Autoregulation of Renal Blood Flow in Streptozocin-Induced Diabetic Rats

YASUKI HASHIMOTO, TERUKUNI IDEURA, ASHIO YOSHIMURA, AND SHOZO KOSHIKAWA

Autoregulation of renal blood flow (RBF) was studied in male Wistar rats. We studied 11 control rats, 11 rats with severe streptozocin (STZ)-induced hyperglycemia (diabetic group), and 10 moderately hyperglycemic rats made diabetic by injection of STZ but given 2–8 U s.c. insulin daily (insulin-treated group). RBF was measured by an electromagnetic flowmeter during stepwise reduction of renal perfusion pressure 4–8 wk after injection of STZ (older group). RBF autoregulation of the diabetic group was impaired compared with the control group. In the insulin-treated group, autoregulatory capability was less attenuated than in the diabetic group. The average autoregulatory index (ARI) of the diabetic group (0.61 ± 0.05) was greater than that of the control (0.24 ± 0.02, $P < .01$) and the insulin-treated (0.33 ± 0.07, $P < .05$) groups. To study the relationship between autoregulation and the duration of diabetes, an autoregulatory study was also made in a group of 22 rats (11 diabetic and 11 control) that were tested 2–3 wk after injection of STZ (younger group). The ARI in the younger diabetic group was smaller than that in the older diabetic group ($P < .05$). The results suggest that in uncontrolled diabetes RBF fluctuates with blood pressure change, and protection against hypertensive injury of glomerular capillaries may be diminished. Autoregulatory disability develops with time, and insulin treatment diminishes impairment of autoregulation. These findings may also explain the adverse consequences of hypertension on the progression of diabetic nephropathy in poorly controlled diabetes. *Diabetes* 38:1109–13, 1989

Characteristic changes in morphology and function of kidneys are associated with diabetes in humans. Recent studies suggest that functional changes may precede the morphological changes in diabetic angiopathy (1). The pathogenic mechanism of diabetic nephropathy remains undefined, but there is some evidence that glomerular hemodynamic changes with increased glomerular filtration rate (GFR) and intraglomerular pressure markedly influence the natural history of diabetic nephropathy (2).

The normal kidney adjusts its vascular resistance so that renal blood flow (RBF) and GFR remain constant in the face of fluctuating arterial pressure. These autoregulatory mechanisms would be expected to protect the glomerular capillaries from barotrauma (3). Clinical studies indicate that when diabetes is complicated by hypertension the rate of decline in kidney function is accelerated (4,5). These observations suggest that diabetic kidneys are associated with impaired autoregulatory capability.

In this study, we examined the autoregulatory capability of rats with streptozocin-induced diabetes (STZ-D) and the effects of insulin treatment on hemodynamic changes in the kidney.

RESEARCH DESIGN AND METHODS

Animals and induction of diabetes. Studies were performed on male Wistar rats weighing 170–190 g that had free access to water and a standard pellet diet. Each animal was assigned to a control, diabetic, or insulin-treated diabetic group. Experimental diabetes was produced by STZ (30 mg · kg^{-1} body wt · day^{-1} i.p. for 5 consecutive days) dissolved in a citrate buffer (pH 4.5) just before injection (6). Age-matched control rats were injected with the vehicle without STZ. The standard pellet diet contained 25.5% protein and 0.4% sodium. The diabetic rats ate nearly twice as much food (mean ± SE 49 ± 3 g/day) as the control rats (23 ± 1 g/day), but insulin treatment abolished this increase in food consumption (29 ± 4 g/day). Diabetes was confirmed by quantitative determination of plasma glucose levels >400 mg/dl, excessive values of daily food and water consumption, large increases in urinary volume, and lower weight

From the Department of Internal Medicine, Showa University Fujigaoka Hospital, Yokohama, Japan.

Address correspondence and reprint requests to Yasuki Hashimoto, MD, Department of Internal Medicine, Showa University Fujigaoka Hospital, 1-30 Fujigaoka, Midori-ku, Yokohama 227, Japan.

Received for publication 22 June 1988 and accepted in revised form 24 April 1988.

gain. Animals assigned to the insulin-treated group received daily subcutaneous injections of zinc-extended insulin suspension (Ultralente, Novo, Copenhagen) from 2 days after injection of STZ. To maintain plasma glucose levels between 200 and 300 mg/dl, the dose (2–8 U/day) was adjusted weekly. Blood samples (0.1 ml/wk) were obtained from the jugular vein, and plasma glucose was assayed by the glucose oxidase method in a multilayer film analyzer (Drichem 1000, Fuji, Saitama, Japan).

