Urinary and Renal Tissue Kallikrein in the Streptozocin-diabetic Rat

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SUMMARY

The renal kallikrein-kinin system is thought to participate in blood pressure regulation and displays abnormalities in human hypertension, as well as in many animal models of hypertension. Urinary excretion and tissue levels of renal kallikrein were measured in streptozocin (STZ)-diabetic rats in relation to blood pressure, glycemia, and insulin treatment.

In study 1, STZ-diabetic rats with marked hyperglycemia showed reduced kallikrein-like esterase excretion, compared with control rats, when first measured after 7 days of diabetes (9.9 ± 2.5 versus 17.5 ± 2.4 EU/24 h, $P < 0.05$). This difference increased with time and, after 210 days, urinary esterase excretion in diabetic and control rats was 6.7 ± 2.1 and 39.0 ± 6.0 EU/24 h, respectively ($P < 0.001$). Urine kallikrein, measured by radioimmunoassay, was similarly reduced in diabetic rats (40.4 ± 8.0 versus 88.0 ± 6.5 μg/24 h, at 30 days, $P < 0.001$). At 120 days, systolic blood pressures were elevated in diabetic rats ($P < 0.05$), and at 180 days over 60% of the diabetic rats had pressures above the highest pressures of control rats.

In study 2, STZ-diabetic rats were treated with insulin for 2 wk (2 U NPH at 0800 h, or 2 U NPH at 0800 and 1600 h). In the single-dose group, with hyperglycemia similar to that of diabetic rats in study 1, kallikrein excretion was reduced as early as day 2, compared with nondiabetic rats (56.0 ± 6.1 versus 109 ± 9.4 μg/24 h, respectively, $P < 0.001$). In the diabetic rats treated with twice-daily insulin, glycemia was reduced and daily urine glucose was not significantly different from that in controls. Kallikrein excretion was normal in these rats.

In study 3, we measured renal tissue kallikrein in untreated and insulin-treated (2.25 U PZI) STZ-diabetic rats. After 2 wk, untreated diabetic rats had decreased renal kallikrein compared with nondiabetic rats (22.8 ± 1.6 versus 29.7 ± 1.4 ng/mg protein, $P < 0.001$). In the insulin-treated diabetic rats with near-normal plasma glucose levels, renal kallikrein (35.8 ± 2.1 ng/mg protein) was increased above that of untreated diabetic ($P < 0.001$) or nondiabetic rats ($P < 0.05$).

These studies confirm that STZ-diabetic rats develop systolic hypertension as assessed by tail cuff measurement. There is an early reduction in kallikrein excretion, which reflects reduced renal enzyme content. These abnormalities in kallikrein can be prevented by insulin treatment. Relationships of these changes in urinary and renal kallikrein to the function of the diabetic kidney and development of hypertension in this model can now be explored. DIABETES 1985; 34:22–28.

Renal kallikrein and its kinin products are thought to have a role in blood pressure regulation and electrolyte homeostasis.[1] Support for this notion comes from studies demonstrating that urinary excretion of this tissue kallikrein is altered in several forms of human hypertension, as well as in many animal models of hypertension.[1] Furthermore, changes in excretion rates are also noted after antihypertensive and diuretic drug treatment.[2-4]

The prevalence of hypertension is said to be significantly increased in patients with diabetes mellitus.[5,6] Hypertension compounds the risk of cardiovascular events due to macrovascular disease in the diabetic patient,[7] and the progression of microvascular disease of the retina and kidney are also accelerated by hypertension.[8,9] It is uncertain whether this increased prevalence of hypertension among diabetic subjects represents an increased incidence of essential hypertension, or occurs as the result of specific abnormalities in

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the diabetic state that raise blood pressure.[7] Studies in diabetic patients[10] and animal models[11,12] of factors known to participate in blood pressure regulation have not resolved the question.

We have recently reported that urinary kallikrein is significantly increased in uncontrolled, insulin-dependent diabetic humans without renal disease or hypertension, and can be reduced with strict glycemic control.[13] We have now extended our studies to the streptozocin (STZ)-diabetic rat, a model that has been shown to develop glomerulopathy and, possibly, hypertension.[12,14] In the first of the studies reported here, urinary kallikrein excretion was measured in relation to the diabetic state and systolic arterial pressure over several months. The striking and persistent changes in kallikrein excretion, which occurred before the development of hypertension, prompted subsequent studies that examined the early course of these kallikrein changes in both urine and renal tissue, and the effects of insulin treatment on kallikrein.

