Mechanisms for Renal Blood Flow Control Early in Diabetes as Revealed by Chronic Flow Measurement and Transfer Function Analysis

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The purpose of this study was to establish the roles of the myogenic response and the TGF mechanism in renal blood flow (RBF) control at the very earliest stages of diabetes. Mean arterial pressure (MAP) and RBF were measured continuously, 18 h/d, in uninephrectomized control and diabetic rats, and transfer function analysis was used to determine the dynamic autoregulatory efficiency of the renal vasculature. During the control period, MAP averaged 91 ± 0.5 and 89 ± 0.4 mmHg, and RBF averaged 8.0 ± 0.1 and 7.8 ± 0.1 ml/min in the control and diabetic groups, respectively. Induction of diabetes with streptozotocin caused a marked and progressive increase in RBF in the diabetic rats, averaging 10 ± 6% above control on day 1 of diabetes and 22 ± 3 and 34 ± 1% above control by the end of diabetes weeks 1 and 2. MAP increased approximately 9 mmHg during the 2 wk in the diabetic rats, and renal vascular resistance decreased. Transfer function analysis revealed significant increases in gain to positive values over the frequency ranges of both the TGF and myogenic mechanisms, beginning on day 1 of diabetes and continuing through day 14. These very rapid increases in RBF and transfer function gain suggest that autoregulation is impaired at the very onset of hyperglycemia in streptozotocin-induced type 1 diabetes and may play an important role in the increase in RBF and GFR in diabetes. Together with previous reports of decreases in chronically measured cardiac output and hindquarter blood flow, this suggests that there may be differential effects of diabetes on RBF *versus* nonrenal BF control.

J Am Soc Nephrol 17: 2184–2192, 2006. doi: 10.1681/ASN.2006030216

Type 1 diabetes is characterized consistently by increases in GFR and renal blood flow (RBF). There is evidence that the glomerular hyperfiltration and increases in glomerular hydrostatic pressure early in diabetes may be responsible for the progression of diabetic nephropathy (1–5), but the mechanisms for controlling renal vascular resistance, particularly at the very earliest stages of diabetes, still are not defined.

It is understood that autoregulation of the afferent arteriole plays an important role in determining glomerular capillary hydrostatic pressure and glomerular filtration (6–8). Furthermore, it protects downstream glomerular capillaries from the damaging effects of increases and fluctuations in arterial BP (9,10). In diabetes, renal autoregulation may be impaired (11–14), but the relative roles of myogenic and tubuloglomerular feedback (TGF) mechanisms in controlling RBF and the time course of their involvement are not known. This is due in part to limitations caused by the effects of anesthesia; by the acute, single-point assessment of autoregulation in conscious or anesthetized animals; and by the inability to perform repeated measurements in the same animal. In addition, very little is known about autoregulatory mechanisms at the very onset of diabetes, before there has been time for renal structural changes to become manifest.

With this in mind, we modified our rat model of streptozotocin (STZ)-induced type 1 diabetes to enable long-term, continuous measurement of RBF using Transonic flow probes (Transonic Systems Inc., Ithaca, NY). Coupled with our continuous measurement of mean arterial pressure (MAP), we are able to use transfer function analysis to determine the relationship between MAP (input) and RBF (output). This type of analysis examines the dynamic ability of the renal vasculature to attenuate, or autoregulate, the influence of the oscillatory power of BP over the range of frequencies at which the myogenic response and the TGF mechanism operate (8,15–18). A major advantage of this model is that the autoregulatory efficiency of the renal vasculature can be determined at the very onset of diabetes by measuring these variables during control conditions and continuing those measurements in the same animal as diabetes is initiated.

Materials and Methods

The experiments were conducted in male Sprague-Dawley rats (350 to 375 g; Harlan Sprague-Dawley, Madison, WI), and protocols were approved by the Institutional Animal Care and Use Committee. Anes-

Received March 9, 2006. Accepted May 16, 2006.

Published online ahead of print. Publication date available at www.jasn.org.

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ISSN: 1046-6673/1708-2184

thesia was induced with pentobarbital (50 mg/kg), and atropine was administered (40 mg per rat, intraperitoneally) to minimize airway secretions. Under aseptic conditions, a laparotomy was performed, the right kidney was removed, and a 1-mm Transonic flow probe was placed on the artery of the left kidney. A nonocclusive polyvinyl catheter then was implanted in the abdominal aorta, below the renal artery, by inserting the catheter tip through a puncture wound in the aortic wall made with the tip of an L-shaped 18-G needle. The insertion point was sealed with cyanoacrylate adhesive, and the catheter and the flow probe cable were exteriorized through the lateral abdominal wall. A femoral vein catheter was implanted through a separate incision. The flow probe leads and catheters were routed subcutaneously to the scapular region and exteriorized through a Dacron-covered plastic button sutured subcutaneously over the scapulae.

