Renal Hemodynamics and Plasma and Kidney Angiotensin II in Established Diabetes Mellitus in Rats: Effect of Sodium and Salt Restriction

Volker Vallon, Lucinda M. Wead, and Roland C. Blantz

Six weeks after the onset of insulin-treated streptozotocin diabetes (STZ) in Munich-Wistar rats, the effect of a low-sodium (LNa) and a low-salt (LNaCl) diet on renal function and on plasma and kidney tissue angiotensin II (AlIp, AlIk) was tested. Clearance experiments were performed in anesthetized rats 7 days after starting on LNa or LNaCl. On a control diet, STZ exhibited an increase in GFR, RBF, and kidney weight (KW) and a reduction in renal vascular resistance (RVR) and AlIk, but no change in AlIp, compared with nondiabetic normal rats (CON). Although sodium restriction reduced and salt restriction increased AlIk in CON, both diets increased AlIp without affecting renal hemodynamics or KW. In diabetic rats, both salt and sodium restriction further increased GFR and RBF by reducing RVR, increased KW, and changed AlIk and AlIp in a similar pattern, but at significantly lower values compared with CON. Daily treatment of STZ-LNa with the All-receptor blocker losartan (20 mg/L, in drinking water) did not affect the reduction in RVR because of a decrease in mean arterial blood pressure and further increased GFR. It was concluded that (1) AlIk but not AlIp is affected differently by LNa compared with LNaCl in both CON and STZ; (2) LNaCl and LNa change AlIp and AlIk in a similar pattern but at significant lower values in STZ compared with CON; and (3) with regard to renal hemodynamics and KW, the response to LNa and LNaCl is different in CON compared with rats 6 wk after the onset of diabetes mellitus, the latter exhibiting a further increase in renal hyperfiltration and KW by a mechanism that is not directly All receptor dependent.

Key Words: Renal hyperfiltration, renal hypertrophy, diabetic nephropathy

Renal hypertrophy and an increase in GFR have been described in early insulin-dependent diabetes mellitus (IDDM) in humans (1) and in animals with experimental diabetes (2,3). The increase in GFR is based on a rise in transglomerular hydrostatic filtration pressure and nephron plasma flow (2). Glomerular hyperfiltration in early IDDM has been associated with a greater risk for the development of diabetic nephropathy and end-stage renal failure (4,5). However, the mechanisms responsible for the described changes in renal structure and function in early IDDM remain incompletely understood.

Alterations in vascular reactivity to vasoactive substances are documented in diabetes (6). Although evidence for normal (7) and abnormal (8) renal vascular responsiveness to exogenous angiotensin II (All) has been described in diabetes mellitus, a previous study has shown that, 5 to 7 days after the onset of streptozotocin (STZ)-induced diabetes mellitus in rats, a low-sodium diet for 4 to 5 days, which is supposed to chronically stimulate endogenous All, can lower glomerular hyperfiltration (9).

Temporal alterations of the renin-angiotensin-aldosterone system (RAAS) have been reported in STZ-diabetic rats. Kikkawa et al. found a biphasic alteration of the RAAS: higher plasma renin activity (PRA) and higher plasma levels of All (AlIp) and aldosterone were found 1 wk after STZ, and lower levels were observed 4 and 8 wk after STZ compared with controls (10). Bank et al. (7) found that the intrarenal infusion of All in rats 1 to 2 wk after STZ caused a reduction in GFR and RPF, indicating that preglomerular, postglomerular, and glomerular contractile cells are able to constrict in response to All at that time. In contrast, Kikkawa et al. (11) reported a reduced contractile responsiveness of glomeruli from diabetic rats to All 8 wk after STZ.

