement of MAP and RBF, and laser-Doppler measurements of SCBF, DCBF, and MBF with an implanted fiber were obtained throughout the experiment. Saline was infused intravenously for two 20-minute control periods. At the end of the second control period, L-NAME was infused at doses of 10 μg/kg per minute during a 20-minute period and then at the fourth period was infused at doses of 50 μg/kg per minute during a 20-minute period. Finally, the intravenous infusion was switched back to saline for 2 additional 20-minute postcontrol periods. A 10-minute period of equili-

Table 1
Plasma glucose levels and UAE in each group

Group	Diabetes	n	Plasma glucose (mg/dL)	UAE (μg/24 h)
1	+	8	341 ± 43[a]	695.4 ± 187.2[a]
2	–	8	139 ± 6.8	195.3 ± 72.4

[a] Significant difference from group 1 ($P < .01$).

bration was allowed before the start of the control period and between each period.

3. Statistical analysis

Values are given as mean ± SE. One-way repeated measures analysis of variance was performed for each group followed by Duncan's multiple-range test, the Wilcoxon test, and the Mann-Whitney *U* test for significance. Values of $P < .05$ were considered significant.

4. Results

As shown in Table 1, diabetic rats showed elevated plasma glucose levels and albuminuria.

4.1. Group 1: diabetic rats (n = 8)

As shown in Table 2 and Fig. 1, MAP averaged 91.3 ± 6.4 mm Hg and RBF averaged 18.1 ± 3.3 mL/min in the second control period; the voltage signals in SCBF, DCBF, and MBF averaged 2.18 ± 0.22, 1.32 ± 0.13, and 0.8 ± 0.17 V, respectively. During low-dose infusion of L-NAME, RBF, SCBF, and DCBF were significantly decreased to 15.3 ± 4.7 mL/min, 1.75 ± 0.27 V, and 1.1 ± 0.21 V, respectively. In contrast, there were no significant changes in MAP or MBF during low-dose infusion of L-NAME. During high-dose infusion of L-NAME, RBF, SCBF, and DCBF were still significantly decreased to 14.8 ± 4.9 mL/min, 1.64 ± 0.33 V, and 0.98 ± 0.17 V, respectively. In contrast, MBF was significantly decreased to 0.51 ± 0.12 V with increased MAP only during high-dose infusion of L-NAME. During the second postcontrol period, RBF, SCBF, DCBF, MBF, and MAP returned to levels not significantly different from the controls.

4.2. Group 2: control rats (n = 8)

As shown in Table 2 and Fig. 2, MAP averaged 90.3 ± 4.9 mm Hg and RBF averaged 14.5 ± 2.7 mL/min in the second control period; the voltage signals in SCBF, DCBF, and MBF averaged 1.55 ± 0.21, 0.99 ± 0.14, and 0.73 ± 0.18 V, respectively. During low-dose infusion of L-NAME, there were no significant changes. During high-dose infusion of L-NAME, MBF was significantly decreased to 0.5 ± 0.14 V with increased MAP. During the second postcontrol period, both MBF and MAP returned to levels not significantly different from the controls.