MATERIALS AND METHODS

Study 1. This study was done in conjunction with a larger study of vascular changes in diabetes, carried out at Research Triangle Park, North Carolina. Subgroups of 16–20 male Sprague-Dawley rats, weighing 170–200 g, were randomly selected for this study from larger cohorts of more than 300 diabetic and control animals that were undergoing studies of arterial wall metabolism (Namm et al., unpublished studies). Diabetes was induced by injecting STZ intravenously, 65 mg/kg body wt, diluted in distilled water just before injection. Glucose was measured in tail-vein serum 1–2 days after injection in both STZ-treated and control animals, and subsequently in both groups on days 12, 25, 50, 90, and 240. Two STZ-treated rats were housed with two control rats and allowed free access to food and water, except on days of urine collection when rats were placed individually in metabolic cages. Twenty-four-hour collections of urine were obtained from diabetic and control rats (N = 6, randomly selected from each subgroup) on days 7, 33, 71, and 210 after STZ for measurement of urinary kallikrein-like esterase activity. After development of a radioimmunoassay for rat urinary kallikrein,[15] kallikrein excretion was reassessed in groups of identically treated diabetic (N = 7) and control (N = 6) rats 30 days after STZ treatment. Urinary kallikrein excretion in these rats was measured with the radioimmunoassay and kallikrein-like activity with the esterase assay.

Systolic blood pressure was measured in each rat, at monthly intervals, with a pneumatic pulse transducer and occluding tail cuff (Narco Bio-Systems, Chicago, Illinois). Rats were placed in plexiglass restrainers in a chamber that was kept at 30°C and measurements were carried out 1 h after rats were placed in the chamber. Blood pressures reported are the means of 4–6 determinations per rat.

Study 2. Male Sprague-Dawley rats, 140–180 g, were studied in Charleston, South Carolina. Diabetes was induced in 12 rats by injecting STZ intraperitoneally, 100 mg/kg body wt, diluted just before injection in Na-citrate buffer, pH 4.5. Diabetes was confirmed 24 h after injection by the presence of 3–4-plus urine glucose (Tes-Tape, Eli Lilly and Company, Indianapolis, Indiana). On the same day, one group of randomly selected diabetic rats (N = 6) was started on insulin (2 U isophane insulin suspension, NPH, Eli Lilly and Company), injected subcutaneously at 1600 h daily. A second diabetic group (N = 6) was injected subcutaneously with 2 U NPH insulin at the same time, again with 2 U NPH at 0800 h the following morning and twice daily thereafter. These regimens were continued daily in each group for 12 days. Diabetic and uninjected control rats (N = 6) were housed individually in metabolic cages throughout the experiment, and had free access to food and water. On day 7, tail-vein blood was sampled by capillary tube at 0800, 1600, and 2400 h for measurement of serum glucose. Twenty-four-hour collections of urine were obtained from all animals on days 2, 5, 7, 9, and 12 for measurement of kallikrein, glucose, Na^+, and K^+. Kallikrein was measured by radioimmunoassay in this study.

Study 3. Male Sprague-Dawley rats, 190–220 g, were used for this study (Charleston). Diabetes was induced in 16 animals by intravenous STZ injection (65 mg/kg body wt), and 8 uninjected rats were used as controls. Tail-vein serum glucose was measured 24 h after injection in STZ-treated rats. On the next day, 8 rats were randomly selected from the diabetic group and begun on insulin treatment, 2.25 U protamine zinc insulin suspension (Protamine Zinc and Iletin, Eli Lilly and Company), injected at 1300 h daily, while 8 diabetic rats remained untreated. All rats were provided free access to food and water and housed 2–3/cage.