On the basis of these findings, we speculated that the character of the renal response to the chronic stimulation of endogenous All may be different in rats 6 wk compared with 1 wk after the onset of diabetes. Because it was not clear whether the sodium-restricted diet used in the study from Bank et al. (9) was also chloride restricted and because a potential influence for chloride on the renin-angiotensin system is described (see Reference 17), we have examined whether sodium and salt restriction exerts similar effects on renal hemodynamics and on AlIp and kidney tissue All (AlIk) under these conditions.
METHODS

All animal experimentation described in this article was conducted in accord with the NIH Guide for the Care and Use of Laboratory Animals. Male Munich-Wistar rats weighing 220 to 240 g were made diabetic by STZ (65 mg/kg ip; Sigma, St. Louis, MO) solved in sodium citrate buffer (pH 4.2). Two days later, the glucose concentration was determined in tail blood samples, and only those animals with blood glucose levels >300 mg/100 mL were included in further experiments. Diabetic rats were treated daily with ultralente insulin (0.5 to 1.5 IU sc in late afternoon; Novo Industry, Copenhagen, Denmark) to adjust blood glucose levels at approximately 350 mg/100 mL. The animals were allowed free access to a regular rat pellet diet (sodium, 0.44%; chloride, 0.63%; potassium, 0.97%) and tap water. Age-matched normal rats fed the same diet served as controls. Six weeks after the onset of STZ diabetes, rats were placed in metabolic cages for 24 h to measure urinary volume and sodium excretion. Subsequently, control and diabetic rats were segregated into three and four groups, respectively: a first group was continued on the standard diet, a second group was begun on a low-salt diet, a third group was begun on a low-sodium diet by eating salt-deficient chow (ICN, Costa Mesa, CA; potassium, 0.36%), and a third group was begun on a low-sodium diet by eating sodium-deficient chow (ICN; chloride, 0.50%; potassium, 0.96%). In diabetic rats, a fourth group was given the AII receptor blocker losartan in the drinking water (20 mg/L) along with a low-sodium diet. Five days later, rats were again housed in metabolic cages for 24 h. Blood glucose levels were measured five to six times during the complete experimental protocol in each animal, including measurements performed at the end of metabolic cage experiments.

Measurement of Renal Hemodynamics

Seven to 8 days after starting on a low-salt or low-sodium diet, rats were anesthetized with Inactin (100 mg/kg ip; Byk-Gulden, Konstanz, Germany) and prepared for clearance experiments. The animals were placed on a servo-controlled heating table to maintain body temperature at 37°C. A tracheostomy was performed to facilitate free breathing. The left femoral artery was cannulated to obtain blood samples and monitor arterial pressure (Statham P23Db transducer; Oxnard, CA). The right jugular vein was cannulated for the infusion of Ringer saline (in millimolar concentrations: NaHCO3, 30; KCl, 4.7; NaCl, 111) at a rate of 1.5 mL/h, containing 20 μCi/mL [3H]inulin as a marker of GFR. Additional Ringer saline was infused to match the urinary flow rate and to maintain arterial hematocrit constant (additional infusion rates [in microliters per minute]: normal rats on standard diet, 11 ± 3; normal rats on sodium or salt restriction, 10 ± 2; diabetic rats on standard diet, 15 ± 3; diabetic rats [1] on sodium restriction, 17 ± 3; [2] on sodium restriction plus losartan, 22 ± 2; and [3] on salt restriction, 20 ± 5). By the use of this infusion protocol, values for arterial hematocrit measured immediately after the insertion of a catheter into the femoral artery, before starting any infusion, were not different from values measured during clearance experiments. The bladder was cannulated for urine collection. An abdominal midline incision was performed to get access to the left renal vein. After the completion of the surgical preparation, the animals were allowed to stabilize for 1 h before the measurements were started.

Renal clearance experiments were carried out for 1 h. Arterial blood samples (160 μL each) were withdrawn at the beginning and end of the timed urine collection period. Simultaneously, renal vein blood samples (8 to 10 μL each) were withdrawn by the direct puncturing of the renal vein with a sharpened micropipette (60 to 80 μm, outer diameter). Inulin clearance was calculated by standard equations. Renal filtration fraction (FF) was calculated according to the following equation:

\[ FF = 1 - \frac{[\text{3H}]\text{inulin}_{\text{plasma ren}}}{[\text{3H}]\text{inulin}_{\text{plasma femoral artery}}} \]

RBF and RVR were calculated as follows:

\[ \text{RBF} = \frac{\text{GFR}}{(\text{FF})(1 - \text{Hct})} \]

\[ \text{RVR} = \frac{\text{MAP}}{\text{RBF}} \]

where Hct is arterial hematocrit and MAP is mean systemic arterial blood pressure.