The rats were allowed to recover from surgery and then were placed in individual metabolic cages in a quiet, air-conditioned room with a 12-h light cycle. The catheters and flow probe leads were passed through a stainless steel spring that was attached to the button, and the opposite end of the spring was connected to a customized adapter on an electrical swivel (Airflyte Electronics, Bayonne, NJ) mounted above the cage. The four flow probe leads were soldered to the swivel wires, and the artery and vein catheters were passed through a hole in the center of the electrical swivel and through a 3-cm section of spring to a dual-channel hydraulic swivel (Instech, Plymouth Meeting, PA) above. The short spring section served to connect the two swivels so that both would turn synchronously with rat movement. The venous catheter was connected, *via* the hydraulic swivel, to a syringe pump (Harvard Apparatus, Holliston, MA) that ran continuously throughout the study. All solutions were infused through a Millipore filter (0.22 μm, Cathivex; Millipore Corp., Bedford, MA). The arterial catheter was filled with heparin solution (1000 USP U/ml) and connected, also *via* the hydraulic swivel, to a pressure transducer for continuous measurement of AP. The flow probe connector was soldered to the electrical swivel wires and connected to a Transonic model T206 flowmeter for continuous measurement of RBF. The pulsatile flow signals from the flowmeter and the amplified pulsatile AP signals (CB Sciences, Dover, NH) were sampled at 100 Hz, 18 h/d throughout the experiment, using Powerlab and a Macintosh computer.

Total sodium intake throughout the experiment was maintained constant at approximately 3.1 mmol/d by continuous intravenous infusion of 20 ml/d sterile 0.9% saline combined with sodium-deficient rat chow (0.006 mmol sodium/g; Teklad, Madison, WI). A sodium-deficient diet ensured that the daily sodium intake could be controlled precisely at normal levels by the infusion. This infusion was begun immediately after placement of the rat in the metabolic cage, and 3 to 5 d were allowed for acclimation.

Experimental Protocol

After recovery from surgery and acclimation, the rats were divided randomly into two groups: Control ($n = 6$) and diabetes ($n = 7$). After an approximate 7-d prediabetic control period, type 1 diabetes was induced in the diabetic group with STZ (40 mg/kg, intravenously). The potential for STZ to affect our primary study variables through side effects, rather than through attenuation of insulin secretion, has been controlled for in previous studies from our laboratory (19–21). Previously, we followed STZ administration with institution of continuous intravenous insulin replacement within 24 h of STZ injection and continued the insulin treatment for approximately 1 wk at a rate that maintained normoglycemia. Diabetes then was induced by removing the intravenous insulin, and the diabetic period was followed by another intravenous insulin replacement recovery period. Those studies revealed no effect of STZ on any renal or systemic variable (including GFR and RBF, measured by clearance methods) that was not corrected by insulin replacement. Because of the extreme difficulty in successfully instrumenting and maintaining rats for our 3-wk RBF studies (approximately 4 wk counting recovery from surgery), we were forced to eliminate this additional control procedure. Although our previous results suggest that side effects of STZ do not contribute to the renal or systemic changes that we measure during hyperglycemia, the potential for side effects on autoregulation *per se* is not tested by our study design.

The diabetic experimental period lasted for 2 wk. Insulin was added to the daily saline infusion syringe when needed to keep blood glucose from exceeding 450 mg/dl. On day 4 of the control period and once per week during the 2-wk diabetic period (days 3 or 4 and 12), 1.3 ml of arterial blood was collected from the arterial catheter for measurement of GFR, plasma renin activity (PRA), hematocrit, protein, plasma electrolytes, osmolarity, and glucose concentrations. Samples were replaced with an equal volume of 0.9% saline.

Statistical Analyses

GFR was measured after a 24-h intravenous infusion of [125I]iothalamate (Glofil, Questcor, Union City, CA; approximately 20 μCi). Because steady state is achieved during the 24-h infusion (22), the isotope infusion rate was substituted for urinary isotope excretion rate to calculate clearance, as we have reported previously in this model (19,23): [$\text{Clearance}_{\text{Glofil}}$ (ml/min) = $\text{Infusion Rate}_{\text{Glofil}}$ (μCi/min)/$\text{Plasma}_{\text{Glofil}}$ (μCi/ml)]. Urinary sodium and potassium concentrations were determined with ion-sensitive electrodes (Synchron El-ise; Beckman-Coulter, Brea, CA), and urine glucose was documented with a Bayer Clinitek 50 dipstick analyzer (Elkhart, IN). PRA was measured by RIA (Diasorin, Stillwater, MN). Data were analyzed by a two-factor ANOVA with repeated measures and with unpaired t test used for supplemental between-group comparisons, and Dunnett test was used for within-group comparisons over time. A value of $P < 0.05$ was considered statistically significant, and data are presented as mean ± SEM.

Transfer Function Analysis. Daily data files that covered the period from approximately 1:00 p.m. to 5:00 a.m. (16 h) subsequently were processed off-line using previously developed software routines (24) written for Matlab (Version 7, Release 14). The 100-Hz data files were digitally low-pass filtered (3.5-Hz cutoff frequency, finite-impulse-response, order 50) and decimated to 5 Hz. These 5-Hz data files were split into blocks of 4096 data points, yielding a frequency discrimination of 0.001 Hz. Power spectral density of AP and RBF was calculated using Welch's method with units of $(\text{mmHg})^2/\text{Hz}$ and $(\text{ml/min})^2/\text{Hz}$, respectively. The transfer function spectra were calculated from AP (input) and RBF (output). The transfer function was taken as the quotient of the cross-spectrum of input and output divided by the power spectrum of the input. This algorithm involved mean detrending and a Hanning window with 50% overlap of the blocks. To permit comparison among rats, the transfer function gain (magnitude) values over the frequency range were normalized to the value at 0 Hz frequency (DC; *i.e.*, to a value of 1). After conversion of the normalized transfer function gain values into decibels (20 log[gain]), a mean spectrum was calculated from the consecutive spectra in each rat, and these subsequently were averaged for all rats.