After 2 wk, tail-vein serum glucose was measured in all rats at 0900 and 2100 h. The following day, kidneys were

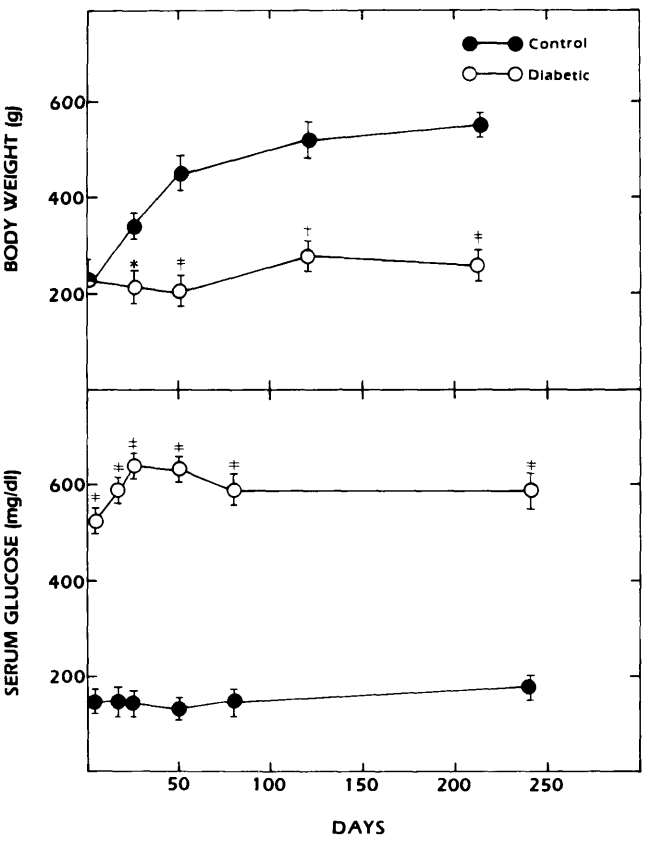

FIGURE 1. Body weight and serum glucose levels of STZ-diabetic and control rats in study 1 (mean ± SEM). Diabetic rats demonstrated marked hyperglycemia and little weight gain during the 240-day study. *P < 0.05, †P < 0.01, and ‡P < 0.001.

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excised from ether-anesthetized diabetic and control animals. Kidneys were perfused via the renal hilus with 10 ml of normal saline to remove blood. Kidneys were minced and homogenized with a Teflon-glass homogenizer (10 strokes) in ice-cold, Dulbecco's phosphate-buffered saline, pH 7.4. Sodium deoxycholate (0.5%) was then added to the tissue homogenate, followed by incubation at room temperature for 30 min. The homogenate was centrifuged at 14,000 $\times g$ for 60 min, and the supernatant, desalted by filtration through Sephadex G-25 (Pharmacia Inc., Piscataway, New Jersey), was assayed for immunoreactive kallikrein and total protein.

Assays. Serum and urine glucose were measured by the glucose-oxidase method[16] in a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, California) or by the Statzyme reagent kit (Worthington Biochemicals, Freehold, New Jersey). Urinary Na^+ and K^+ were measured by flame photometry. Tissue homogenate protein was measured by the method of Lowry[17] with bovine serum albumin used as the standard. Urinary kallikrein-like esterase activity was measured using α-N-tosyl-L-arginine-O-methylester (Tos-Arg-OMe) as substrate.[4] One esterase unit (EU) is defined as the amount of enzyme that hydrolyzes 1.0 μmol Tos-Arg-OMe/min at pH 8.0 and 30°C in a standard titrametric assay.[18] Radioimmunoassays of urinary and tissue kallikrein were performed as previously described.[15] All urine and tissue extracts were centrifugally filtered through Sephadex G-25 before assaying by either method.[4] This was shown to remove more than 90% of urinary ketones measured as beta-hydroxybutyrate,[19] and more than 95% of urinary glucose.[16] Glucose (7 g/dl) produced no interference in the esterase assay when urine was not filtered. Filtration of urine had no effect on the measurement of kallikrein by radioimmunoassay.