Measurement of AIIp and AIIk

AIIp and AIIk were measured in samples according to a protocol previously described (12). Levels of AII may be increased because of anesthesia and surgery in all groups.

Plasma Processing. AIIp was measured in a separate set of animals treated exactly as the other set of groups that was used for metabolic cage experiments, clearance experiments, and kidney AII measurements. Animals were anesthetized with Inactin (100 mg/kg ip), and after an abdominal incision, the abdominal aorta was punctured and 1.0 mL of blood was collected in a syringe containing 20 μL of EDTA (0.16 M) and 10 μL of converting enzyme inhibitor (0.1 mM). Samples were spun at 4°C, and plasma was stored at −70°C until processed. Plasma was extracted using a Bondelut C18 column (Varian, Harbor City, CA) previously washed with methanol and triethyamine formic acid. The column was rinsed with triethyamine formic acid buffer, and AII was eluted off with acetonitrile: triethyamine formic acid (70:30), lyophilized on a Speed-Vac (Savant, Farmingdale, NY) overnight, and kept at −20°C until assayed. This extraction procedure yielded 92% recovery of AII.

Renal Tissue Processing. After the clearance experiments, kidneys were perfused free of blood with 50 mL of a solution containing 4.9 mM 8-hydroxyquinoline hemisulfate, 2.6 mM EDTA, and 3% bovine serum albumin (BSA) administered through an aortic catheter, excised, flash-frozen in liquid nitrogen, and stored at −70°C until further processing. Individual whole kidneys were homogenized (Polytron, Brinkman, Westbury, NY; 10 s at 6 setting) in 2 mL of radioimmunoassay (RIA)-BSA 0.25% buffer, added to 9 mL of homogenizing medium (1N glacial acetic, 0.02N hydrochloric acid, and 0.1% 2-mercaptoethanol), heated to 90°C for 10 min, and then centrifuged at 30,000 × g for 20 min. The supernatant, S1, was removed, and the pellet was resuspended in 4.5 mL of homogenizing medium and centrifuged at 30,000 × g for 20 min. The supernatant was combined with S1 and lyophilized on a Speed-Vac overnight. The resulting lyophilisate was resuspended in 2 mL of RIA-BSA 0.25% buffer, and 500 μL of this was extracted with AII binding protein (BSA) 0.25% buffer, added to 9 mL of BSA 0.25% buffer, and 500 μL of this was extracted with a Bondelut C18 column as described above for plasma. This combined procedure yielded 59% recovery of AII.

RIA. Lyophilisate was resuspended in 500 μL of RIA buffer, and 200 μL of this was added to 100 μL of specific rabbit AII-antibody diluted 1/62,500. After incubation for 2 days at 4°C, 100 μL of [125I]AII (6,000 cpm) (Dupont, NEN Research Products, Boston, MA) was added to each tube and incubated again at 4°C overnight. All bound to antibody was separated from unbound with normal rabbit serum diluted 1/200 (100 μL), goat anti-rabbit immunoglobulin G diluted
buffer added to each tube. Tubes were incubated at 4°C for 2 h and then spun for 30 min, the supernatant was decanted, and the pellet was counted in a gamma counter. All concentrations were calculated with a computer-aided logit/log transformation of the standard curve. Cross-reactivity of this All antibody with angiotensin I is 0.33% and with angiotensin II is 68%.

Analytic Methods

Blood glucose levels were measured with a glucometer (Glucometer 3; Miles Inc., Elkhart, IN). Urinary sodium and serum concentrations of sodium and potassium were measured by flame photometry (FLM3; Radiometer, Copenhagen, Denmark). The concentration of [3H]inulin in plasma and urine was measured by liquid phase scintillation counting.

Statistical Analysis

Data are presented as the means ± SE. Data from metabolic cage experiments were subjected to paired t test, and with data from clearance experiments, were subjected to analysis of variance and unpaired t test with Bonferroni correction for multiple intergroup comparisons. P values <0.05 were considered to be statistically significant.

