Hyperreninemia Due to Increased Renal Renin Synthesis in BioBreeding Worcester Rats

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It is well known that diabetes mellitus is often associated with hypertension. We previously reported the unresponsiveness of renin release to volume depletion with impaired renal prostaglandin E\textsubscript{2} synthesis in rats with streptozotocin-induced diabetes. However, we have found that BioBreeding Worcester rats, spontaneously susceptible to diabetes mellitus either before or after the onset of diabetes, showed a pronounced fourfold to ninefold increase in plasma renin activity in comparison with control Wistar rats. Furthermore, these rats developed mild hypertension as high as 134 mm Hg after the age of 90 days. The hyperreninemia responded to 1-week sodium loading or restriction; the blood pressure increased during sodium loading. Oral administration of captopril (30 mg/kg) for 1 week resulted in a large blood pressure decrease (−47.1±5.9 mm Hg, n=10) in comparison with controls (−17.0±4.7 mm Hg, n=12). Vascular response to angiotensin II was also attenuated. Plasma angiotensin II levels were 5.7-fold higher and associated with a 1.5-fold increase of plasma aldosterone concentration compared with control rats, whereas angiotensinogen-plasma concentrations were lower than in control rats. The renal renin content determined enzymatically or histochemically was more enhanced in BioBreeding Worcester rats than in control rats, but the renal renin messenger RNA levels did not differ. These results suggest that the strain-specific hyperreninemia in BioBreeding Worcester rats might be due to posttranscriptional abnormalities of renal renin synthesis. Further work is needed to elucidate the specific mechanism or mechanisms responsible.

Considerable efforts have been made to clarify the pathophysiological roles of the renin-angiotensin axis in diabetes mellitus (DM). Plasma renin activity (PRA) is reportedly suppressed together with an increase in vascular responsiveness to angiotensin II (Ang II) in alloxan-induced DM in the rat.\textsuperscript{1} In contrast, diabetics with ketoacidosis usually have pronounced associated secondary hyperaldosteronism, which is rapidly normalized with metabolic control.\textsuperscript{2} Decreased PRA, however, has commonly been found in diabetic patients with orthostatic hypotension and in those patients with renal disease.\textsuperscript{3} Additionally, DM has frequently been found in association with hyporeninemic hypoaldosteronism.\textsuperscript{4} Hyporeninemia can be due to volume expansion after hyperglycemia,\textsuperscript{1} impaired renin release,\textsuperscript{5} inadequate conversion of prorenin to renin,\textsuperscript{4} and diminished catecholamine secretion.\textsuperscript{6} We recently reported that PRA in rats with streptozotocin-induced DM did not respond to volume reduction 1 week after onset, and that renal renin release in vitro was stimulated by isoproterenol but not by prostaglandin E\textsubscript{2}.\textsuperscript{7}

In most of the experimental studies previously cited, rats with chemically induced DM have been used. However, with the establishment of a special strain of BioBreeding Worcester (BB/W) rat susceptible to DM,\textsuperscript{8} which is a model of insulin-dependent DM, more information should become available about the pathophysiological roles of the renin-angiotensin axis in diabetic renal function and blood pressure regulation. The present study was performed to characterize the renin-angiotensin axis in this rat model.

**Methods**

BB/W rats were obtained from the Department of Pathology, University of Massachusetts Medical School (courtesy of A. Like). A colony of these BB/W rats was maintained by sister-brother mating and
grown under specific pathogen-free conditions. The rats were maintained on normal laboratory chow (0.7% NaCl, wt/wt) and tap water ad libitum except where otherwise specified. Daily glycosuria was monitored with Keto-Diastix (Miles Laboratories, Inc., Elkhart, Indiana). The onset of overt DM was defined as the day on which glycosuria was first observed. DM usually developed after 65–75 days, and the incidence of DM reached 90% at the age of 90 days. After the onset of DM, 2–4 units of ultralente insulin was injected subcutaneously according to the severity of glycosuria to avoid ketoacidosis and to maintain normal growth. Rats that did not develop glycosuria by 105 days were considered to be non-DM. Blood glucose levels during ad libidum food intake were 134.3 ± 5.5 (±SEM) mg/dl in 70-day-old normal Wistar rats (n = 6), 131.2 ± 4.0 in 50-day-old BB/W rats before onset of DM (n = 19), and 134 ± 7.0 mg/dl in 120-day-old non-DM BB/W rats (n = 7); whereas seven 70-day-old or six 120-day-old DM BB/W rats demonstrated significantly higher blood glucose levels of 474.0 ± 47.4 or 515 ± 36.6 mg/dl (p < 0.01 vs. controls or non-DM controls), respectively.

