Renin and Angiotensinogen Expression During the Evolution of Diabetes

Allen D. Everett, Joanne Scott, Neysa Wilfong, Bradley Marino, Roberto P. Rosenkranz, Tadashi Inagami, and R. Ariel Gomez

The expression of renin and angiotensinogen genes and their proteins were studied during the progression of diabetes using adult BioBreeding spontaneously diabetic rats at 1 day and 2–12 months of diabetes. The number of renin-stained cells per juxtaglomerular apparatus was determined by immunocytochemistry. Initially, at 2 months of diabetes the number of renin-stained cells per juxtaglomerular apparatus increased significantly \( p < 0.0001, 2 \text{ months versus resistant groups} \) and was followed by a decrease in the number and intensity of renin-stained cells after 12 months of diabetes \( p = 0.007, 2 \text{ months versus 12 months} \). A significant negative correlation was observed between the number of renin-containing cells and the duration of diabetes \( r = 0.99, p = 0.014 \). Immunoreactive angiotensinogen was restricted to the proximal tubule and appeared increased after 4 and 8 months of diabetes as compared with the 2- and 12-month diabetic groups. Renin messenger RNA (mRNA) levels increased with the onset of diabetes and decreased markedly during chronic diabetes. At 1 day of diabetes, renin mRNA levels were 700% higher than at 12 months of diabetes. Angiotensinogen mRNA levels were unchanged. We conclude that diabetes results in an initial increase in renin gene expression, and as the duration of diabetes lengthens, there is a progressive decrease in renin gene expression and in the number of cells containing renin. These findings suggest that as the duration of diabetes and the age of the animal lengthens, there is a decrease in the number of cells expressing the renin gene. (Hypertension 1992;19:70–78)

Chronic diabetes mellitus is frequently complicated by hypertension and renal disease.1,2 Physiological studies using the streptozocin rat model have suggested that the altered glomerular hemodynamics, namely, increased glomerular capillary pressure and decreased ultrafiltration coefficient, seen in diabetes are responsible for subsequent glomerular injury and decreased renal function.3 Angiotensin converting enzyme inhibition corrects the glomerular hemodynamic changes observed in diabetes and may arrest the progression of glomerular damage and renal injury.4 Thus, it has been suggested that the functional and morphologic changes observed in the diabetic kidney may be, in part, secondary to activation of the intrarenal renin-angiotensin system.3,4 The intrarenal distribution of renin may be of importance in the regulation of glomerular hemodynamics. Although the presence of immunoreactive renin has been demonstrated in the human end-stage diabetic kidney,5 the time-dependent alterations in kidney renin gene expression and the intrarenal localization of immunoreactive renin during chronic diabetes have remained unexplored. Similarly, the effect of diabetes progression on the intrarenal distribution of angiotensinogen protein and expression of the angiotensinogen gene is unknown.

To determine whether the intrarenal distribution of renin and angiotensinogen and expression of their respective genes are altered during evolution of the disease, we used the spontaneously diabetic BioBreeding (BB) rat, which displays clinical and pathological features closely resembling type 1 (insulin-dependent) diabetes in humans.6

Methods

Animals

Diabetes-prone BB rats were obtained as weanlings from the inbred colony at the University of Massachu-
sets (Worcester) and were maintained in the BB rat facility at the University of Virginia. Animals were kept in an air-conditioned room with a 12-hour light/dark cycle and were fed standard Purina Lab Chow 5012 ad libitum. Approximately 75% of these animals develop a syndrome of severe hyperglycemia secondary to insulinopenia, are nonobese, and require daily insulin injections to prevent ketoacidosis and death.\(^7\) Males and females are equally susceptible to the syndrome, which develops at a mean age of 90 days (range, 60–120 days).