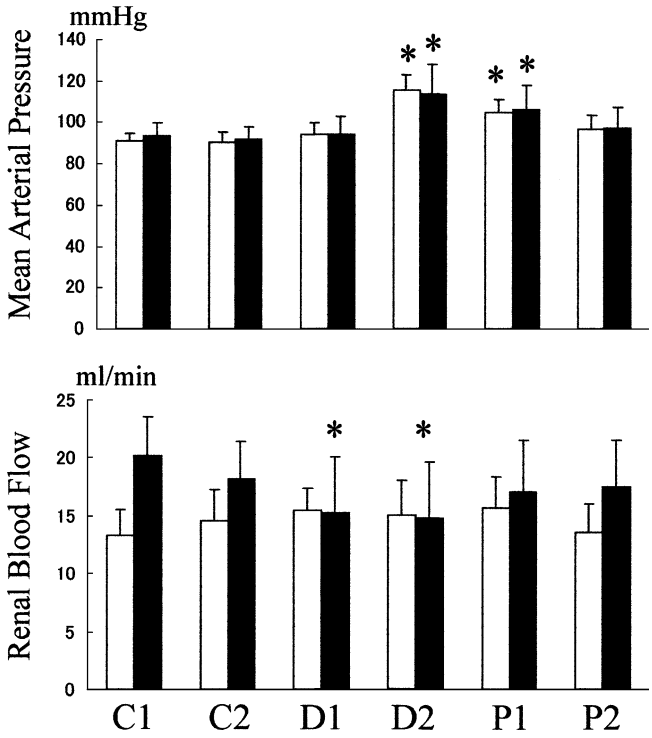

Fig. 1. Effects of intravenous infusion of L-NAME on MAP (top) and RBF (bottom) in control (□) and diabetic rats (■). C1, C2, basal values before drug administration; D1, D2, values obtained during 2 consecutive 20-minute intervals beginning at 10 minutes after drug administration; P1, P2, basal values after drug administration. *Significant difference from the second control period ($P < .05$).

5. Discussion

Recently, several articles have been devoted to the study of the hypothesis that NO-mediated renal vasodilatation contributes to glomerular hyperfiltration in diabetic rats [2,4,7,8,16,17]. As in previous studies, in this experiment L-NAME decreased the RBF and CBF in early diabetic

Table 2
MAP, RBF, SCBF, DCBF, and MBF in the second control and L-NAME (low and high) periods

Group	Diabetes	L-NAME	MAP (mm Hg)	RBF (mL/min)	SCBF (V)	DCBF (V)	MBF (V)
1	+	–	91.3 ± 4.6	18.1 ± 3.3	2.18 ± 0.22	1.32 ± 0.13	0.80 ± 0.17
1	+	Low	94.0 ± 9.0	15.3 ± 4.7*	1.75 ± 0.27*	1.10 ± 0.21*	0.70 ± 0.50
1	+	High	113.8 ± 14.1*	14.8 ± 4.9*	1.64 ± 0.33*	0.98 ± 0.17*	0.51 ± 0.12*
2	–	–	90.3 ± 4.9	14.5 ± 2.7	1.55 ± 0.21	0.99 ± 0.14	0.73 ± 0.18
2	–	Low	94.1 ± 5.8	15.4 ± 1.9	1.52 ± 0.20	1.03 ± 0.21	0.70 ± 0.14
2	–	High	115.3 ± 7.4*	15.0 ± 3.1	1.39 ± 0.26	0.84 ± 0.15	0.50 ± 0.14*

* Significant difference from the second control period ($P < .05$).

nephropathy. The low dose of L-NAME decreased SCBF, DCBF, and RBF in diabetic rats, whereas this dose did not affect MBF or MAP in diabetic rats or the renal hemodynamics in control rats. The medullary circulation is normally protected from potent vasoconstrictor actions of circulating hormones which in turn are protected by the existence of multiple counterregulatory paracrine systems much more strongly expressed in the renal medulla than in the renal cortex. These include the production of NO, CO, kinins, prostaglandins, and adenosine. Nitric oxide is functionally the best characterized of these systems and has been shown to play an important role in the maintenance of the medullary circulation [18-21]. In this experiment, a high dose of L-NAME decreased MBF without altering CBF in the control rats, which is consistent with a previous chronic study [9]. The results of the L-NAME infusion in this experiment are interesting and novel, indicating that basal blood flow in the superficial and deep cortical region of the kidney in the diabetic rat is more dependent upon NO production. Blood flow to the cortex in normal rats is far less responsive to inhibition of NOS than in the medullary flow. In contrast, it is shown that in diabetic rats both cortical and medullary flow respond to L-NAME, suggesting an important role for excess NO production in determining cortical blood flow in this model of diabetes. An interesting finding of the present study is that in control rats the regulation of MBF is more sensitive to NOS inhibition than CBF, whereas in diabetic rats the regulation of CBF is more sensitive to NOS inhibition than MBF.