Coherence is a frequency domain estimate of a linear correlation (*i.e.*, squared coherence, akin to coefficient of determination) between two signals that indicates the degree to which the variance in one signal can be explained by a linear operation on the other signal. The coherence spectra were calculated from AP (input) and RBF (output) during both control and experimental periods. The coherence function was taken as the quotient of the square of the cross-spectrum of input and output

divided by the product of the power spectrum of the input times the power spectrum of the output. The algorithm involved mean detrending and a Hanning window with no overlap of blocks of 256 data points yielding a frequency discrimination of 0.02 Hz. To determine the threshold for coherence above which it exceeds zero with a certain significance level, we used the method described by Koopmans (25), which depends on the total number of samples, the total number of blocks, and the nature of the tapering window. In this study with very large sample numbers, coherence values >0.01 are significantly different from zero at $P < 0.001$.

Results

Blood glucose was not different between the two groups of rats during the control period, averaging 126 ± 4 and 126 ± 6 mg/dl in the control and diabetic rats, respectively, but induction of diabetes with STZ increased blood glucose in the diabetic rats to average levels of 431 ± 16 and 477 ± 12 mg/dl during diabetic weeks 1 and 2, respectively (Table 1), and caused significant appearance of glucose in the urine. Note that one control rat was removed from the study on day 10 of diabetes. MAP averaged 91 ± 0.5 and 89 ± 0.4 mmHg in the control and diabetic groups, respectively, during the control period. As we have reported previously (20), MAP increased modestly during the diabetic period, averaging 98 ± 1 mmHg in the diabetic group by the end of the first week of diabetes (Figure 1, top).

Figure 1, bottom, shows the 18-h/d RBF measurements in both groups during the control and experimental periods. RBF averaged 8.0 ± 0.1 and 7.8 ± 0 ml/min in the control and diabetic groups, respectively, during the control period and did not change significantly in the control group. Conversely, induction of diabetes caused a marked and progressive increase in RBF in the diabetic rats, averaging 10 ± 6% above control on day 1 and 22 ± 3 and 34 ± 1% above control by the end of diabetes weeks 1 and 2, and renal vascular resistance decreased significantly during the diabetic period.

During the control period, GFR averaged 2.1 ± 0.1 and 1.7 ± 0.1 ml/min in the control and diabetic groups, respectively. GFR did not change during the experiment in the control rats but increased significantly in the diabetic group, averaging 54 ± 21 and 52 ± 19% above control during diabetic weeks 1 and 2 (Figure 2). There were no significant changes in filtration fraction in either group. PRA was not different between groups during the control period, averaging 0.8 ± 0.2 and 1.7 ± 0.8 ng angiotensin I/ml per h in the control and diabetic groups, respectively. PRA increased significantly during week 1 of diabetes in the diabetic group and returned toward control in week 2, similar to previous reports (20) (Figure 2).

Power spectral density of AP and RBF, transfer function gain from AP to RBF, and coherence between AP and RBF for both control and diabetic animals are shown in Figures 3 through 6. Within each rat in the control and diabetic groups, the values for the above-mentioned variables did not differ over control period days C1 through C7. Similarly, within each of control period days C1 through C7, these values did not differ among rats. Figure 3 represents overall mean data for all rats in the control and diabetic groups for control period days C1 through C7. Figure 4 is experimental period day D1, Figure 5 is experimental period day D7, and Figure 6 is experimental day D14.

Table 1. Blood glucose, hematocrit, plasma protein, urine albumin, and urinary Na + excretion during each study period in control and diabetic rats[a]

Variable/Group	Experimental Period		
	Control	Diabetic Week 1	Diabetic Week 2
Blood glucose (mg/dl)			
control	126 ± 4.0	121 ± 3.3	123 ± 3.2
diabetes	126 ± 6.1	431 ± 16.6[b,c]	477 ± 12.7[b,c]
Hematocrit (%)			
control	40 ± 0.5	40 ± 1.4	41 ± 1.2
diabetes	38 ± 0.9	43 ± 0.9	38 ± 1.7
Plasma protein (g/dl)			
control	6.1 ± 0.1	6.3 ± 0.2	6.5 ± 0.0
diabetes	6.0 ± 0.3	7.0 ± 0.2	6.8 ± 0.2
Urine albumin (mg/d)			
control	5.3 ± 1.2	4.9 ± 1.2	4.6 ± 0.8
diabetes	4.9 ± 1.0	5.0 ± 1.2	10.8 ± 3.0
$U_{NA}V$ (mEq/d)			
control	2.9 ± 0.3	2.7 ± 0.1	2.7 ± 0.2
diabetes	3.3 ± 0.3	3.5 ± 0.2[b]	3.4 ± 0.3

[a]Data are means ± SEM. $U_{NA}V$, urinary sodium excretion.
[b]$P < 0.05$ for within-group comparisons *versus* the control period value.
[c]For between-group comparisons at $P < 0.05$ within a given period, diabetes *versus* control; $n = 5$ in control group for diabetic week 2.

