Vascular and Adrenal Reninlike Activity in Chronically Diabetic Rats

MARIANO UBEDA, ISABEL HERNANDEZ, FRANCISCO FENOY, AND TOMAS QUESADA

SUMMARY The aim of this work was to investigate, in an experimental model of diabetes mellitus, the levels of renin activity in vascular and adrenal tissues and their relationship to several circulating renin-angiotensin system components. Rats with chronic (12 weeks) streptozocin-induced diabetes showed a significant decrease in plasma renin activity (PRA), plasma renin concentration, and plasma aldosterone. However, plasma trypsin activatable inactive renin concentration was increased (11.65 ± 1.40 vs 6.73 ± 0.57 ng angiotensin I/ml/hr; p < 0.001), as were aortic reninlike activity (p < 0.001) and adrenal renin, both in the zona glomerulosa (p < 0.01) and the fascicular-reticular-medullary portion (p < 0.001) with respect to an age-matched control group. After bilateral nephrectomy, plasma renin-angiotensin system components (PRA and plasma active and inactive renin concentrations) as well as aortic and fascicular-reticular-medullary renin activity significantly decreased in both control and diabetic rats. However, glomerular renin activity increased in control nephrectomized rats to the levels observed in diabetic animals but did not change in diabetic nephrectomized rats. The parallel changes of aortic and fascicular-reticular-medullary renin activity and plasma inactive renin concentration in diabetes and nephrectomy suggest an interdependent relationship, whereas the increase of glomerular renin activity in diabetic and nephrectomized animals, both with low levels of PRA, suggests the existence of a local autonomic renin-angiotensin system regulated by plasma feedback. Tissue renin-angiotensin system alterations in diabetes could mean that a pathogenic factor is involved in long-term diabetic complications or that only a compensatory physiological process is at work. (Hypertension 11:339–343, 1988)

KEY WORDS • diabetic rats • tissue renin • inactive renin • adrenal renin • vascular renin

ALL the components of the renin-angiotensin system (RAS) have been detected in vascular1–5 and adrenal tissues.6–9 Naruse et al.9,10 have recently confirmed that the reninlike activity present in adrenal gland of rats is due to true renin since it is completely inhibited by specific anti-rat renin antibodies. Moreover, immunohistological studies have shown the presence of renin in the zona glomerulosa and reticularis of the human adrenal gland.11 Dzau12 hypothesized that the RAS consists of two compartments, one in circulation and the other in local tissues. The traditional circulating RAS is an endocrine system whose principal function is short-term cardiorenal homeostasis, and the tissue RAS may function as a paracrine or autocrine system that regulates some long-term tissue functions.

It is generally accepted that microvascular alterations could be an important factor in the genesis of long-term diabetic complications,13 and some authors have found a close association between high levels of plasma inactive renin and the presence of microvascular complications in human diabetic patients.14 An adrenal dysfunction consisting of hypoaldosteronism with low levels of plasma renin activity (PRA) is frequently associated with human diabetes15 and has been demonstrated in experimental models of diabetes.16,17 As has the hyposecretion of aldosterone induced by angiotensin II.18 Thus, in the present study we examined whether changes in vascular and adrenal RAS might occur in experimental diabetes.

Materials and Methods

Male Wistar rats (age, 8 weeks; Panlab, Spain) weighing 250 ± 10 g were used in this work. Diabetic state was induced by intravenous administration of a
single dose (65 mg/kg) of streptozocin (Zanosar; Upjohn). Control rats were injected with vehicle (saline solution). In the nephrectomized group of animals, bilateral nephrectomy was performed in ether-anesthetized rats 24 hours before the experiments. At the end of the experimental period (3 months after streptozocin or vehicle injection), rats were killed by decapitation.