Measurements of urinary kallikrein-like activity by esterase assay and radioimmunoassay were previously compared.[15] The esterase assay is somewhat nonspecific, measuring a small portion of the activity of an enzyme called rat urinary esterase-A.[20] However, urinary esterase activity and kallikrein immunoreactivity are highly correlated: r = 0.95.[15] No detectable cross-reactivity of other esterases, including esterase-A, occurs in the radioimmunoassay.[15,20] Renal tissue homogenates produce displacement curves in the radioimmunoassay parallel to purified urinary kallikrein standard,[15] and renal tissue kallikrein measurement by radioimmunoassay and kininogenase activity are highly correlated.[21]

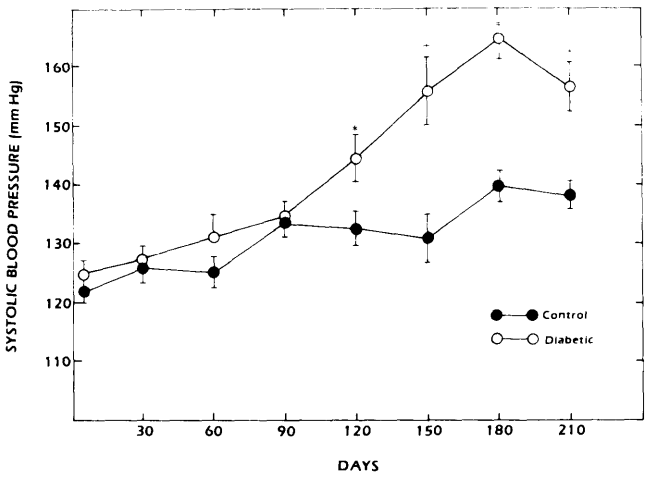

FIGURE 2. Tail systolic blood pressures at monthly intervals in STZ-diabetic and control rats in study 1 (mean ± SEM). Readings in each rat represent the average of 4–6 determinations made on the same day. Diabetic rats showed significantly raised pressures after 120 days. *P < 0.05, †P < 0.01, and ‡P < 0.001.

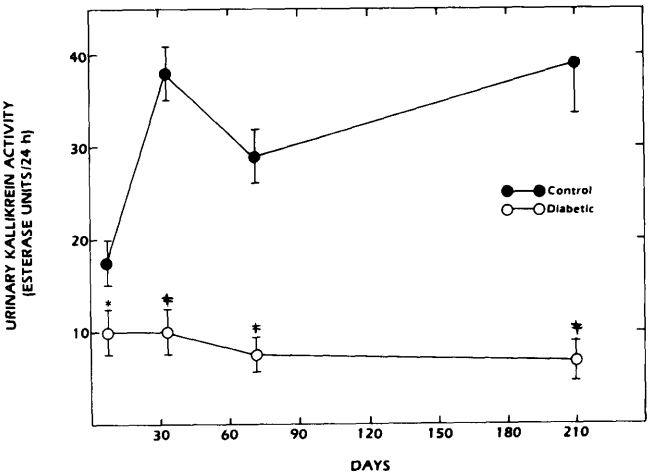

FIGURE 3. Urinary kallikrein excretion in STZ-diabetic and control rats in study 1, measured as esterase activity using α-N-tosyl-L-arginine-O-methylester as substrate (mean ± SEM). In control rats, excretion increased from day 7 to 30. Excretion was reduced in diabetic rats on day 7 and remained low throughout the study. *P < 0.05 and ‡P < 0.001.

Statistical analysis. Data are expressed as means ± SEM. Differences were determined by Student's *t*-test for paired or unpaired observations and considered significant at P < 0.05. Correlations were determined by regression analysis with the Hewlett-Packard 67 curve-fitting program.

RESULTS

STUDY 1. EFFECTS OF LONG-TERM DIABETES

Diabetic state. Diabetes in these STZ-treated rats was characterized by marked hyperglycemia from day 3 through day 240 of observation (Figure 1). In contrast to the steady weight gain of the control rats (>300 g during 7 mo), diabetic rats had little weight gain over the duration of the experiment (Figure 1). In addition to persistent polyuria, the diabetic rats also developed pronounced cataracts after 3 mo.

Blood pressure. Systolic blood pressures of diabetic rats were not different from controls for the first 90 days after STZ treatment (Figure 2). At 120 days, systolic pressures in the diabetic rats became significantly elevated compared with controls (P < 0.05). This difference persisted over the remaining 3 mo of observation. At 180 days, over 60% of the diabetic rats had systolic pressures greater than the highest pressures in control animals.