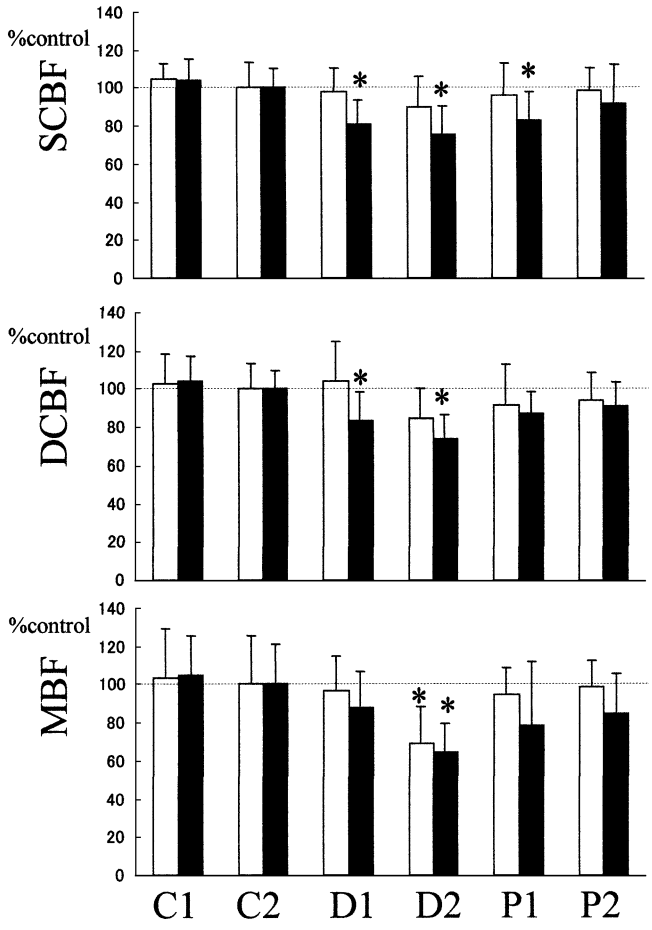

Fig. 2. Effects of intravenous infusion of L-NAME on SCBF (top), DCBF (middle), and MBF (bottom) in control (□) and diabetic rats (■). C1, C2, basal values before drug administration; D1, D2, values obtained during 2 consecutive 20-minute intervals beginning at 10 minutes after drug administration; P1, P2, basal values after drug administration. *Significant difference from the second control period ($P < .05$).

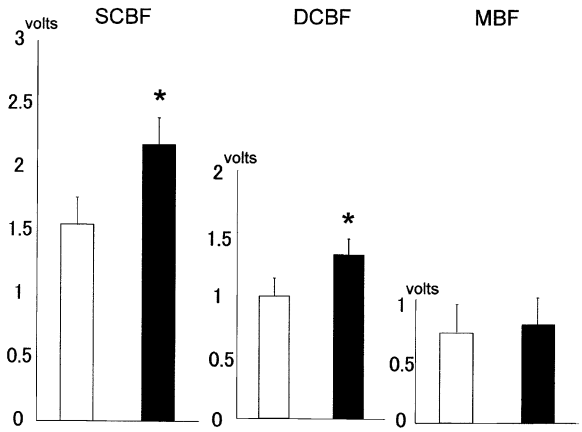

Fig. 3. SCBF, DCBF, and MBF in control (□) and diabetic rates (■) under baseline condition. *Significant difference from the control group ($P < .05$).

It is inappropriate to use laser Doppler flowmetry as a measurement of absolute blood flow in this experiment, as this system cannot be calibrated in absolute blood flow units. This method is not suitable for evaluating the differences in CBF and MBF between different animals. However, in diabetic rats the SCBF and DCBF were significantly increased to a greater extent compared with the MBF (Fig. 3). The result supports the conclusion that the cortical blood flow in diabetic rats is more dependent upon NO production. The regional blood flow distribution in the kidney could be altered in early diabetic nephropathy.

In summary, this study reports the regional renal blood distribution in STZ-treated diabetic rats under baseline conditions and after NOS inhibition. The results indicate that CBF is increased more than MBF in diabetic rats. This increase is thought to be mediated, in part, by increased NO, and the regulation of CBF is more sensitive to NOS inhibition than MBF because a lower dose of L-NAME, which did not alter MBF, decreased CBF in diabetic rats but not in control rats.

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