A blood sample was collected into tubes containing NaEDTA and centrifuged at 4°C, and plasma then was stored at −20°C for biochemical determinations. To avoid tissue contamination of plasma renin, animals were completely exsanguinated through an incision made in the right atrium and the vascular bed was perfused with 125 ml of ice-cold saline through a needle inserted into the left ventricle. Then, adrenals and aorta were removed and cleaned of surrounding tissues. The capsular portion of adrenal gland was separated using dissecting microscopy. The separation of the zona glomerulosa from the fascicular-reticular-medullary (FRM) portion was verified by light microscopic examination. Aorta, zona glomerulosa, and the FRM portions were stored at temperatures below −20°C until assayed for renin.

**Tissue Reninlike Activity**

For the renin activity determination, aorta, zona glomerulosa, and FRM portions were frozen and thawed four times. Aorta was cut into small pieces with scissors. Then, tissues were homogenized in 0.1 M sodium phosphate buffer, pH 7 (10 μl/mg wet weight), containing a mixture of protease inhibitors: 2 mM phenylmethylsulfonyl fluoride (PMSF; Sigma), 1 mM diisopropylfluorophosphate (Sigma), and 10 mM EDTA (Sigma). Extracts were centrifuged at 3000 g for 30 minutes, and 0.1 ml of supernatant was coincubated at 37°C for 20 hours with 0.1 ml of bilaterally nephrectomized rat plasma and 0.1 ml of 0.1 M sodium phosphate buffer (pH 7) containing the following protease inhibitors: 2 mM diisopropylfluorophosphate (Sigma), and 10 mM EDTA (Sigma). Extracts were centrifuged at 3000 g for 30 minutes, and 0.1 ml of supernatant was coincubated at 37°C for 20 hours with 0.1 ml of bilaterally nephrectomized rat plasma and 0.1 ml of 0.1 M sodium phosphate buffer (pH 7) containing the following protease inhibitors: 2 mM diisopropylfluorophosphate, 2 mM PMSF, 10 mM EDTA, and 2 mM 8-hydroxyquinoline (Sigma), and 0.1% neomycin sulfate (Sigma). Under these conditions angiotensin I (Ang I) recovery was 74.44, 80.43 and 76.14%, respectively, when aorta, zona glomerulosa, and FRM extracts were present in the incubation medium. From each tube an aliquot was drawn out before the incubation was stopped at 10 minutes by adding soybean trypsin inhibitor, 10 mg/ml. Inactive renin was defined as the difference between total renin (trypsin treatment) and active renin. Plasma aldosterone concentration was determined by radioimmunoassay of Ang I as described by Haber et al. Plasma renin concentration (PRC) was estimated by the Ang I generated in 1 hour when samples were incubated with nephrectomized rat plasma as a source of excess renin substrate. Plasma inactive renin was measured by trypsin activation based on the method of Barret et al. Briefly, trypsin (Type IX, Sigma) freshly dissolved in 0.2 M phosphate buffer, pH 7.4, was added to 0.1-ml aliquots of rat plasma. Trypsin was appropriately diluted to reach a final concentration of 5 mg/ml of plasma. The reaction was stopped at 10 minutes by adding soybean trypsin inhibitor, 10 mg/ml. Inactive renin was defined as the difference between total renin (trypsin treatment) and active renin. Plasma aldosterone concentration was determined by direct radioimmunoassay (Biodata).

**Statistical Analysis**

Results are expressed as means ± SEM. Differences between groups were analyzed by the nonpaired t test.

**Results**

As shown in Table 1, the blood glucose concentration was increased in chronically diabetic rats to 471.55 ± 22.81 mg/dl compared with 111.57 ± 4.68 mg/dl for normal controls (p < 0.001). Plasma creatinine was not different among groups. PRA was decreased in diabetic rats (0.82 ± 0.17 vs 4.32 ± 0.76 ng Ang I/ml/hr for normal controls; p < 0.001). Plasma aldosterone was also decreased (91.62 ± 15.44 pg/ml for normal controls; p < 0.001). Plasma renin concentration was increased in chronically diabetic rats to 471.55 ± 22.81 mg/dl compared with 111.57 ± 4.68 mg/dl for normal controls (p < 0.001). Plasma inactive renin concentration was also increased in diabetic rats (0.82 ± 0.17 vs 4.32 ± 0.76 ng Ang I/ml/hr for normal controls; p < 0.001). Plasma aldosterone was also decreased (91.62 ± 15.44 pg/ml for normal controls; p < 0.001).