Beginning at approximately 50 days of age, urinary glucose and ketone levels were determined twice weekly with reagent strips (Keto-Diastix, Ames Division, Elkhart, Ind.) as previously described.\(^7,8\) Individual rats were classified as diabetic on the basis of positive, sustained glycosuria as described previously.\(^7,8\) Each diabetic rat was subsequently maintained on a single daily dose of PZI insulin (Eli Lilly and Co., Indianapolis, Ind.), the quantity of which was determined by the daily assessment of glycosuria and ketonuria.\(^8\) Thus, diabetic animals were maintained in a state of moderate glycosuria with normal weight gain in the absence of ketoacidosis, as we previously reported.\(^7,8\)

Diabetic rats were divided into five litter-matched and sex-matched groups to be killed within 1 day after diabetes onset, or at 2, 4, 8, or 12 months after onset, respectively, as shown in Table 1. To serve as a control, Wistar-Furth (WF) rats at 4 and 8 months of age, diabetes-resistant BB rats (a subline of the BB rat strain; less than 1% become diabetic; group resistant) at 4 months of age, and nondiabetic littermates of overtly diabetic BB rats (group prone) at 4 months of age were studied. Animals were decapitated; the kidneys immediately were removed and were placed in liquid nitrogen and stored at −70°C until processed for renin or angiotensinogen messenger RNA (mRNA) determinations or fixed in 10% buffered formalin for renin or angiotensinogen immunochemistry as detailed below.

**Immunocytochemical Studies**

As shown in Table 1, immunocytochemical analysis of kidney sections were examined from BB rats after 2 (n=6), 4 (n=5), 8 (n=7), and 12 (n=5) (5, 7, 11, and 15 months of age, respectively) months of diabetes and for control, WF rats, resistant BB rats at 4 months of age, and prone BB rats at 7 months of age. After fixation, tissues were dehydrated, embedded in paraffin blocks, and serially sectioned (5–7 µm) in the sagittal plane perpendicular to the renal cortex. Immunohistochemical staining for renin was performed as previously described.\(^9,10\) Briefly, kidney sections were incubated with a specific primary anti-renin or angiotensinogen polyclonal rabbit antibodies (1:1,000 final dilution). The high specificity and characterization of the renin antibody has been documented previously.\(^11,12\) The secondary antibody (biotin-conjugated anti-rabbit immunoglobulin G made in a goat) was then added and incubated with avidin-biotinylated horseradish peroxidase complex (Vectastain ABC Kits, Vector Lab, Burlingame, Calif.). As a source of peroxidase substrate, sections were exposed to 0.1% diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, Mo.) and 0.02% H2O2. Slides were then counterstained with hematoxylin and/or with PAS (periodic acid and Schiff's reagent) (Sigma) such that renin or angiotensinogen-containing cells were stained brown in a blue background and the brush border of proximal tubules were stained pink. As a negative control, sections were incubated with nonimmune rabbit serum (as a substitute for the primary antibody) with no staining observed. In addition, to detect possible interstaining variability, positive control tissue sections from a 5-day-old kidney were used as a tissue standard at each staining session. A minimum of two sections per animal were examined by direct microscopic visualization. All renin immunostained and total juxtaglomerular apparatuses (JGAs) were counted in each section. JGAs were defined by the presence of at least an afferent arteriole entering a glomerulus. In addition, the number of cells per JGA stained for renin were counted as a more sensitive measure of renin distribution. Because the number of JGAs may vary from section to section the following ratios were determined and compared among groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (mo)</th>
<th>Immunocytochemistry</th>
<th>mRNA</th>
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<tr>
<td>1 day diabetic</td>
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<td>12 mo diabetic</td>
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<tr>
<td>Resistant</td>
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<td>7</td>
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<td>Prone</td>
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<tr>
<td>Wistar-Furth</td>
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Resistant, diabetic resistant subline of BB rats; prone, nondiabetic littermates of overtly diabetic BB rats; Wistar-Furth, inbred control strain of rat. n, Number of animals studied in each experimental group.

\[ \text{%JGA} = \frac{\text{number of stained JGA} \times 100}{\text{total number of JGA}} \]

\[ \text{JGCI} = \frac{\text{number of stained JGA} \times \text{number of stained cells/JGA}}{\text{total number of JGA}} \]

where %JGA is the percent of total JGA that immunostained and JGCI, the juxtaglomerular cell index, is the number of renin-staining cells per section normalized to the total JGA per section. The ratios thus obtained from each slide were averaged for each animal. Comparisons were made using the final ratios obtained for each animal group.

To determine whether glomerular fibrosis/sclerosis was present, kidney sections from diabetic and dia-
beates-resistant animals were stained using Masson’s trichrome stain (Sigma). Using this technique, collagen-containing material will appear green with a red background. All sections were coded and examined in a blinded manner by a single examiner (A.D.E.).