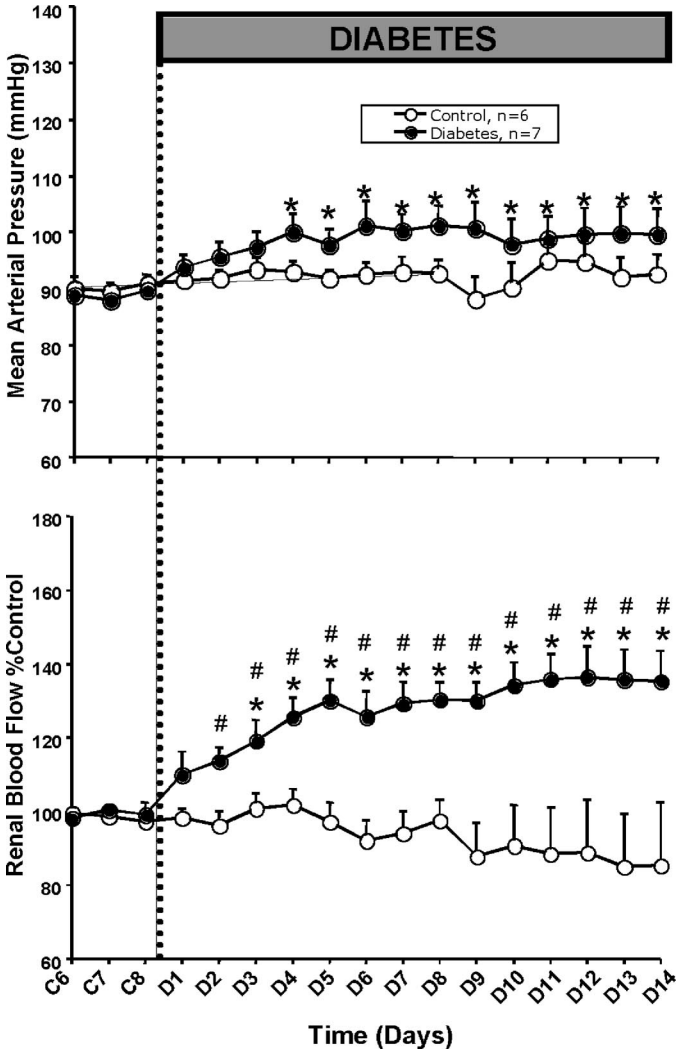

Figure 1. Mean arterial pressure (top) and renal blood flow (RBF) expressed as percentage of control (bottom) measured in control (○) and diabetic (●) rats during the last 3 d of the control (C) period and the 14-d diabetic (D) period. $^{*}P < 0.05$ compared within-group with the C period; $^{\#}P < 0.05$ *versus* between group; $n = 5$ in control group from D10 thru D14.

During the control period, power spectral density of AP and RBF, as well as transfer function gain, did not differ between control and diabetic groups. In both control and diabetic groups, the pattern of transfer function gain was characterized by negative values for gain over the frequency range of 0.001 to 0.1 Hz, a noticeable dip in gain values in the frequency range of 0.02 to 0.06 Hz, and a plateau of slightly positive gain values in the frequency range 0.15 to 0.3 Hz. The dip at 0.02 to 0.06 Hz (cycle length of 17 to 50 s) is the signature of the slower tubuloglomerular (TGF) component of RBF autoregulation. The plateau at 0.15 to 0.3 Hz (cycle length of 3 to 7 s) reflects the faster myogenic component of RBF autoregulation. Coherence for both control and diabetic groups was statistically significantly different from 0 and showed a broad peak centered at 0.4 Hz. Coherence in the diabetic group was significantly higher than that in the control group above 0.2 Hz.