RESULTS

Metabolic Cage Experiments

The results of metabolic cage experiments are shown in Table 1. In normal rats, salt restriction reduced body weight (BW) by 2 ± 1%, food intake by 40 ± 5%, and urinary sodium excretion (UNaV) by 97 ± 1% and tended to reduce urinary volume (UV). Sodium restriction in normal rats reduced BW by 2 ± 1%, UV by 47 ± 7%, and UNaV by 98 ± 1%. In diabetic rats on standard diet, BW was reduced and food intake, blood glucose level, UV, and UNaV were increased compared with normal rats. Salt restriction in diabetic rats reduced BW by 7 ± 1% and food intake by 31 ± 6%, increased UV by 69 ± 14%, and decreased UNaV by 98 ± 1%. Sodium restriction in diabetic rats reduced BW by 6 ± 1% and food intake by 24 ± 6%, increased UV by 58 ± 15%, and decreased UNaV by 88 ± 3%. Sodium restriction plus losartan in diabetic rats reduced BW by 4 ± 1%, doubled UV, and decreased UNaV by 97 ± 1%. These results show that hyperglycemic diabetic rats can reduce sodium excretion efficiently in response to salt restriction and to a significant lower extent in response to sodium restriction. The finding that the addition of losartan to sodium-restricted diabetic rats further reduced sodium excretion differs from the natriuretic effect of the All receptor antagonist saralasin in sodium-depleted normal rats (13).

Clearance Experiments

The results of clearance experiments are depicted in Table 2. In both normal and diabetic rats, sodium and salt restriction reduced serum sodium concentration without significantly affecting serum potassium concentration. Sodium and salt restriction in normal rats did not affect renal hemodynamics and KW. Diabetic rats showed an increase in GFR, RBF, and KW and a reduction in mean arterial blood pressure and RVR compared with normal rats. Sodium and salt restriction in diabetic rats significantly increased GFR, RBF, and KW and reduced FF and RVR. The simultaneous treatment of sodium-restricted diabetic rats with losartan did not affect the reduction in RVR and the increase in KW but slightly reduced RBF because of a decrease in mean arterial blood pressure and further increased GFR. The response to sodium restriction was not different than that to salt restriction either in

<table>
<thead>
<tr>
<th>TABLE 1. Metabolic cage experimentsa</th>
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<tbody>
<tr>
<td><strong>Group</strong></td>
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<td>-------</td>
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<tr>
<td>CON</td>
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<td>CON + Low NaCl</td>
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<tr>
<td>CON + Low Na</td>
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<td>STZ</td>
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<td>STZ + Low NaCl</td>
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<td>Losartan + Low NaCl + Losartan</td>
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a Values are mean ± SE. BGL, blood glucose level; CON, normals.
b P < 0.05 versus Week 6.
c P < 0.01 versus CON.
d P < 0.01 versus STZ + LowNaCl.
e P < 0.01 versus STZ + LowNa.

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normal or in diabetic rats; pooled data are presented in Figure 1.

AlIp

On a standard diet, AlIp was not significantly different in normal compared with diabetic rats (15 ± 2 versus 12 ± 1 pg/mL; not significant). In normal rats, AlIp was increased in response to both salt and sodium restriction (37 ± 4 and 33 ± 4 pg/mL; each P < 0.01 versus standard diet). Also in diabetic rats, AlIp was increased in response to both salt and sodium restriction (25 ± 3 and 20 ± 2 pg/mL; each P < 0.01 versus standard diet). AlIp was significantly lower in response to sodium restriction in diabetic compared with normal rats (each P < 0.01). The response to sodium restriction was not different than that to salt restriction either in normal or in diabetic rats; pooled data are presented in Figure 2.