Three weeks after injection of STZ, animals were placed in individual metabolic cages for 24-h urine collection, and a venous blood sample was taken to determine creatinine clearance. Urine and plasma creatinine concentrations were measured by autoanalyzer (JEOL VS-700, Nihon-Denshin, Tokyo).

Kidney function measurements. Measurements of RBF autoregulation were made on 11 control, 11 diabetic, and 10 insulin-treated rats 4–8 wk after STZ injection. The rats were anesthetized with injection of pentobarbital sodium (30 mg/kg body wt i.p.) and placed on a heating table that maintained body temperature between 36.5 and 38.0°C. After tracheostomy, a polyethylene (PE 50) catheter was inserted via a femoral artery into the abdominal aorta, and the tip was positioned near the renal artery for monitoring renal arterial pressure (RAP). RAP was measured with a transducer (Statham P23Db, Oxnard, CA) and recorded on a polygraph (model RM-6200, Nihon-Koden, Tokyo). A catheter was also placed in the left femoral vein, and 0.15 M NaCl (0.2–0.4 ml · kg^{-1} · min^{-1} for control and insulin-treated groups, 0.3–0.5 ml · kg^{-1} · min^{-1} for diabetic group) was infused through the catheter for replacement of surgical (~1% of body wt) and ongoing losses. These replacement doses were determined by our preliminary data on hematocrit (n = 5 for each group). There was no statistically significant change between initial and final hematocrit for each group in the paired t test (control, 45.9 ± 0.4 to 45.4 ± 0.4; diabetic, 45.7 ± 0.5 to 46.0 ± 0.4; insulin treated, 44.4 ± 0.3 to 44.7 ± 0.3). After the femoral vein was cannulated, the rest of the experiments were completed within 60 min. The left kidney was exposed through a midline abdominal incision. RBF was measured in the left renal artery by a small-diameter probe (model F1-007T, Nihon-Koden) connected to an electromagnetic flowmeter (model MFV-1100, Nihon-Koden). RAP, reduced by an adjustable clamp placed around the aorta just above the right renal artery, was maintained constant for 30-s intervals during 10-mmHg stepwise decrements down to 40 mmHg. Renal vascular resistance (RVR) was calculated as RVR = (RAP − RVP)/RBF, where RVP is renal venous pressure (assigned value of 5 mmHg) (7). To compare the effectiveness of RBF autoregulation, we calculated an autoregulatory index (ARI) for each animal, suggested by Semple and de Wardener (8), as

$$\mathrm{ARI} = \frac{(F_1 - F_2)F_1^{-1}}{(P_1 - P_2)P_1^{-1}}$$

where F_1 and P_1 are basal RBF and basal RAP, respectively, and F_2 and P_2 are RBF and RAP at 80 mmHg, which is the pressure that is thought to be the lower limit of autoregulation in the rat (9). ARI ≥1 indicates lack of autoregulation, and an index approaching 0 indicates efficient autoregulation.

Autoregulation and duration of diabetes. To study the relationship between autoregulatory capability and duration of diabetes, RBF autoregulation measurements were also made 2–3 wk after STZ injection in a separate, younger group of 22 rats (11 untreated diabetic and 11 control rats). The ARI was also calculated for this group.

Statistics. Data are presented as means ± SE. Statistical analysis was performed by one-way analysis of variance with significance at $P < .05$. Further specific group differences were determined by Tukey's method.

RESULTS

Plasma glucose concentrations averaged 121 ± 3, 541 ± 23, and 247 ± 11 mg/dl in the control, diabetic, and insulin-treated groups, respectively (Table 1). Whole-kidney GFR with creatinine clearance in the insulin-treated group was significantly higher than in the other two groups (Table 1). Diabetic rats had significantly lower body weights than control ($P < .01$) or insulin-treated ($P < .05$) rats, but body weight did not differ significantly between the latter two groups (Table 1). Despite the similar body weights, the average weight of the left kidney in the insulin-treated group was higher than that in the control rats (Table 1). There was no statistically significant difference in the baseline values of RAP, RBF, or RVR among the three groups (Table 1).