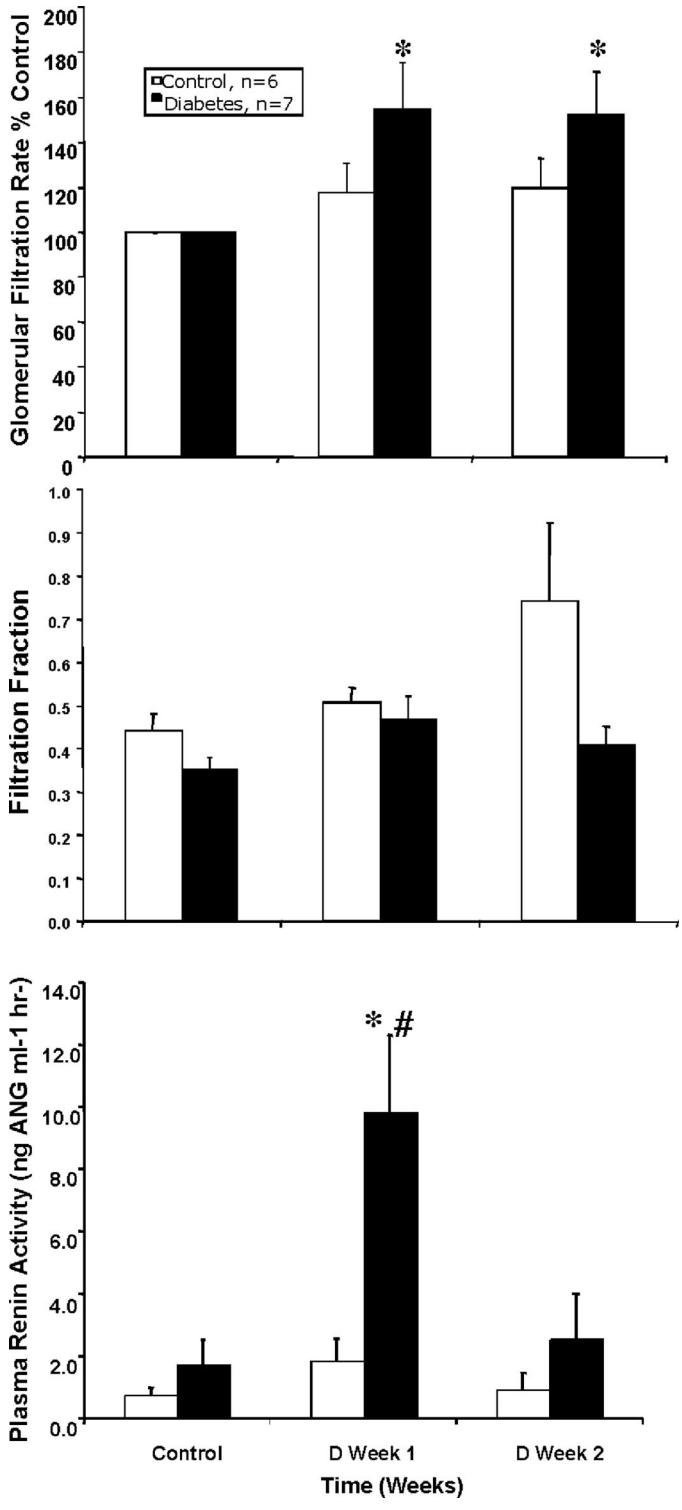

Figure 2. GFR (top), filtration fraction (middle), and plasma renin activity (bottom) measured on day 4 of the C period and days 4 and 12 of the D period in the C (□) and D (■) rats. $^{*}P < 0.05$ compared within-group with the C period; $n = 5$ in C group for D week 2.

The induction of diabetes with STZ caused rapid and marked impairment of these indexes of autoregulation. Figure 4 (D1) shows that the control rats had the same response pattern as during the control period, with negative values for

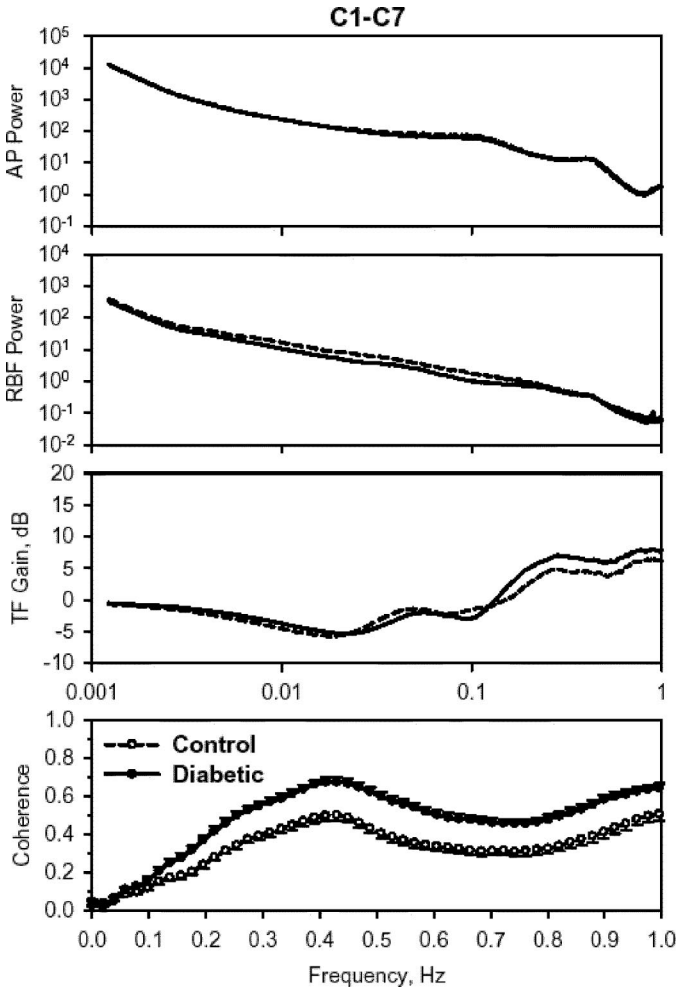

Figure 3. Results of frequency domain analysis of C period days C1 through C7. From top down, power spectral density of AP, $(mmHg)^2/Hz$; power spectral density of RBF, $(ml/min)^2/Hz$; transfer function gain, dB; and coherence. C group, ○ and dashed lines; D group, ● and solid lines.