Renin Messenger RNA Determinations

As shown in Table 1, BB rats were studied after 1 day (n=4), 4 (n=6), 8 (n=6), and 12 months (n=5) of diabetes (aged 3, 7, 11, and 15 months, respectively). Control WF rats at 4 and 8 months (n=4) of age and nondiabetic littersmates of overtly diabetic BB (n=2) and resistant BB rats at 4 months of age (n=7) were also studied. Total RNA was extracted as previously described following the method of Chirgwin et al. Total kidney RNA samples from each group were pooled and submitted to Northern blot analysis as previously described. Membranes were hybridized by the method of Church and Gilbert using a full length (1.4 kb) rat renin complementary DNA (cDNA) or 1.6 kb angiotensinogen cDNA (gift of Dr. K. Lynch, University of Virginia) labeled with phosphorus-32 by nick translation as we previously described. Renin and angiotensinogen mRNA were detected by autoradiography using an intensifying screen. In addition, to analyze interanimal variability, dot blots were performed as previously described by diluting 2 µg total RNA from each animal (such that each dot represents a single animal) and transferring it to a membrane by vacuum. Membranes were hybridized as described above and were quantified by densitometry (Ultrascan XL Laser Densitometer, LKB, Bromma, Sweden). Three scans of each dot were performed, and the results were averaged for each animal. Multiple exposures of Kodak AR and RP film were made to determine linearity. As a control, blots used for renin and angiotensinogen mRNA determinations were also hybridized to β-actin cDNA prepared from a rat aorta cDNA library (a gift of Dr. Gary Owens and Marcie Corjay, University of Virginia).

Statistical Analysis

The %JGA and the number of immunoreactive cells/JGA between the different groups are presented as mean±SD with comparisons performed using the Mann-Whitney U test. In addition, to observe the entire distribution of immunoreactive cells/JGA, frequency distribution polygons were constructed and compared by x² analysis. To determine the correlation between the decrease in JGCI and the duration of diabetes, a regression line and associated correlation coefficient were computed by the least-squares formula. The densitometric absorbance of renin or angiotensinogen mRNA from dot blots are presented as mean±SEM. A value of p<0.05 was defined as significant.

Results

Table 1 shows the characteristics of the different groups studied and number of animals included in each (immunocytochemistry, mRNA) determination. Diabetic animals were not obese and demonstrated appropriate weight gain. Initial and final body weights (g) were as follows: 235±27, 298±36 (2 months); 218±17, 316±40 (4 months); 237±16, 328±36 (8 months); 243±21, 326±36 (12 months).

Effect of Diabetes on Glomerular Histology

Microscopic examination of the diabetic kidneys demonstrated no evidence of glomerular sclerosis or fibrosis with conventional hematoxylin and eosin or trichrome staining in any study group (data not shown).

Effect of Diabetes on the Immunocytochemical Distribution of Renin and Angiotensinogen

The mean number of total (renin positive and renin negative) JGAs per section (±SD) were not different among the different groups of diabetic animals (42±18, 45±8, 44±7, 37±11, duration of diabetes 2, 4, 8, and 12 months, respectively; p=0.76, analysis of variance). However, the intrarenal distribution of renin changed markedly as the duration of diabetes increased. Photomicrographs of representative glomeruli and afferent arterioles stained for renin from diabetes-resistant BB rats, and 2- and 12-month diabetic kidneys are shown in Figure 1. As shown, as diabetes duration lengthened, the number of JG cells containing renin as well as the intensity of immunostaining was decreased. The %JGA stained for renin increased after 4 months of diabetes (80±2%) as compared with the control resistant (42±13%) group (p<0.01) and decreased after 12 months of diabetes (56±16%, p<0.01). The number of cells per JGA stained for renin was increased at 2 months of diabetes (3.46±0.9) when compared with the control prone and resistant groups (2.46±0.3 and 2.2±0.1, p<0.02) but not the WF group (3.5±0.5). In addition, the number of renin-containing cells per JGA decreased with evolution of diabetes. To illustrate this point and to provide a more accurate representation of the population of immunoreactive cells in the JGA, frequency distribution polygons were constructed (Figure 2). As shown, after 2 months of diabetes there is a significant rightward shift in the distribution, indicating a significant increase (p<0.0001) in the number of renin-stained cells per JGA as compared with the resistant group. Whereas, after 12 months of diabetes there is a significant (p=0.0007) leftward shift in the distribution and thus a decrease in renin-stained cells per JGA with prolonged diabetes as compared with the 2-month diabetic group. Furthermore, as illustrated in Figure 3, a significant negative correlation was obtained between the number of cells per JGA and the JGCI (r=-0.986, p=0.014) demonstrating that the overall number of renin-containing cells decreased with the duration of diabetes.