**Determination of Blood Pressure, Plasma Renin Activity, and Plasma Levels of Angiotensin II, Aldosterone, and Angiotensinogen**

Systolic blood pressure and heart rates were determined by a tail-cuff method with BB/W rats and normal Wistar rats obtained from Labo Animals (Tokyo, Japan) at various ages and under conditions of normal sodium intake as well as high or low sodium intake or captopril administration. Blood samples (0.2 ml) were also withdrawn from the jugular vein without anesthesia into a chilled tube containing 2 mg EDTA. Plasma was kept frozen until analysis of PRA.

Because systolic blood pressure and PRA were not different irrespective of the presence of DM, 60–70-day-old BB/W rats before the onset of DM were used except where specified. For sodium loading or restriction, 2.5% NaCl solution (wt/vol) instead of tap water (n = 7) or a low sodium rat chow containing 0.07% NaCl (wt/wt) (n = 6) was given for 7 days. For angiotensin converting enzyme inhibition, captopril 30 mg/kg/day was administered orally to 10 BB/W rats in the morning for 1 week. During this period, age-matched normal Wistar rats (n = 12) served as controls. During the 2 days before or the last 2 days of sodium loading or restriction or captopril administration, some of the rats were housed in metabolic cages, and two 24-hour urine samples were collected. Urine was kept frozen until analysis of urinary sodium and potassium concentrations by flame photometry.

In some diabetic or nondiabetic 120-day-old BB/W rats (n = 6 each) and in age-matched control rats (n = 5), plasma concentrations of Ang II, angiotensinogen, and aldosterone were measured by using blood samples obtained from the trunk after decapitation. Plasma angiotensinogen levels were estimated by determination of angiotensin I (Ang I) after incubation of plasma with an excess amount of rat cortical homogenate in the presence of 5 mM phenylmethylsulfonyl fluoride and 5 mM EDTA for 60 minutes at 37°C. Usually, 100 μl of cortical homogenate (20 mg/ml) added to 1 μl of plasma resulted in a plateau within 60 minutes.

**Vascular Response to Angiotensin II**

Vascular pressor response to a single intravenous bolus injection of 10, 20, or 50 ng of Ang II was determined in BB/W rats (n = 6) and age-matched control Wistar rats (n = 6). Blood pressure changes in response to a continuous infusion of [Sar1, Ile8]Ang II (Bachem, Torrance, California) at 0.4 μg/kg/min for 10 minutes followed by 1.0 μg/kg/min for another 10 minutes were also recorded in both strains (n = 5 each). Catheters implanted into the femoral artery and vein while the rats were under pentobarbital anesthesia on the preceding day were connected to a transducer (T4812AD, Gould-Statham, Medical Products Division, Oxnard, California), and blood pressure was recorded on a polygraph (RM 6100, Nihon Kohden, Tokyo, Japan) before and after administration of Ang II or Ang II analogue without anesthesia.

**Quantification of Renal Renin Messenger RNA and Renin Content**

BB/W rats (four pre-DM and 11 DM) and age-matched control Wistar rats (four 60-day-old and six 106-day-old) were killed by decapitation. Blood was collected from the trunk for PRA determination, and the kidneys were removed quickly and snap-frozen in liquid nitrogen. The renal cortex (200–400 mg) was homogenized in 4 M guanidine thiocyanate, 0.5% sodium-N-lauryl sarcosine, 25 mM sodium citrate (pH 7.0), and 0.1 M β-mercaptoethanol, and total RNA was extracted according to the method of Chomczynski and Sacchi. The precipitated RNA was purified with an oligo dT cellulose column. The poly-A rich fraction (3 μg) was lyophilized, denatured with 72% formamide and 9.6% formaldehyde in 32 mM 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (pH 7.0) at 60°C for 10 minutes, and size-separated through 1.2% agarose gel in 20 mM MOPS buffer (pH 7.0). The gel was transblotted by capillary action with 10× standard saline citrate (SSC) for 20 hours, and then baked at 80°C in a vacuum oven for 2 hours. A solution of 50% formamide, 10% dextran sulfate, 1% sodium dodecyl sulfate (SDS), and 80 μg/ml salmon sperm DNA was used for prehybridization for 10 minutes at 60°C. The blots were then hybridized overnight in the same buffer, to which was added nick-translated rat renin complementary DNA (cDNA) (PRRn23 corresponding to nucleotides 308–1,005 of rat renin messenger RNA [mRNA]). After hybridization, the blots were washed in 2×SSC containing 0.1% SDS followed by 0.1×SSC with 0.1% SDS at room temperature. The blots were then dried and subsequently exposed to x-ray film (Fuji HR, Fuji Medical Systems Co. Ltd., Tokyo, Japan).
FIGURE 1. Bar graphs of time course of plasma renin activity (PRA), systolic blood pressure (SBP), and heart rate (HR) in spontaneously diabetic BioBreeding Worcester (BB/W) rats and control (Cont.) Wistar rats (stippled column) at various ages. Pre-diabetic or nondiabetic BB/W rats are indicated by clear columns, and diabetic BB/W rats are indicated by hatched columns. Each value represents mean±SEM. Numbers in the columns indicate numbers of rats. *p<0.05, **p<0.01.