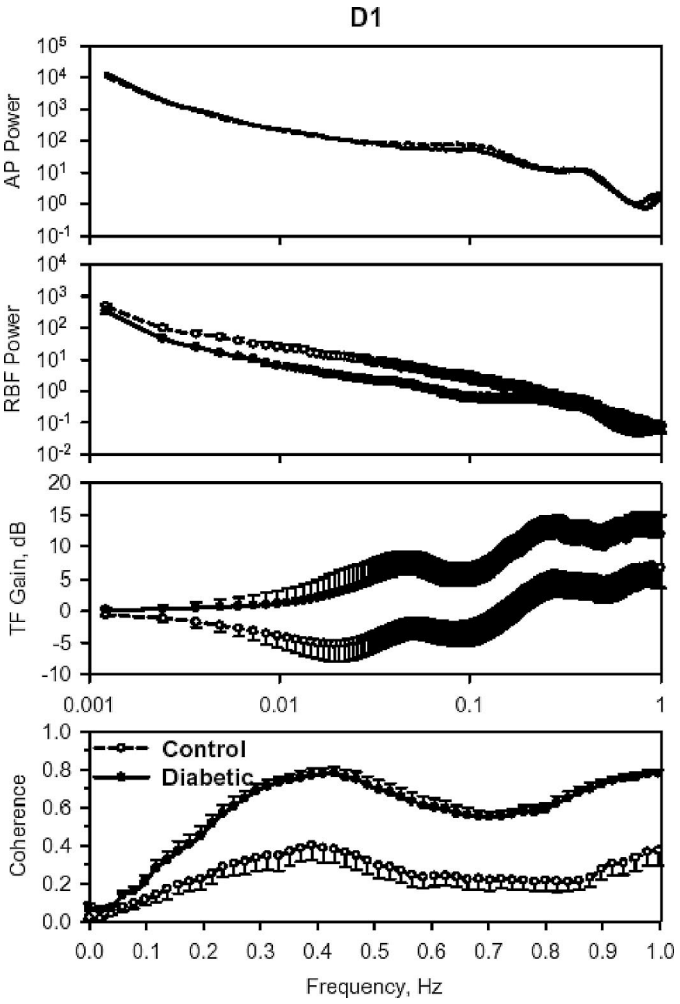

Figure 4. Results of frequency domain analysis of experimental period day D1. From top down, power spectral density of AP, $(mmHg)^2/Hz$; power spectral density of RBF, $(ml/min)^2/Hz$; transfer function gain, dB; and coherence. C group, ○ and dashed lines; D group, ● and solid lines.

transfer function gain up to slightly above 0.1 Hz, a dip in the TGF frequency range of 0.02 to 0.06 Hz, and a plateau of slightly positive gain values in the frequency range 0.15 to 0.3 Hz. In the diabetic rats, however, transfer function gain was significantly different from that in control rats, showing positive gain values throughout and lacking the TGF signature. In addition, the myogenic plateau was increased to more positive values of gain, and coherence increased markedly, being significantly higher in the diabetic group *versus* the control group above 0.15 Hz.

Figure 5 (D7) shows the continued stability of the control group and maintenance of significantly increased transfer function gain and coherence in the diabetic group. On D14 (Figure 6), power spectral density of RBF was slightly higher, although not significantly, in the diabetic *versus* the control rats, and the diabetic group continued to exhibit positive gain values throughout, lacking the TGF signature and having a more elevated myogenic plateau than in the control group.

Discussion

In this study, BP and RBF were measured continuously, 18 h/d, throughout control conditions and continuing in the same animals as they developed diabetes. Our results showed that the onset of STZ-induced type 1 diabetes caused a very rapid increase in RBF and a progressive rise throughout the 2-wk diabetic period. Moreover, transfer function analysis of the AP and RBF power spectra from every rat during the course of the experiment revealed an abrupt change in transfer function gain in the diabetic group, which began on experimental day D1 and was sustained through to experimental day D14. The rapid and sustained increases in gain over the TGF and myogenic frequency ranges, combined with the decrease in renal vascular resistance, suggest that renal autoregulation was impaired rapidly with onset of hyperglycemia early in type 1 diabetes and played a major role in the increase in RBF and GFR.

Diabetes consistently is characterized by hyperfiltration and an increase in RBF, but it is not known how diabetes causes

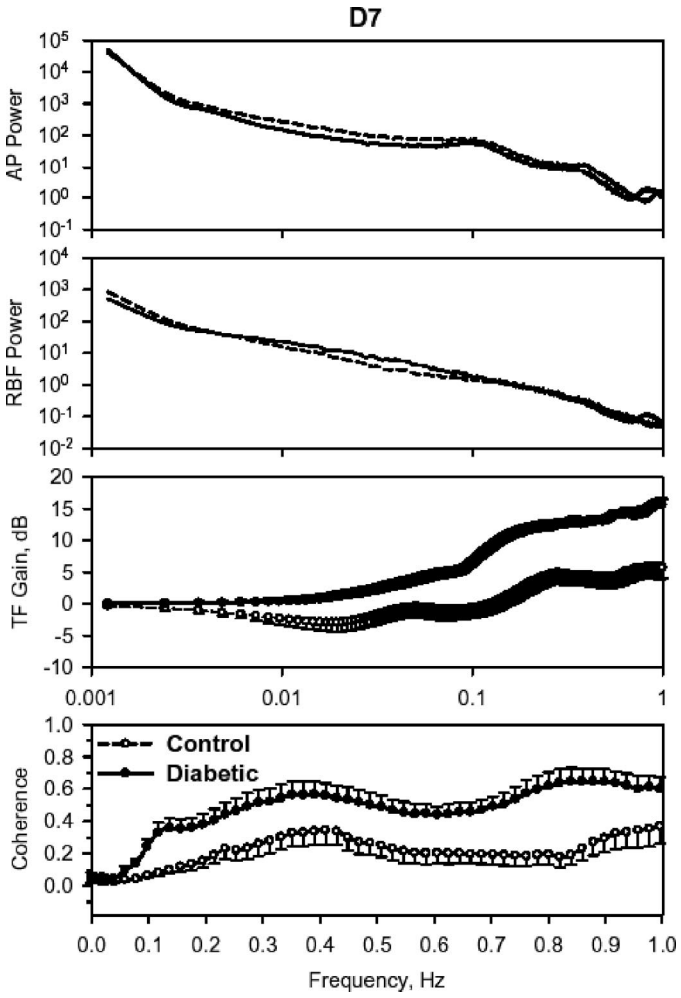

Figure 5. Results of frequency domain analysis of experimental period day D7. From top down, power spectral density of AP, $(mmHg)^2/Hz$; power spectral density of RBF, $(ml/min)^2/Hz$; transfer function gain, dB; and coherence. C group, ○ and dashed lines; D group, ● and solid lines.