Urinary kallikrein excretion. Kallikrein-like esterase excretion in diabetic and control rats is shown in Figure 3. Excretion rates were significantly reduced in diabetic compared with control rats when first measured 7 days after induction of diabetes (P < 0.05). These differences persisted at 33, 71, and 210 days (P < 0.001 for each). Control rats showed a seemingly abrupt increase in urinary esterase excretion between 7 and 33 days. The reason for this change is un-

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TABLE 1
Study 2: serum glucose levels in diabetic rats treated with insulin, single dose (diabetic-2U) or twice-daily dose (diabetic-4U), and in control rats (mean ± SEM)

	Serum glucose (mg/dl)		
Time (h)	0800	1600	2400
Diabetic-2U	*797 ± 53†,‡*	*668 ± 65*,‡*	*356 ± 53*,‡*
Diabetic-4U	474 ± 71‡	119 ± 49	46 ± 4‡
Control	118 ± 3	102 ± 2	108 ± 2

*P < 0.001 compared with diabetic-4U; †P < 0.01 compared with diabetic-4U; ‡P < 0.001 compared with control.

known. Diabetic rats showed no change in excretion over the entire period of the study. Correction of kallikrein-like esterase excretion for body weight (EU/24 h · 100 g body wt) did not eliminate the differences between diabetic and control rats, except on day 7 (5.0 ± 0.9 versus 7.5 ± 1.1 EU/24 h · 100 g, respectively, P = NS). On days 33, 71, and 210, respectively, urinary esterase in diabetic rats was 4.2 ± 0.4, 3.3 ± 0.6, and 2.7 ± 0.3 EU/24 h · 100 g, compared with 9.4 ± 0.8, 6.1 ± 0.7, and 6.7 ± 0.7 EU/24 h · 100 g in control rats (P < 0.02 or less for each).

After the availability of a specific radioimmunoassay for urinary kallikrein,[15] additional groups of diabetic (N = 7) and control rats (N = 6) were studied 30 days after STZ injection. Both urinary kallikrein-like esterase and immunoreactive kallikrein excretion were measured on two consecutive days. Average esterase excretion was 7.7 ± 1.4 EU/24 h in diabetics, compared with 16.1 ± 1.2 in controls (P < 0.001). By radioimmunoassay, excretion was 40.4 ± 8.0 μg/24 h in diabetic rats and 88.0 ± 6.5 in controls (P < 0.001). Correction of excretion rates for body weight reduced, but did not eliminate, these significant differences. Thus, in two separate experiments and by two methods of measurement, urinary kallikrein excretion was reduced in STZ-diabetic rats. This reduction occurred before the onset of systolic hypertension in the initial experiment.

STUDY 2. EFFECTS OF INSULIN TREATMENT

In this study, kallikrein excretion was measured during the first 2 wk after STZ treatment in diabetic rats treated with insulin. Daily (2 U NPH at 1600 h) or twice daily (2 U NPH at 0800 and 1600 h) subcutaneous insulin injections were given to maintain different hyperglycemic states.

Diabetic state. Tail-vein serum glucose levels measured at 8-h intervals on day 7 are shown in Table 1 for the diabetic rats treated with either a single or twice-daily dose of 2 U insulin and for control rats. Serum glucose levels were markedly elevated throughout the 24-h period in the diabetic rats receiving the single dose, compared with the control or twice-daily-treated diabetic rats (P < 0.01–0.001). The diabetic rats treated with twice-daily insulin had measured glucose levels above those of control animals only at 0800 h (P < 0.001).

Average weight gain during the 12 days in the twice-daily insulin-treated group was similar to that of the control group (102 ± 6 versus 86 ± 6 g, P = NS). In the single-dose group, weight increase (32 ± 4 g) was less than one-half of that of the twice-daily-treated or control rats (P < 0.001).

On all days of measurement, diabetic rats given a single insulin dose had greatly increased urine volume (P < 0.01 or less) and glucose excretion (P < 0.001) compared with the other diabetic and control rats (Table 2). They also showed progressively increasing urine volume and glucose from day 2 through day 9. On 4 of 5 days, the twice-daily-injected diabetic rats also had greater urine output than did controls (P < 0.05 or less); however, urinary glucose excretion was not significantly increased, except on day 9 (Table 2).