Figure 1 shows the pressure-flow autoregulation curves of the three groups. RBF in each 10-mmHg interval was expressed as the percentage of RBF recorded at 100 mmHg. In the control group, RBF was effectively autoregulated at mean RAP >80 mmHg. However, in the diabetic group, RBF readily fluctuated as blood pressure changed, and autoreg-

TABLE 1
Summary of kidney function

Group	n	Body wt (g)	Left kidney wt (g)	Plasma glucose (mg/dl)	Ccr (ml/min)	RAP (mmHg)	RBF (ml/min)	RVR (mmHg · ml^{-1} · min^{-1})	ARI
Control	11	399 ± 19*	1.23 ± 0.05	121 ± 3*	1.12 ± 0.05	114 ± 3	5.4 ± 0.3	20.4 ± 1.1	0.24 ± 0.02*
Diabetic	11	285 ± 26†	1.33 ± 0.05†	541 ± 23‡	1.16 ± 0.11‡	107 ± 4	4.6 ± 0.3	23.7 ± 2.5	0.61 ± 0.05†
Insulin treated	10	373 ± 11	1.56 ± 0.06§	247 ± 11§	1.78 ± 0.15§	115 ± 2	5.7 ± 0.4	19.2 ± 1.0	0.33 ± 0.07

Values are means ± SE by analysis of variance and Tukey's method for multiple comparisons. Ccr, creatinine clearance measured 3 wk after injection of streptozocin (see RESEARCH DESIGN AND METHODS); RAP, renal arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance; ARI, autoregulatory index.
*$P < .01$ vs. diabetic.
†$P < .05$, ‡ $P < .01$, vs. insulin treated.
§$P < .01$ vs. control.

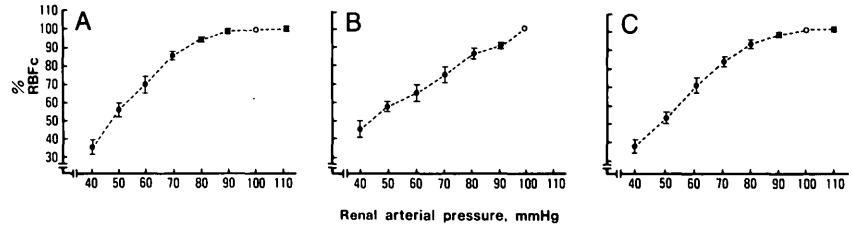

FIG. 1. Relationship between renal blood flow (RBF) and renal arterial pressure. ○, percentage of RBF at 100 mmHg. Values are means ± SE. *n* = 11 for control (*A*), *n* = 11 for diabetic (*B*), and *n* = 10 for insulin-treated (*C*) rats.

ulation of RBF was impaired. In the insulin-treated group, autoregulatory capability was less attenuated than in the untreated diabetic group. The average ARI in diabetic rats was greater than in the control ($P < .01$) or insulin-treated ($P < .05$) rats. There was no statistically significant difference in ARI between the control and the insulin-treated groups (Table 1). RVR in the control and insulin-treated groups fell progressively as renal perfusion pressure was lowered to 70–80 mmHg, whereas RVR remained relatively constant in the diabetic group (Fig. 2).

Changes in ARI at different times after injection of STZ are shown in Fig. 3. Differences in ARI between the control and diabetic groups were not statistically significant in the younger (2–3 wk after injection of STZ) groups (0.27 ± 0.03 vs. 0.31 ± 0.06) but became significant in the older (4–8 wk after injection of STZ) groups (0.24 ± 0.02 vs. 0.61 ± 0.05, $P < .01$). There was also an age-related difference in the ARI of the two diabetic groups ($P < .05$).

DISCUSSION

This study was designed to examine the pressure-flow characteristics of the diabetic rat kidney with electromagnetic flow techniques. Continuous monitoring of small changes in RBF during stepwise reduction of RAP, although extremely difficult in humans, is quite feasible in experimental animals by use of an electromagnetic flowmeter.

The glucose level of our diabetic group may correspond to severe uncontrolled hyperglycemia in humans (10), whereas the glucose level of the insulin-treated group may be commensurate with that of humans whose glucose levels are less elevated.

The results show that autoregulation of RBF in diabetic rats is strongly impaired, but insulin treatment remits this impairment. The autoregulatory capability of rats in the STZ-D group that received insulin was not impaired (Table 1; Fig.1). We therefore concluded that autoregulatory defects in the diabetic group were not caused by direct toxic effects of STZ on tubular cells.