Tokyo, Japan), and the autoradiographs were scanned with a Shimadzu densitometer (Tokyo, Japan). The recovery of total RNA from BB/W rats (2.19±0.19 µg/mg tissue) was not different from that of control rats (2.48±0.11 µg/mg tissue).

The renocortical renin concentration was also determined by radioimmunoassay (RIA) of Ang I generated in the presence of an excess of renin substrate obtained from plasma of a nephrectomized rabbit, as described previously, and expressed as nanograms of Ang I per hour per milligram of tissue.

Histochemical Studies

Seventy-day-old control Wistar rats (n=5) and BB/W rats at various stages (n=19) were killed by decapitation. The renal cortex was removed immediately and used for microscopic and immunohistochemical examination. Cortical tissues were fixed in 10% formalin, dehydrated in alcohol, and embedded in paraffin. Thin sections (4 µm) were stained with periodic acid-Schiff reagent (PAS), and the numbers of PAS-positive granules were counted according to the method of Hartroft and Hartroft. Briefly, 200 serial glomeruli were observed microscopically, and the degree of granulation in the juxtaglomerular cells was rated as 0 or + to ++++. The totals recorded under scores of zero, one-plus, two-plus, three-plus, and four-plus were multiplied by factors of 0, 1, 2, 3, and 4, respectively. The weighted totals were then expressed per 100 glomeruli as the indexes of juxtaglomerular cell granulation. For immunohistochemistry, the sections were rehydrated and incubated with antisera against mouse submandibular gland renin at 1:100 dilution. In the subsequent steps, biotinylated sheep antiserum against rabbit immunoglobulin G and avidin-biotin-peroxidase complex (Vector Labs, Burlingame, California) were used to visualize renin-containing granules.

Assays

PRA was determined by using a specific Ang I RIA. Anti-Ang I antibody was obtained from Miles Laboratories (Elkhart, Indiana). The plasma concentration of Ang II or aldosterone was measured by a specific direct RIA for Ang II or aldosterone. Cross-reactivity of anti-Ang II antibody with Ang I was 0.1%, and that of antialdosterone antibody with corticosterone was 0.0086%. Interassay and intra-assay variation were 13% and 8% for Ang I RIA, and less than 10% for Ang II or aldosterone assay. Corresponding iodinated tracers and 32P-deoxycytidine 5'-triphosphate (dCTP) were purchased from New England Nuclear (Boston, Massachusetts).

Statistics

All data were expressed as mean±SEM. Statistical difference was analyzed by paired or unpaired Student's t test or Mann-Whitney U test and considered to be significant when the p value was less than 0.05.

Results

PRA in BB/W rats was elevated in comparison with control Wistar rats, irrespective of whether they
TABLE 2. Effects of Seven-Day Sodium Loading or Restriction on Blood Pressure, Heart Rate, Plasma Renin Activity, Plasma Aldosterone Concentration, Urine Volume, and Urinary Excretion of Sodium and Potassium

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sodium loading (n=7)</th>
<th>Sodium restriction (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>108±3.6</td>
<td>126±7.7*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>394.5±4.6</td>
<td>384.6±14.0</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>57.3±17.0</td>
<td>23.8±4.7*</td>
</tr>
<tr>
<td>Urine volume (ml/100 g body wt)</td>
<td>2.5±0.5</td>
<td>18.7±0.9†</td>
</tr>
<tr>
<td>Sodium excretion (meq/100 g body wt)</td>
<td>0.30±0.03</td>
<td>1.07±0.69†</td>
</tr>
<tr>
<td>Potassium excretion (meq/100 g body wt)</td>
<td>0.82±0.06</td>
<td>0.65±0.07*</td>
</tr>
</tbody>
</table>

Values represent mean±SEM. PRA, plasma renin activity.