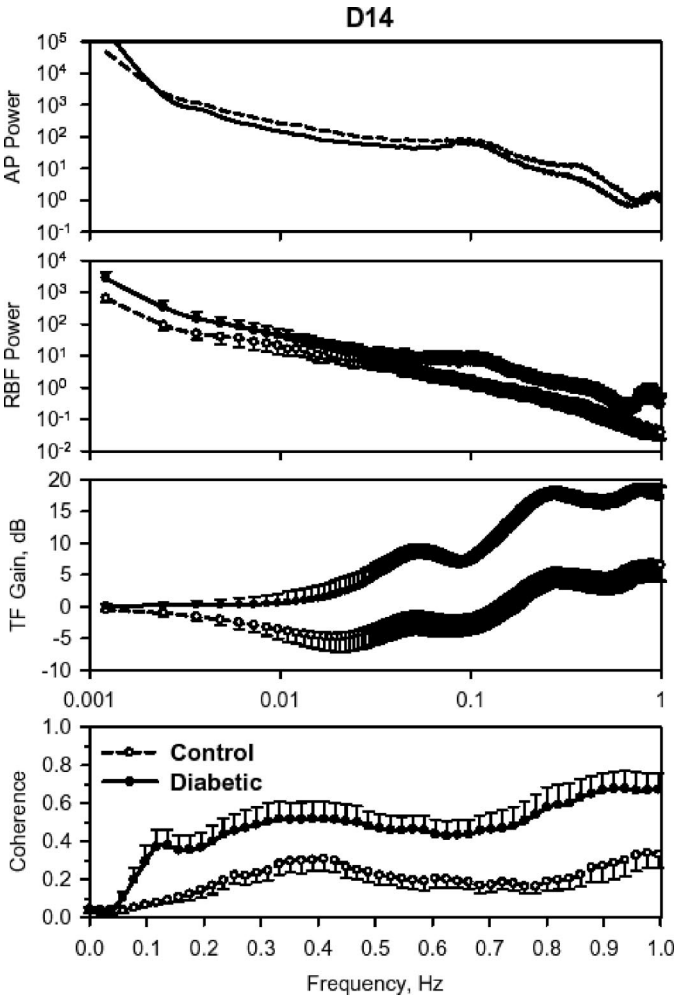

Figure 6. Results of frequency domain analysis of experimental period day D14. Results of frequency domain analysis of C period days C1 through C7. From top down, power spectral density of AP, $(mmHg)^2/Hz$; power spectral density of RBF, $(ml/min)^2/Hz$; transfer function gain, dB; and coherence. C group, ○ and dashed lines; D group, ● and solid lines.

those changes in renal hemodynamics. We reported previously that the onset of diabetes causes significant and progressive decreases in cardiac output (19) and hindquarter blood flow (21). Both were measured continuously, 18 h/d, using Transonic flow probes as done in this study. Our results also showed that the onset of diabetes was characterized by significant decreases in sodium and water balance, which contributed to the decrease in cardiac output (19). The onset of hyperglycemia very early in type 1 diabetes is a volume contracted, vasoconstricted condition overall, suggesting that the renal vasodilation is due to unique effects of the hyperglycemic state on the control of renal vascular resistance.

Our finding that transfer function gain increased significantly in the frequency range controlled by TGF is important in that regard, because TGF is a renal-specific control system for regulating organ blood flow. In general, changes in RBF lead to parallel changes in renal tubular sodium chloride delivery to the macula densa. TGF describes the response of the macula densa cells then to signal a change in renal vascular resistance, primarily at the afferent arteriole, that brings RBF back toward normal levels (7). Increased renal perfusion, for example, typically induces TGF-mediated increases in afferent arteriolar resistance to prevent or counteract increased GFR and RBF. In diabetes, however, the increased tubular glucose load increases fractional proximal tubular sodium reabsorption because of increased glucose-linked sodium transport (26,27). One potential consequence of that may be the sensing of decreased sodium chloride by the macula densa, even though total delivery and tubular flow are increased (26–28). This is similar to the effect of protein intake and filtered amino acids on renal hemodynamics (29,30), and this effect of hyperglycemia has been shown to be independent of an osmotic diuretic action *per se* (31). Therefore, this could be a mechanism through which hyperglycemia increases RBF and GFR in diabetes *via* TGF (26,27).

Blunting of TGF sensitivity also could contribute to the renal vasodilation (32–35) on the basis of evidence that renal nitric

oxide (NO) is increased early in diabetes (36–41). However, angiotensin II (AngII) has an opposite effect by increasing TGF sensitivity (7,33,42), and we measured significant increases in PRA by day 3 of diabetes, consistent with other reports in animals and patients with diabetes (43,44). Likewise, other factors that play a role in modulating or mediating the sensitivity of TGF to increases in AP, such as sodium intake and distal delivery (26), may be altered by diabetes, and this study cannot determine their relative contributions to controlling TGF. Nonetheless, there is evidence that TGF may be engaged by the diabetic state and contribute to the renal vasodilation.