We could observe no statistical differences between the baseline values of RBF in the insulin-treated group and those in the other two groups (Table 1). This lack of statistical difference probably reflects the large standard error of the mean of the RBF data. There is also no correlation between GFR with creatinine clearance and RBF (Table 1). These results may be causally related to the inaccuracy of measurement of creatinine clearance in diabetic rats and the fact that GFR was not measured on the experimental day.

Impaired autoregulation of cerebral, retinal, subcutaneous, and visceral tissue blood flow has also been documented in long-term diabetic patients with clinical microangiopathy (11–14). Sometimes there is dissociation between RBF and GFR autoregulation (15–18), but these two parameters usually change in parallel. Thus, we speculated that in untreated diabetic rats GFR autoregulation will also be impaired because of deterioration of RBF autoregulation, although this was not tested in our experiments.

The precise mechanism for RBF autoregulation has not been fully characterized, but most investigators agree that it involves a tubuloglomerular-feedback (TGF) mechanism and myogenic response (19–21). Neurogenic factors seem to be of no importance because the autoregulatory response is still present in denervated animals (22). Normal autoregulatory responses to decreases in perfusion pressure would be expected to result in proportional vasodilation of preglomerular vessels such that RBF and GFR would remain constant within the autoregulatory range (22,23). The lower limit of autoregulation seems to be ~80–90 mmHg in rats (9), and further reduction in perfusion pressure sharply reduces RBF and GFR. The TGF system is a control system that maintains a balance between hemodynamic inputs that control the filtrated load and the reabsorptive function of the tubules (24). From the unique morphology of the juxtaglomerular complex, it might be speculated that the macula densa can sense some aspect of fluid composition in the distal tubule and transmit a signal to the preglomerular vessels. Studies have shown that hyperglycemia, extracellular volume expansion, high salt intake, and high-protein diet attenuate the TGF loop (25–28). Because our diabetic models also demonstrated severe hyperglycemia and extremely high daily consumption of water and food, it would seem that autoregulation of diabetic rats was impaired as a consequence of attenuated TGF sensitivity. The insulin-treated group consumed less water and food, and therefore, TGF activity may be less attenuated in this group.

Woods et al. (29) reported that acute hyperglycemia by infusion of 10–30% glucose solutions impaired kidney autoregulation, but in our study, the RBF autoregulation in the younger group of diabetic rats reflected by the ARI was not impaired regardless of the existence of severe hyperglycemia. Hyperglycemia does not always have significant ef-

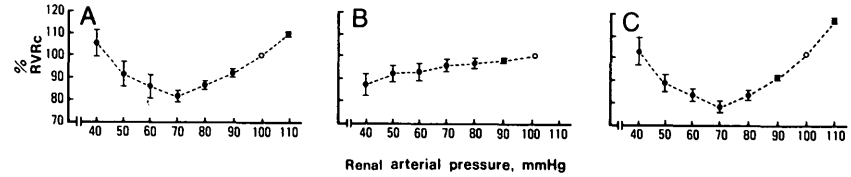

FIG. 2. Relationship between renal vascular resistance (RVR) and renal arterial pressure. ○, percentage of RVR at 100 mmHg. Values are means ± SE. *n* = 11 for control (*A*), *n* = 11 for diabetic (*B*), and *n* = 10 for insulin-treated (*C*) rats.

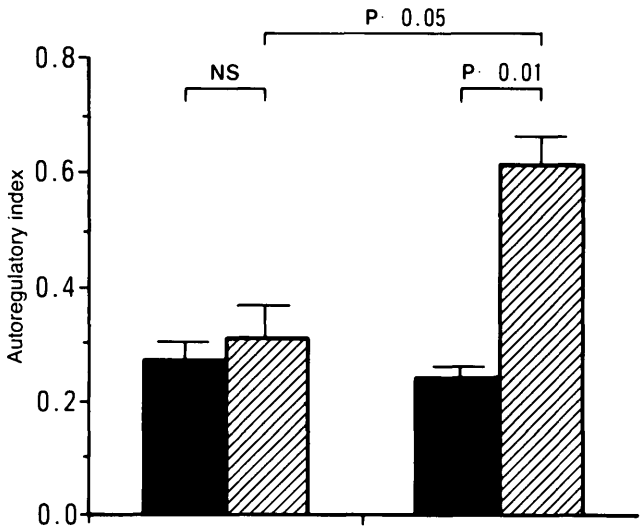

FIG. 3. Change in autoregulatory index (ARI) in control (*solid bars*) and diabetic (*hatched bars*) rats 2–3 wk (*left*) and 4–8 wk (*right*) after injection of streptozocin. Values are means ± SE.

fects on kidney autoregulatory capability. These discrepancies may have their origins in the difference between acute hyperglycemia induced by glucose infusion and chronic hyperglycemia induced by STZ.