*p<0.05 vs. controls before sodium loading or restriction.

Typical Northern blot patterns of renal renin mRNA are shown in Figure 3. The probe was highly specific for renin mRNA, detecting a single species of approximately 1.6 kb, which is in agreement with previous estimates of the size of rat renin mRNA. Comparison of PRA, renal renin contents, and renin mRNA levels between control rats and BB/W rats is
FIGURE 3. Typical Northern blot analysis of rat renal renin messenger RNA (mRNA) levels from age-matched 106-day-old control Wistar rats and diabetic BioBreeding Worcester (BB/W) rats. Renin mRNA signals correspond to fragments of approximately 1.6 kb.

FIGURE 4. Bar graphs of plasma renin activity (PRA), renal renin concentrations (RRC), and renal renin messenger RNA (mRNA) levels determined by Northern blot analysis in control Wistar rats (unfilled bars, n=10) and BioBreeding Worcester (BB/W) rats (filled bars, n=15). Blood glucose levels were 153±3.8 mg/dl in control rats and 154±17.5 or 289±40.5 mg/dl (p<0.01) in non-diabetes mellitus (DM) or DM BB/W rats, respectively.

Discussion

The present study clearly demonstrates pronounced hyperreninemia in BB/W rats, irrespective of whether they were young or old, or DM or non-DM, on the basis of higher PRA levels and elevated Ang II concentrations after the age of 30 days. Furthermore, mild hypertension developed after 90 days. In fact, captopril, an angiotensin converting enzyme inhibitor, produced a massive blood pressure decline in comparison with control rats. Taken together with the responsiveness of the hyperreninemia to 1-week sodium loading, an elevated
plasma Ang II level might be involved in blood pressure maintenance in BB/W rats. This hypothesis was further supported by the attenuated vascular pressor response to exogenously administered Ang II and by the less significant blood pressure increase in response to Ang II analogue observed in this rat model that may be due to desensitization of blood vessels because of the augmented renin-angiotensin axis. In contrast, plasma aldosterone concentrations in BB/W rats were also higher than in control Wistar rats. Ang II is known to modulate Ang II receptor density in adrenal glomerulosa cells. A positive correlation between PRA and plasma aldosterone levels in BB/W rats might indicate an augmented response of adrenal glomerulosa cells to endogenous Ang II, possibly through up-regulation as reported under conditions such as sodium restriction in both humans and rats.\(^{18,19}\)

It seems unlikely that the hyperreninemia in this strain was due to an increase in renin substrate because plasma angiotensinogen levels in BB/W rats were lower than in control rats. Instead, it might have been due to augmented synthesis or secretion of renin, or augmented synthesis and secretion of renin, by juxtaglomerular cells. In fact, the renal renin content determined enzymatically or histochemically did show a twofold increase in BB/W rats in comparison with controls. It is likely that BB/W rats store more renin and release more renin in response to stimuli. No difference, however, was observed in renin mRNA levels between hyperreninemic BB/W rats and normoreninemic control rats although there exists substantial variability in the amount of RNA detected. Such nonproportional changes in plasma renin concentration, renal renin concentration, and renin mRNA levels have been reported previously.\(^{15,20}\) For example, Nakamura et al\(^{15}\) reported that 15-day sodium restriction and captopril treatment produced a 46-fold increase in plasma renin concentration associated with only a 1.5-fold or 2.8-fold increase in the renal renin concentration or renin mRNA level, respectively. In such cases, a small temporal increase of renin gene transcription might explain the observed increase in plasma renin concentration. No difference in renin mRNA levels in BB/W rats, however, might indicate an appreciable degree of posttranscriptional control, possibly at the step of translation or prorenin processing, or both. Such translational regulation was reported in several genes, such as proinsulin, ovalbumin, and \(\alpha_1\)-acid glycoprotein. Further studies will be needed to determine which site or sites are responsible for the

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**FIGURE 5.** Photomicrographs of typical immunohistochemical staining patterns in renal cortex of a 70-day-old normal Wistar rat (left panel) and a BioBreeding Worcester (BB/W) rat (right panel) by using the avidin-biotin-peroxidase technique with antiserum against mouse submandibular gland renin. (Original magnification, \(\times 100\).)
strain-specific hyperreninemia observed in spontaneously diabetic BB/W rats.

Acknowledgments

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