Despite increased urine volume in both diabetic groups, and markedly increased glucose excretion in the single-dose group, there were no consistent differences in Na^+ or K^+ excretion among any of the rats. Average daily excretion of Na^+ in the insulin, single- and twice-daily-treated, and control rats was 1.94 ± 0.13, 2.05 ± 0.12, and 1.74 ± 0.09 meq/day, respectively (P = NS between any groups). Excretion of K^+ in these groups was 4.73 ± 0.21, 4.91 ± 0.25, and 4.30 ± 0.08 meq/day, respectively (P = NS between any groups).

Urinary kallikrein excretion. Immunoreactive urinary kallikrein excretion was reduced 2 days after STZ injection in the rats receiving the single insulin dose compared with control rats (Figure 4). This significant decrease was observed on all subsequent days except day 4. In contrast, the twice-daily-treated diabetic group had excretion rates

TABLE 2
Study 2: urine volume and glucose excretion in diabetic rats treated with insulin, single dose (diabetic-2U) or twice-daily dose (diabetic-4U), and in control rats (mean ± SEM)

	Day				
	2	4	7	9	12
Urine volume (ml/day)					
Diabetic-2U	47 ± 4†,‡	83 ± 7†,‡	121 ± 4*,‡	138 ± 8*,‡	123 ± 14*,‡
Diabetic-4U	24 ± 4‖	34 ± 9	42 ± 4§	55 ± 9§	33 ± 4§
Control	13 ± 1	15 ± 1	20 ± 2	15 ± 1	13 ± 2
Urine glucose (g/day)					
Diabetic-2U	3.4 ± 0.3*,‡	4.8 ± 0.6*,‡	6.0 ± 0.6*,‡	9.0 ± 0.6*,‡	5.7 ± 0.8*,‡
Diabetic-4U	0.8 ± 0.4	0.7 ± 0.4	0.2 ± 0.1	1.4 ± 0.5‖	0.4 ± 0.2
Control	0.009 ± 0.002	0.01 ± 0.03	0.01 ± 0.003	0.009 ± 0.003	0.009 ± 0.003

*P < 0.001 compared with diabetic-4U; †P < 0.01 compared with diabetic-4U; ‡P < 0.001 compared with control; §P < 0.01 compared with control; ‖P < 0.05 compared with control.

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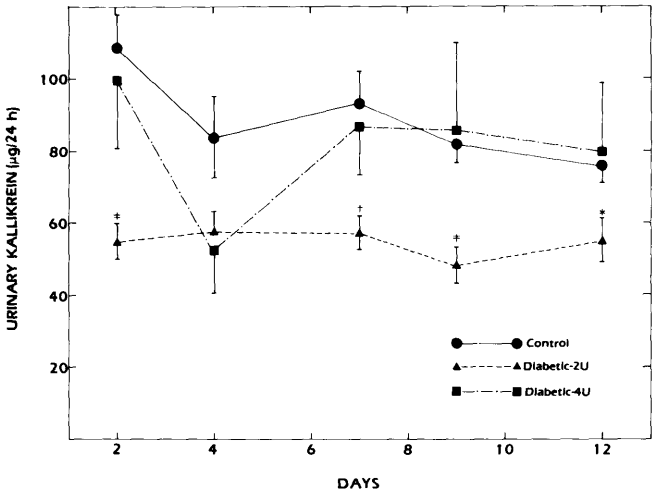

FIGURE 4. In study 2, urinary kallikrein excretion in STZ-diabetic rats treated with a single daily insulin dose (diabetic-2U) or twice-daily dose (diabetic-4U), and in control rats. Kallikrein was measured by radioimmunoassay. Excretion was reduced in diabetic-2U rats as early as day 2. In diabetic-4U rats, kallikrein excretion was not different from that in controls. *P < 0.05, †P < 0.01, and ‡P < 0.001.

not significantly different from controls. In all diabetic rats, average kallikrein excretion during the study correlated inversely with average urinary glucose excretion ($r = 0.84$, $P < 0.005$). This relationship between kallikrein and glucose excretion is best described by the equation: kallikrein (μg/day) = 74.0 − 13.6 [ln glucose (g/day)]. Average kallikrein excretion during the 2-wk period also correlated inversely ($r = 0.66$, $P < 0.02$) with the average serum glucose measured midway through the study on day 7: kallikrein (μg/day) = 220 − 26 [ln serum glucose (mg/dl)].