Another possible explanation of our findings is that severe vascular dysfunction may have developed as diabetes persisted, and this caused impaired responsiveness to changing renal perfusion pressure. The autoregulatory behavior seen in diabetic rats may be caused by morphological change or metabolic disturbance of the smooth muscle cells in the preglomerular vessels or both. Most of the evidence suggests abnormal vascular responses in experimentally diabetic rats. Mueller (30) reported that severe vascular dysfunction (decreased maximal vascular-dilator and vascular-constrictor capacity) develops with time in alloxan-induced diabetes and that this can be prevented by insulin treatment. This vascular dysfunction was not just observed after induction of experimental diabetes. The increased cross-sectional area with a poorly developed coat of vascular smooth muscle cells of the smallest arteries and abnormal development of the vessel walls were also previously reported in juvenile STZ-D mice (31). These vascular abnormalities could contribute to the impaired kidney autoregulatory response. It was also suggested that insulin treatment normalized the kidney autoregulatory response through prevention of functional or structural diabetic vascular alterations or both.

Our results demonstrated that kidney autoregulation was not impaired in the hyperfiltrating, large kidneys seen in the insulin-treated group. Insulin-treated moderately hyperglycemic rats were reported to have marked afferent arteriolar vasodilation and increased intraglomerular pressure (10). The capacity for further autoregulatory dilation in preglomerular vessels seems to be preserved even though the afferent arterioles are already dilated in insulin-treated diabetic rats (10). Our results are consistent with the report that kidney autoregulation is preserved in the pregnant rabbit despite markedly vasodilated hyperfiltering kidneys (32). However, hormonal effects on regulating glomerular vascular tone, as possible mediators of the responses that were observed, remain to be examined.

Regardless of the precise mechanism for the failure of autoregulation, the clinical significance of impaired autoregulation of RBF in an uncontrolled diabetic state is lack of or diminished protection against hyper- or hypoperfusion induced by alteration in blood pressure. In other words, there is increased vulnerability to hypertensive or ischemic injuries to preglomerular vessels and glomerular capillaries in diabetic patients, and the control of blood pressure, although quite difficult in diabetic patients, is important for preventing severe vasculopathy. We suggest that insulin treatment may prevent further increases in glomerular pressure through protection of kidney autoregulatory capacity when RAP is elevated. These findings might help explain the well-recognized adverse consequence of hypertension on the progression of diabetic nephropathy in poorly controlled diabetes.

ACKNOWLEDGMENTS

We thank Masako Morito, Yoko Matsuzaki, and Kiyoko Inui for expert technical help, and Dr. Albert Simpson for assistance in the preparation of this manuscript.

This study was supported by a Research Grant for Health Sciences from the Ministry of Health and Welfare, Japan.