During the evolution of diabetes, immunoreactive angiotensinogen was localized exclusively to the proximal tubule of the kidney as evidenced by positive PAS staining of the proximal tubule brush border. As demonstrated in Figure 4, granules contain-
FIGURE 1. Representative photomicrographs of kidney sections from diabetes-resistant BioBreeding (BB) control (panel A), 2-month (panel B), and 12-month (panel C) diabetic BB rats. Renin-containing cells are stained brown and are localized to the juxtaglomerular apparatus/afferent arteriole. Number and intensity of renin-stained cells are increased after 2 months of diabetes (panel B) and then decrease after 12 months of diabetes (panel C). Original magnification, panels A and B, ×128; panel C, ×160.
JGCI correlates significantly with the duration of diabetes in months; ordinate, mean JGCI (see Figure 3, linear correlation between the juxtaglomerular apparatus (JGA) in kidney sections from diabetes-resistant BioBreeding (BB) rats (resistant, n=7) and in BB rats after 2 (2 months, n=6) and 12 (12 months, n=5) months of diabetes. Abscissa, mean JGCI (see Figure 3, linear correlation between the juxtaglomerular apparatus (JGA) in kidney sections from diabetes-resistant BioBreeding (BB) rats (resistant, n=7) and in BB rats after 2 (2 months, n=6) and 12 (12 months, n=5) months of diabetes. Abscissa, renin-stained cells/JGA; ordinate, percent of stained JGAs corresponding to each cell number interval x axis analysis 2 months versus 12 months, p=0.0005.

Effect of Diabetes on Renal Renin and Angiotensinogen Messenger RNA Levels

The relative levels of renin and angiotensinogen mRNA are shown by the Northern blots in Figures 5 and 6 (left panels) and the subsequent densitometric analyses in Figures 5 and 6 (right panels), respectively. Renin mRNA levels declined with duration of diabetes (Figure 5). As demonstrated by Figure 5 (right panel), at the onset (1 day) of diabetes renin mRNA levels were 700% higher than at 8 and 12 months of diabetes (0.43±0.02 versus 0.06±0.006 and 0.065±0.03 mean absorbance units, respectively) (p<0.05). In addition, renin mRNA levels after 1 day and 4 months of diabetes were 500-1,000% higher, respectively, than in the resistant and prone groups (0.43±0.02 and 0.29±0.09 versus 0.084±0.009 and 0.045±0.005; p<0.05). Thus, renin mRNA levels increase initially with the onset of diabetes and then decline gradually as the duration of diabetes lengthens. Also, renin mRNA levels were not different between the WF 4- and 8-month-old control (0.24 and 0.29) and the 1-day (4 months of age) and 4-month (8 months of age) (0.43±0.02 and 0.29±0.09) diabetic groups (Figure 5, right panel).

Angiotensinogen mRNA levels are demonstrated in Figure 6. As demonstrated by the dot blot of Figure 6 (right panel), there is a great deal of interanimal variability in angiotensinogen mRNA levels from, particularly, the 4- and 8-month diabetic animals. Angiotensinogen mRNA levels after 1 day, 4 months, 8 months, or 12 months of diabetes as compared with the prone and resistant groups, respectively (0.05±0.004, 0.18±0.09, 0.51±0.35, 0.09±0.03 versus 0.17±0.11 and 0.21±0.07), were not statistically different due to the variability between animals. Thus, kidney angiotensinogen mRNA levels were not significantly altered by chronic diabetes.

b-ACTIN mRNA demonstrated the appropriate electrophoretic mobility without degradation. b-ACTIN mRNA levels were not altered during the evolution of diabetes and were comparable within the study groups (data not shown).

Discussion

The present study demonstrates that with the onset of diabetes, there is a marked increase in kidney renin gene expression. As the duration of diabetes and the age of the animal progress, there is a downregulation of kidney renin gene expression, accompanied by a corresponding decrease in the number of juxtaglomerular cells containing renin. Unlike renin, angiotensinogen gene expression is not altered, whereas angiotensinogen protein expression appears to increase at 4 and 8 months followed by a decrease after 1 year of diabetes.