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>PRA (ng Ang I/ml/hr)</th>
<th>PRC (ng Ang I/ml/hr)</th>
<th>PIRC (ng Ang I/ml/hr)</th>
<th>Aldosterone (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>111.57 ± 4.68</td>
<td>0.46 ± 0.09</td>
<td>4.32 ± 0.76</td>
<td>5.60 ± 0.98</td>
<td>6.73 ± 0.57</td>
<td>233.05 ± 31.45</td>
</tr>
<tr>
<td>NX</td>
<td>87.00 ± 4.08*</td>
<td>—</td>
<td>0.18 ± 0.05†</td>
<td>0.12 ± 0.07†</td>
<td>1.87 ± 0.39†</td>
<td>—</td>
</tr>
<tr>
<td>DIAB</td>
<td>471.55 ± 22.81†</td>
<td>0.41 ± 0.03</td>
<td>0.82 ± 0.17†</td>
<td>1.42 ± 0.33†</td>
<td>11.65 ± 1.40†</td>
<td>91.62 ± 15.44*</td>
</tr>
<tr>
<td>DIAB + NX</td>
<td>378.87 ± 27.87†</td>
<td>—</td>
<td>0.30 ± 0.07†</td>
<td>0.15 ± 0.07†</td>
<td>2.37 ± 0.44†</td>
<td>—</td>
</tr>
</tbody>
</table>

*Values are means ± SE. PRC = plasma renin concentration; PIRC = plasma inactive renin concentration; NX = 24 hours after bilateral nephrectomy; DIAB = chronically diabetic; DIAB + NX = 24-hour nephrectomized diabetic rats.

*p < 0.01, †p < 0.001, compared with control group values; ‡p < 0.05, §p < 0.001, compared with diabetic group values.
for diabetic animals and 233.05 ± 31.45 pg/ml for controls; p < 0.01).

**Active and Inactive Plasma Renin Concentrations**

As shown in Table 1, PRC was decreased in chronically diabetic rats (1.42 ± 0.33 vs 5.60 ± 0.98 ng Ang I/ml/hr for controls; p < 0.001). Bilateral nephrectomy decreased PRC to almost undetectable levels: 0.12 ± 0.07 ng Ang I/ml/hr for nondiabetic rats (p < 0.001) and 0.15 ± 0.07 ng Ang I/ml/hr for diabetic rats (p < 0.001). In all experimental groups PRA changes were similar to those observed in PRC levels (Table 1). Table 1 shows the plasma inactive renin concentrations in control rats and in diabetic rats. The increase in plasma inactive renin concentration of diabetic rats was statistically significant (p < 0.001) compared with that for the control group. Twenty-four hours after bilateral nephrectomy, the plasma inactive renin concentration decreased to 1.87 ± 0.39 ng Ang I/ml/hr for nondiabetic rats (p < 0.001) and to 2.37 ± 0.44 ng Ang I/ml/hr for diabetic animals (p < 0.001).

**Adrenal Reninlike Activity**

Glomerular (capsular) reninlike activity in diabetic rats was increased about fivefold (p < 0.01) compared with that for normal controls (Figure 1). Bilateral nephrectomy increased glomerular reninlike activity in nondiabetic animals (22.5 ± 3.73 vs 5.09 ± 0.99 ng Ang I/mg protein/20 hr; p < 0.01) but failed to increase it in diabetic rats (see Figure 1).

On the other hand, the FRM portions showed a statistically significant increase (p < 0.001) in reninlike activity in diabetic rats compared with their controls (7.13 ± 0.83 vs 2.63 ± 0.25 ng Ang I/mg protein/20 hr). Bilateral nephrectomy significantly decreased (p < 0.01) reninlike activity in FRM portions in both nondiabetic and diabetic rats (Figure 2).