STUDY 3. RENAL TISSUE KALLIKREIN

In this experiment, renal tissue kallikrein was measured 2 wk after STZ treatment in diabetic rats either untreated or treated with protamine zinc insulin (2.25 U PZI daily), and in control rats.

Diabetic state. One day after STZ, before starting insulin, tail-vein serum glucose levels at 1600 h in the untreated and subsequently treated diabetic rats were not different: 404 ± 18 and 384 ± 26 mg/dl, respectively. Serum glucose levels in all groups after 2 wk are shown in Table 3. Serum glucose in the untreated diabetic rats was similar to the untreated or low-dose insulin-treated rats in studies 1 and 2. The insulin-treated group had serum glucose levels similar to those of controls. Weight gain over 2 wk in the untreated diabetic rats was 51 ± 6 g, significantly less ($P < 0.001$) than in insulin-treated (99 ± 3 g) or control rats (112 ± 4 g).

Renal kallikrein. Two weeks after STZ, immunoreactive renal tissue kallikrein in untreated diabetic rats was 22.8 ± 1.6 ng/mg protein. This was significantly lower ($P < 0.01$) than in control rat kidneys (29.7 ± 1.4 ng/mg protein). In diabetic rats treated with insulin, renal tissue kallikrein (35.8 ± 2.1 ng/mg protein) was significantly higher than that in either untreated diabetic ($P < 0.001$) or in control rats ($P < 0.05$).

DISCUSSION

These studies show that the urinary excretion and renal tissue levels of kallikrein are acutely and chronically reduced in STZ-diabetic rats. Within 2 days of STZ administration, urinary kallikrein is reduced significantly, and after 2 wk of diabetes, when kallikrein excretion remains low, renal tissue levels are also reduced. Insulin, in doses that lower glycemia and glycosuria significantly, not only prevents this fall in urinary kallikrein but also increases the renal levels of the enzyme above those found in nondiabetic kidneys. Furthermore, in diabetic rats the reduction of kallikrein excretion precedes the development of hypertension and persists for several months.

During the course of these studies in Sprague-Dawley rats, Hayashi et al.[22] reported that STZ-diabetic Wistar rats also show reduced kallikrein excretion after 1 wk of diabetes and increased systolic blood pressures after 4 wk. In a preliminary screening effort, we have also found that urinary kallikrein excretion is reduced in the BB-Wistar spontaneously diabetic rat compared with nondiabetic BB-Wistar controls: 1.1 ± 0.1 versus 6.1 ± 0.5 μg/mg creatinine, respectively (samples provided by Dr. Michael Appel, University of Massachusetts, Worcester, Massachusetts).

All of these findings in markedly hyperglycemic diabetic rats contrast with our findings in studies of diabetic humans.[13] Uncomplicated insulin-dependent diabetic patients in poor control ($HbA_{1c} > 11\%$) show significantly increased urinary kallikrein excretion compared with excretion rates in patients with HbA_{1c} levels <11%, or in normal subjects. However, like the kallikrein abnormality in diabetic rats, the abnormality of excretion seen in patients was reversed with glycemic control, suggesting that in both cases the changes in renal kallikrein are related to the diabetic state.

The reasons for the qualitative difference between the kallikrein abnormalities in diabetic patients and these animal models are uncertain. Although the experimental diabetic rat model develops several of the renal changes and complications found in diabetic patients,[14,23,24] some important differences have been noted. In particular, the glomerular hyperfiltration found early in insulin-dependent patients is not found in diabetic rats with severe hyperglycemia.[25,26] In fact, glomerular plasma flow and nephron filtration in these rats are reduced; however, rats with moderate hyperglycemia, while receiving some insulin, do demonstrate glomerular hyperfiltration.[26,27] Since kallikrein excretion rate has been previously correlated with renal blood flow[28] and glomerular filtration rate,[29] the differences we have observed in renal kallikrein between clinical and experimental diabetes might be explained by differences in the severity of hyperglycemia and related renal hemodynamic differences.

TABLE 3
Study 3: serum glucose and renal kallikrein levels in untreated diabetic (diabetic), insulin-treated diabetic (diabetic-insulin), and control rats (mean ± SEM)

	Serum glucose (mg/dl)		Renal kallikrein (ng/mg protein)
	9 a.m.	9 p.m.	
Diabetic	601 ± 34*,†	562 ± 36*,†	22.8 ± 1.6*,‡
Diabetic-insulin	145 ± 24	78 ± 14‡	35.8 ± 2.1§
Control	125 ± 9	130 ± 4	29.7 ± 1.4

*P < 0.001 compared with diabetic-insulin; †P < 0.001 compared with control; ‡P < 0.01 compared with control; §P < 0.05 compared with control.