The renin immunocytochemical data of the present study is in close agreement with the findings of Faraggiana and collaborators. Using human kidney tissue from three individuals with longstanding diabetes, those investigators demonstrated a paucity of immunoreactive renin and only occasional cells within a given JGA stained positive for renin. Those findings in humans are strikingly similar to those observed after 1 year of diabetes in our BB rats. The results of the Northern blot analysis and the statistical analysis of individual animals by dot blot demonstrate increased kidney renin mRNA levels initially, followed by a gradual decline with the evolution of diabetes. These findings together with the immunocytochemical results suggest that as the duration of diabetes increases, there is a decrease in the number...
FIGURE 4. Representative photomicrographs of kidney angiotensinogen immunostaining during the evolution of diabetes. Angiotensinogen-containing granules are stained brown and are seen near the luminal surface of the proximal tubule cells. Number of angiotensinogen-containing granules appears higher after 4 (panel B) and 8 (panel C) months of diabetes as compared with groups after 2 (panel A) and 12 (panel D) months of diabetes. Original magnification, x400.
FIGURE 5. Representative Northern blot analysis of renin gene expression during chronic diabetes (left panel) and dot blot densitometric analyses (right panel). Left panel: Total RNA from each group was pooled Wistar-Furth (WF, n=4) rats, nondiabetic littermates of overtly diabetic BioBreeding (BB) rats (P, n=2), and diabetes-resistant BB rats (R, n=7) control groups and each diabetic BB (Diabetic, 1 day, n=4; 4 months, n=6; 8 months, n=6; 12 months, n=5 animals) group. Upper row of numbers indicates the ages in months of the WF, R, and P groups and the duration of diabetes (1 day and 4–12 months in the Diabetic groups. Exposure, 4 days with Kodak AR film with an intensifying screen. Right panel: Dot blot densitometric determinations of renin messenger RNA (mRNA) levels from single animals. Ordinate, densitometric absorbance units (mean±SEM) from dot blot autoradiograph for renin; abscissa, control groups: Wistar-Furth 4 months (WF4) n=1 and 8 months (WF8) old n=1, nondiabetic littermates of overtly diabetic BB (P) n=2 and diabetes-resistant BB (R) n=7. Experimental groups, diabetic BB rats at 1 day (1 D) n=4, 4 months (4 M) n=6, 8 months (8 M) n=5, and 12 months (12 M) n=5 of diabetes. *, Mean absorbance units for each diabetic group; O, for each control group. As shown, the mean absorbance, indicating the concentration of renin mRNA, is increased with the onset of diabetes and decreases as the age of animals and the duration of diabetes increases. (–)p<0.05.

of JG cells expressing the renin gene. Although we have not measured actual renin synthesis, the decrease in immunoreactive renin in the diabetic kidney suggests that renin synthesis may be decreased. Alternatively, an increase in renin or prorenin release by the regulated or constitutive pathways may explain the paucity of intrarenal renin stores. However, since renin mRNA levels are low, these mechanisms seem less likely because it would also require an increase in translational efficiency.

Recently Katayama et al,16 using the BB/Worcester rat, demonstrated that during early stages of
diabetes, plasma renin activity is elevated without changes in renin mRNA levels when compared with Wistar rats as controls. In the present study, kidney renin mRNA levels also were not different between the diabetic and Wistar-Furth groups; however, when nondiabetic littermates of diabetic animals and diabetes-resistant BB rats were studied as controls, renin gene expression markedly increased at the onset of diabetes. This elevation in renin gene expression and the reported increase in circulating renin would suggest that improved translational efficiency is not the major mechanism regulating renin synthesis in early diabetes. Thus, the increases in plasma renin activity observed in early stages of diabetes seem to be due to an increase in renin gene transcription; however, this hypothesis remains to be tested.

The mechanisms responsible for changes in kidney renin gene and protein expression during diabetes are not well understood. Fluid and salt balance are known to affect the activity of the renin-angiotensin system. In addition, low or high salt diets and excess volume expansion are known to alter plasma renin activity in the diabetic BB rat. The diabetic animals in our study that demonstrated a consistent weight gain were nonketotic and were allowed free access to food and water. The sodium and volume status was not determined in our study. However, in similarly treated animals (average blood glucose 551 ± 19 mg/dl) Cohen et al demonstrated that after 100–150 days of diabetes, BB rats developed elevated plasma volumes without alterations in serum or urinary sodium excretion. Thus, although speculative, increased plasma volume and positive sodium balance may have contributed to decreased renin gene expression in our study.