**Aortic Reninlike Activity**

Figure 3 shows the levels of reninlike activity determined in aortic extracts. Diabetic animals showed an increase in aortic reninlike activity compared with controls (p < 0.001). After nephrectomy aortic reninlike activity decreased from 1.44 ± 0.10 to 0.56 ± 0.05 ng Ang I/mg protein/20 hr (p < 0.001) in nondiabetic animals and from 3.38 ± 0.7 to 1.56 ± 0.56 ng Ang I/mg protein/20 hr for diabetic rats (p < 0.05).

**Discussion**

In the present study PRA, PRC, and plasma aldosterone decreased in streptozocin-induced diabetic rats 12 weeks after induction of diabetes. Other authors have also observed that chemically induced diabetes in rats is associated with low levels of renin and aldosterone in plasma. The marked decrease in plasma renin of diabetic rats remains unexplained. Though a plasma volume expansion may be sufficient to suppress renin secretion in these animals, other factors cannot be excluded. The plasma aldosterone decrease in diabetic rats probably is secondary to a deficiency of angiotensin II.16,17

We also found that chronically diabetic rats show an increase in plasma inactive renin. This inactive renin probably originated from the kidneys because it decreased after nephrectomy (as it did in control rats). Alternatively, the increase in inactive renin in diabetic rats could be due to an impairment in its clearance. It has been shown that another glycoprotein, dopamine β-hydroxylase, has an increased half-life in plasma of streptozocin-induced diabetic rats because glucose competes with its carbohydrate portion for its hepatic catabolic receptor.23

We found that aortic and FRM reninlike activity increased in diabetic rats compared with control animals and decreased after bilateral nephrectomy in both control and diabetic rats. In normal rats, Swales et al. also showed a decrease of renin activity in aorta after

![Figure 1](http://hyper.ahajournals.org/fig-1.png)
bilateral nephrectomy. Furthermore, it has been demonstrated that aorta can obtain renin from the circulation when renin is administered exogenously. Thus, vascular and FRM renins may originate, at least in part, from plasma. However, our data do not entirely support this concept because diabetic animals showed an increase in vascular and FRM reninlike activity when plasma renin was decreased. Moreover, in all experimental groups the changes in vascular and FRM renin activity were followed by similar variations of plasma inactive renin, suggesting that these tissues can take up inactive renin from plasma and convert it into active renin. This hypothesis is supported by the existence of a neutral protease on endothelial cell membrane capable of activating prorenin. Locally generated angiotensin in vascular wall could affect vascular tone, contracting smooth muscle cells and facilitating adrenergic transmission, as well as increase vascular permeability by contracting endothelial cells and opening interendothelial junctions. Therefore, an abnormality in vascular renin generation could contribute to the microvascular complications of diabetes. The physiological or pathophysiological interest of FRM reninlike activity remains to be established, although it is known that angiotensin II can stimulate catecholamine release from chromaffin cells and angiotensin receptors have been shown in adrenal fasciculata cells.

We have shown an increase in reninlike activity in the glomerular (capsular) portion of adrenal gland in diabetic rats. This high level of renin activity did not decrease after nephrectomy, and in nephrectomized control rats it increased to values observed in diabetic animals. These results support the idea that glomerular renin is of local origin and related to plasma renin in an inverse way. The pathophysiological role of the increment of renin activity in the zona glomerulosa of diabetic adrenal gland remains to be determined. Circulat-
ing angiotensin II derived from the kidney could be directly inhibiting synthesis of renin by the adrenal glomerulosa. In normal Wistar rats Doi et al. found a positive correlation between adrenal renin and adrenal aldosterone, suggesting a regulatory role of adrenal renin on aldosterone production. In diabetic rats plasma aldosterone is decreased, thus it is difficult to support the idea that glomerular renin stimulates aldosterone production. On the other hand, renin in the zona glomerulosa could be involved in other tissue functions. For example, it has been suggested that increases in adrenal angiotensin II may lead to down-regulation of angiotensin II receptors. In fact, adrenal zona glomerulosa cells from streptozocin-induced diabetic rats showed a markedly reduced sensitivity to angiotensin II and lower maximal response of aldosterone production.

References
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