The transfer function analysis in this study supports that assertion by showing that onset of STZ-induced type 1 diabetes causes very rapid blunting of autoregulation of RBF *via* TGF. The control period was characterized by negative values for gain over the frequency range of 0.001 to 0.1 Hz and a noticeable dip in gain values in the frequency range of 0.02 to 0.06 Hz, which is the hallmark of TGF-mediated autoregulation. That hallmark was lost on the first day of diabetes, and gain in the TGF frequency range reached positive values. It is important to recall that these were serial daily measurements in each rat over 7 control days and 14 post-STZ diabetic days, so the consistency of results in each rat could be followed to alleviate potential concerns that diabetes induced unpredictable changes in the frequency at which TGF was operating. Together with the difference in coherence and in RBF on day 1, these data provide strong evidence that the contribution of TGF to autoregulation was impaired and contributed significantly to the renal vasodilation. The measurement of increased GFR with no change in filtration fraction is consistent with primarily an afferent arteriolar site of the renal vasodilation, which also is consistent with the TGF mechanism for controlling renal vascular resistance. In addition, it is important to note the slight increase in MAP that occurred in the diabetic rats, because that indicates that vasodilation was not a generalized, whole-body response to diabetes, consistent with our previous reports (19–21,23).

The rapid increase in gain in the frequency range of the myogenic mechanism is particularly interesting in that regard. Others have shown that diabetes impairs the myogenic responsiveness of the afferent arteriole (13), and our data reveal impairment as soon as poor glycemic control begins. Impairment of myogenic autoregulation also is implicated as a significant contributor to the increase in RBF at the onset of diabetes. However, if myogenic autoregulation were impaired throughout the body to the extent that it was in the kidneys, then it might be difficult to reconcile that with the increase in BP that we measured. We did not assess blood flow autoregulation in other tissues, but because our MAP data and previous results (19,21) suggest that there is vasoconstriction in many nonrenal vascular beds, the impaired myogenic mechanism and increased blood flow in the kidneys may suggest that myogenic autoregulation in the kidneys is subject to unique modification by the diabetic state.

These results make it interesting to speculate on a potential role for NO. There is considerable debate on the role of NO in controlling GFR and RBF in diabetes. Our study was not designed to test the role of NO, but we have shown in several studies (23,45) that the induction of diabetes in rats that are pretreated chronically with N^G-nitro-L-arginine methyl ester fails to increase GFR. Those previous data provide evidence that NO plays a major role in renal vasodilation at the very earliest stages of type 1 diabetes. It is interesting, however, that we also have shown that there is no impairment in acetylcholine-induced skeletal muscle vasodilation on either the second or sixth day of STZ-induced diabetes (21), which again points to potential differences in RBF *versus* nonrenal BF control in diabetes. Other laboratories also have reported dependence of diabetic renal vasodilation on NO (37,40,41,46–48), and a unique aspect of blood flow control in the kidneys *versus* most other tissues is the potential involvement of NO synthase (NOS). Blockade of neuronal NOS has been shown to blunt hyperfiltration in diabetes (37,38,40,46). Although this is not a universal finding, and our new dynamic autoregulation data do not shed light on the role for NO or any specific NOS isoform in controlling renal vascular resistance in diabetes, this may contribute to our observation that myogenic autoregulation of RBF is attenuated significantly and RBF is increased, yet our previous BP, total peripheral resistance, and skeletal muscle blood flow data suggest that the vasculature in other tissues is not dilated.

It will be important to block NOS, as well as AngII, in future studies and perform transfer function analysis, because NO and AngII have powerful and opposing effects on vascular tone throughout the body, on TGF sensitivity, and on BP control and overall renal function in diabetes (41,49,50). It also will be interesting in future studies to determine whether the changes in transfer function gain that we measured early in diabetes can be reversed by restoring normoglycemia, because, although our previous reports have shown reversibility of renal and systemic hemodynamic variables, renal hypertrophy during diabetes might alter the ability of autoregulation to return to normal. The effect of more modest hyperglycemia and the effect of hyperglycemia in type 2 diabetes need to be explored as well. Nonetheless, the results from this study show that dynamic autoregulation of RBF, in the frequency ranges of the myogenic and TGF mechanisms, is impaired significantly immediately after induction of STZ-induced type 1 diabetes. These changes may contribute to the immediate increase in continuously measured RBF, and our filtration fraction data suggest that the renal vasodilation is focused predominantly on the afferent arterioles. In addition, because MAP is increased and our previous reports show that total peripheral resistance and skeletal muscle vascular resistance are increased, these data suggest that diabetes may affect myogenic control of RBF by mechanisms that are different from those involved in myogenic control in other organs and tissues.

Acknowledgments

This work was supported by National Heart, Lung, and Blood Institute grants HL56259 and HL75625 and American Heart Association predoctoral fellowship AHA00038 and the Adlerbertska Forskningsstiftelsen, The Royal Society of Arts and Sciences, Gôteborg, Sweden.

We acknowledge the assistance of Dr. Sam L. Robinson (Augusta

State University) and the technical assistance of and Cassandra Fleming-Henry.

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