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Another difference between the diabetic rats with reduced renal and urinary kallikrein and patients with raised urinary kallikrein is their insulin treatment. These rats were receiving little or no insulin. The rats treated with higher doses of insulin, adequate to reduce glycemia toward normal, had normal urinary kallikrein and elevated renal kallikrein. The patients were also receiving considerable amounts of insulin. In both cases, with peripheral administration of insulin, the kidney may have been exposed to higher-than-normal circulating insulin concentrations. It is possible that insulin has a direct influence on renal tissue kallikrein. In study 2, twice-daily insulin treatment was sufficient to result in hypoglycemia at 2400 h. The implications of this hypoglycemia to renal kallikrein are unknown.

Other factors known to influence renal kallikrein-kinin system activity include Na^+ and K^+ intake and excretion,[30,31] sodium-retaining steroids,[30] and renal parenchymal disease and hypertension.[4,32] Although Na^+ and K^+ intake were not measured in the present studies, daily urinary excretion rates in study 2 were not different between rats with normal or low kallikrein excretion. Urine volumes were elevated in the diabetic rats with low kallikrein excretion. Early studies found a direct correlation between kallikrein excretion and urine volume, but other studies have failed to show such a relation.[33-35] An osmotic diuresis induced by a glucose infusion for several hours produces no significant change in urinary kallikrein.[36] In view of many studies of renal kallikrein content and urinary kallikrein excretion rates,[37] as well as recent measurement of renal kallikrein synthesis rate,[38] it seems unlikely that a washout of kallikrein from the kidney accounts for any sustained change in kallikrein excretion. Total renal kallikrein content at any point accounts for <10% of total daily excretion (e.g., approximately 3–4 μg of immunoreactive kallikrein is found in both kidneys compared with total daily excretion of 50–100 μg). In study 2 (Figure 4), diabetic-2U rats with reduced urinary kallikrein had excretion rates on day 1 (data not shown) that were nearly identical to those on day 2. This suggests that no significant early washout of renal kallikrein occurred. Furthermore, in diabetic-4U rats with normal kallikrein excretion, urine volumes increased by day 7 to levels seen on day 2 in diabetic-2U rats. This increase produced no change in urinary kallikrein in these rats.

Plasma mineralocorticoid levels or excretion were not determined, but Hayashi et al.[22] reported that plasma aldosterone was decreased in STZ-diabetic rats with low urinary kallikrein. Relative hypoaldosteronism has been found in alloxan-diabetic rats as well.[23] Decreased aldosterone levels could explain reduced renal kallikrein activity.[30] The fact that kallikrein excretion falls soon after induction of diabetes excludes structural diabetic nephropathy as causative, and the fact that insulin prevents this fall is evidence that streptozocin toxicity is not responsible either.

Our studies confirm previous reports that STZ-diabetic rats develop systolic hypertension as measured by a tail cuff pressure.[12,22,39,41] The relation between raised tail cuff pressures and direct arterial pressure measurements in STZ-diabetic rats has been studied. Hayashi et al.[22] found that tail cuff pressures reflect raised mean arterial pressures, measured directly in the carotid artery in unanesthetized rats. Buñag et al.[12] also found raised femoral artery diastolic pressures in awake STZ-diabetic rats with tail cuff systolic hypertension. A recent preliminary report failed to find elevated direct arterial blood pressure in STZ-diabetic rats with tail cuff systolic hypertension.[41] Thus, it is generally agreed that STZ-diabetic rats become hypertensive, although this conclusion will likely be subject to further study. Regardless, in the present study, the development of hypertension follows the decrease in renal kallikrein by several weeks and, therefore, neither the hypertensive state itself nor renal hypertensive changes can be responsible for the reduced kallikrein levels we have found. It seems likely that further study of the STZ-diabetic rat or, perhaps, the BB-Wistar rat, will help clarify the relations between tissue kallikrein, the diabetic state, insulin, and renal function.

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