Multiple factors other than plasma volume, such as circulating atrial natriuretic peptide and prostaglandin synthesis, are altered during diabetes and are known to affect renin release and possibly synthesis in the nondiabetic animal. In addition, autonomic regulation of renin release during diabetes may be impaired as reported in the BB rat and humans. Whether these physiological changes may in turn be responsible for alterations in renin gene transcription and/or mRNA stabilization during diabetes are not known and require further study.

As previously demonstrated, angiotensinogen mRNA is present in the kidney with the angiotensinogen peptide localized to the proximal tubule. As described, angiotensinogen mRNA levels appear stable during chronic diabetes, whereas angiotensinogen protein expression increased after 4 and 8 months and then decreased after 12 months of diabetes. This discrepancy between the lack of change in angiotensinogen gene expression and the fluctuation in angiotensinogen protein could be due to changes in translational efficiency, transcript degradation, or increased uptake of circulating angiotensinogen by the proximal tubule. Previously, the presence of extrahepatic angiotensinogen gene transcripts in multiple organs has served as evidence for local renin-angiotensin systems, and recently a tubular angiotensin generating system was proposed. Whether the presence and alteration of angiotensinogen gene and protein expression in the diabetic kidney reflects an alteration in tubular function/transcriptional activity of renin and angiotensinogen may be regulated in an uncoordinated fashion and that different mechanisms may control the expression of the renal renin and angiotensinogen gene during chronic diabetes.

The effects of aging on the renin-angiotensin system have not been well studied. Previous reports have centered around enzymatic determinations of circulating renin, which appears decreased after the sixth decade of life in humans. We and others have demonstrated that renin gene expression and protein distribution changes dramatically with ontogeny, resulting in a decrease in renin mRNA in the adult rat (90-day-old) when compared with the fetal or newborn rat. It is unknown whether there is further alteration in the intrarenal distribution and expression of renin with aging beyond 90 days of life. We attempted to control for the possible effects of aging by using control animals of different ages, but a time control for every animal group was not available during the course of our study. The present study does clearly demonstrate that renin gene expression is higher after 1 day and 4 months of diabetes (3 months and 7 months of age, respectively) than in either the diabetes-resistant (4-month-old) or the nondiabetic littermates of overtly diabetic animals (7-month-old). In addition, there is no difference in the renin mRNA expression at 4 or 8 months of age in the Wistar-Furth animals. Taken together, these data suggest that although a biologic effect of aging cannot be completely excluded, a chronologic effect of aging during the first 8 months of diabetes is small. The possible effects of aging on the regulation of the intrarenal renin-angiotensin system requires further study.

In the present study, we demonstrated a lack of glomerulopathy in the kidney of the BB rat after 12 months of diabetes. This is in agreement with previous studies demonstrating lack of glomerulopathy in the diabetic BB rat kidney as opposed to the streptozocin diabetic model. Brown et al demonstrated that the light microscopic exam of the diabetic BB rat kidney was normal. The significant finding was a thickening of the glomerular basement membrane without alteration in the mesangial cell number or area or capillary wall area after 30 weeks of diabetes. In addition, the animals excreted normal amounts of albumin. These findings were confirmed by Cohen et al when diabetic BB rat kidneys were studied after 12 months of diabetes and were examined by electron microscopy. Thus, it appears that glomerular basement membrane thickening is the only histologic abnormality seen in the diabetic BB

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rat kidney. This suggests that possible genetic factors may influence the pathological expression of diabetic nephropathy in rats.

In summary, the onset of diabetes results in increased kidney renin gene expression and, with progression of diabetes, a decrease in the number of renin-containing cells and an associated decrease in renin gene expression. Kidney angiotensinogen gene expression is relatively unchanged whereas angiotensinogen protein expression appears increased after 4 and 8 months of diabetes. Therefore, the onset of diabetes appears to result in an activation of the renal renin-angiotensin system followed by a marked downregulation as the duration of diabetes and the age of the animal increase. We suspect that these significant alterations in the intrarenal renin-angiotensin system may have an important impact on the functional and morphologic changes observed in the diabetic kidney. In addition, these temporal changes in the activity of the intrarenal renin-angiotensin system should be taken into account when experiments with diabetic animals are planned.

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**References**


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