REFERENCES

1. White NH, Santiago JV: Reversibility of the vascular complications of diabetes by intensive therapy. *Pediatr Adolesc Endocrinol* 15:272–84, 1986
2. Hostetter TH, Rennke HG, Brenner BM: The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. *Am J Med* 72:375–80, 1982
3. Bidani AK, Schwartz MM, Lewis EJ: Renal autoregulation and vulnerability to hypertensive injury in remnant kidney. *Am J Physiol* 252:F1003–10, 1987
4. Mogensen CE: High blood pressure as a factor in the progression of diabetic nephropathy. *Acta Med Scand Suppl* 602:29–32, 1976
5. Christlieb AR, Warram JH, Królewski AS, Busick EJ, Ganda OP, Asmal AC, Soeldner JS, Bradley RF: Hypertension: the major risk factor in juvenile-onset insulin-dependent diabetics. *Diabetes* 30 (Suppl. 2):90–96, 1981
6. Like AA, Rossini AA: Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. *Science* 193:415–17, 1976
7. Arendshorst WJ, Finn WF, Gottschalk CW: Autoregulation of blood flow in the rat kidney. *Am J Physiol* 228:127–33, 1975
8. Semple SJG, de Wardener HE: Effect of increased renal venous pressure on circulatory "autoregulation" of isolated dog kidneys. *Circ Res* 7:643–48, 1959
9. Hope A, Clausen G, Rosivall L: Total and local renal blood flow and filtration in the rat during reduced renal arterial blood pressure. *Acta Physiol Scand* 113:455–63, 1981
10. Hostetter TH, Troy JL, Brenner BM: Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int* 19:410–15, 1981
11. Bentsen N, Larsen B, Lassen NA: Chronically impaired autoregulation of cerebral blood flow in long-term diabetics. *Stroke* 6:497–502, 1975
12. Atherton A, Hill DW, Keen H, Young S, Edwards EJ: The effect of acute hyperglycemia on the retinal circulation of the normal cat. *Diabetologia* 18:233–37, 1980
13. Kastrup J, Nørgaard T, Parving H-H, Henriksen O, Lassen NA: Impaired autoregulation of blood flow in subcutaneous tissue of long-term type 1 (insulin-dependent) diabetic patients with microangiopathy: an index of arteriolar dysfunction. *Diabetologia* 28:711–17, 1985
14. Parving H-H, Kastrup H, Smidt UM, Andersen AR, Feldt-Rasmussen B, Christiansen JS: Impaired autoregulation of glomerular filtration rate in type 1 (insulin-dependent) diabetic patients with nephropathy. *Diabetologia* 27:547–52, 1984
15. Hall JE, Guyton AC, Cowley AW Jr: Dissociation of renal blood flow and filtration rate autoregulation by renin depletion. *Am J Physiol* 232: F215–21, 1977
16. Abe Y, Dixon F, McNay JL: Dissociation between autoregulation of renal blood flow and glomerular filtration rate. *Am J Physiol* 219:986–93, 1970
17. Duchin KL, Peterson LN, Burke TJ: Effect of furosemide on renal auto-

regulation. *Kidney Int* 12:379–86, 1977
18. Baer PG, Navar LG: Renal vasodilation and uncoupling of blood flow and filtration rate autoregulation. *Kidney Int* 4:12–21, 1973
19. Thurau K: Renal hemodynamics. *Am J Med* 36:698–719, 1964
20. Navar LG, Burke TJ, Robinson RR, Clapp JR: Distal tubular feedback in the autoregulation of single nephron glomerular filtration rate. *J Clin Invest* 53:516–25, 1974
21. Bayliss WM: On the local reaction of the arterial wall to changes in internal pressure. *J Physiol* (Lond) 28:220–31, 1902
22. Shipley RE, Study RS: Changes in renal blood flow, extraction of insulin, glomerular filtration rate, tissue pressure and urine flow with acute alterations of renal artery blood pressure. *Am J Physiol* 167:676–88, 1951
23. Navar LG: Renal autoregulation: perspectives from whole kidney and single nephron studies. *Am J Physiol* 234:F357–70, 1978
24. Navar LG, Bell PD, Burke TJ: Role of a macula densa feedback mechanism as a mediator of renal autoregulation. *Kidney Int* 22 (Suppl. 12):157–64, 1982
25. Blantz RC, Peterson OW, Gushwa L, Tucker BJ: Effect of modest hyperglycemia on tubuloglomerular feedback activity. *Kidney Int* 22 (Suppl. 12):206–12, 1982
26. Thurau K: Modification of angiotensin-mediated tubulo-glomerular feedback by extracellular volume. *Kidney Int* 8:202–207, 1975
27. Kaufman JS, Hamburger RJ, Flamenbaum W: Tubuloglomerular feedback: effect of dietary NaCl intake. *Am J Physiol* 231:1744–49, 1976
28. Seney FD Jr, Wright FS: Dietary protein suppresses feedback control of glomerular filtration in rats. *J Clin Invest* 75:558–68, 1985
29. Woods LL, Mizelle HL, Hall JE: Control of renal hemodynamics in hyperglycemia: possible role of tubuloglomerular feedback. *Am J Physiol* 252:F65–73, 1987
30. Mueller SM: Insulin treatment prevents vascular dysfunction in early juvenile alloxan-induced diabetes mellitus. *Am J Physiol* 247:H132–38, 1984
31. Bohlen HG, Niggl BA: Arteriolar anatomical and functional abnormalities in juvenile mice with genetic or streptozotocin-induced diabetes mellitus. *Circ Res* 45:390–96, 1979
32. Woods LL, Mizelle HL, Hall JE: Autoregulation of renal blood flow and glomerular filtration rate in the pregnant rabbit. *Am J Physiol* 252:R69–72, 1987