Table 2. Clearance experiments

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Na&lt;sub&gt;serum&lt;/sub&gt; (mM)</th>
<th>K&lt;sub&gt;serum&lt;/sub&gt; (mM)</th>
<th>KW (g)</th>
<th>Hct (%)</th>
<th>MAP (mm Hg)</th>
<th>UV (µL/min)</th>
<th>GFR (mL/min)</th>
<th>RBF (mL/min)</th>
<th>FF (%)</th>
<th>RVR (mm Hg/mm per mL)</th>
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</thead>
<tbody>
<tr>
<td>CON</td>
<td>6</td>
<td>148 ± 2</td>
<td>4.9 ± 0.2</td>
<td>1.20 ± 0.05</td>
<td>51 ± 1</td>
<td>112 ± 5</td>
<td>9 ± 2</td>
<td>2.58 ± 0.10</td>
<td>20 ± 1</td>
<td>26 ± 2</td>
<td>5.52 ± 0.11</td>
</tr>
<tr>
<td>CON-LowNaCl</td>
<td>6</td>
<td>140 ± 1±</td>
<td>4.3 ± 0.2</td>
<td>1.25 ± 0.05</td>
<td>50 ± 1</td>
<td>104 ± 4</td>
<td>7 ± 1</td>
<td>2.64 ± 0.10</td>
<td>24 ± 3</td>
<td>24 ± 3</td>
<td>4.95 ± 0.45</td>
</tr>
<tr>
<td>CON-LowNa</td>
<td>6</td>
<td>141 ± 2±</td>
<td>4.5 ± 0.2</td>
<td>1.27 ± 0.07</td>
<td>49 ± 1</td>
<td>108 ± 4</td>
<td>7 ± 1</td>
<td>2.57 ± 0.10</td>
<td>20 ± 1</td>
<td>26 ± 2</td>
<td>5.26 ± 0.19</td>
</tr>
<tr>
<td>STZ</td>
<td>9</td>
<td>147 ± 2</td>
<td>4.4 ± 0.3</td>
<td>1.64 ± 0.05</td>
<td>49 ± 1</td>
<td>99 ± 3±</td>
<td>17 ± 1±</td>
<td>2.92 ± 0.05</td>
<td>26 ± 1±</td>
<td>23 ± 1</td>
<td>3.82 ± 0.13</td>
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<tr>
<td>STZ-LowNaCl</td>
<td>6</td>
<td>133 ± 2±</td>
<td>4.2 ± 0.2</td>
<td>1.85 ± 0.06</td>
<td>51 ± 1</td>
<td>98 ± 3</td>
<td>22 ± 2±</td>
<td>3.49 ± 0.16</td>
<td>44 ± 2±</td>
<td>17 ± 1±</td>
<td>2.23 ± 0.15</td>
</tr>
<tr>
<td>STZ-LowNa</td>
<td>9</td>
<td>137 ± 2±</td>
<td>4.5 ± 0.2</td>
<td>1.80 ± 0.03</td>
<td>51 ± 1</td>
<td>93 ± 2</td>
<td>19 ± 1</td>
<td>3.36 ± 0.10</td>
<td>50 ± 3±</td>
<td>14 ± 1±</td>
<td>1.85 ± 0.14</td>
</tr>
<tr>
<td>STZ-LowNa-Isoartan</td>
<td>6</td>
<td>135 ± 1±</td>
<td>4.6 ± 0.2</td>
<td>1.90 ± 0.07</td>
<td>48 ± 1</td>
<td>80 ± 2±</td>
<td>24 ± 2±</td>
<td>3.76 ± 0.09</td>
<td>41 ± 2±</td>
<td>18 ± 1±</td>
<td>1.99 ± 0.13</td>
</tr>
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</table>

* Values are mean ± SE. Hct, hematocrit; MAP, mean arterial pressure; CON, normals; Na<sub>serum</sub>, serum sodium concentration; K<sub>serum</sub>, serum potassium concentration.

** P < 0.01 versus CON.

* P < 0.01 versus STZ.

* P < 0.01 versus STZ-LowNa.

Figure 1. Effect of sodium or salt restriction on renal hemodynamics and KW in normal (CON) and diabetic (STZ) rats. Because the response to sodium restriction was not different than that to salt restriction either in CON or in STZ, pooled data are presented (lowNa + lowNaCl). *P < 0.01 versus standard diet; **P < 0.01 versus CON-LowNaCl.

Figure 2. Effect of sodium or salt restriction on AlIp in normal (CON) and diabetic (STZ) rats. Because the response to sodium restriction was different than that to salt restriction either in CON or in STZ, pooled data are presented (lowNa + lowNaCl). *P < 0.01 versus standard diet; **P < 0.01 versus CON-LowNaCl/lowNaCl.

AlIk

Results are depicted in Figure 3. AlIk was reduced in diabetic compared with normal rats for all three diets. In both normal and diabetic rats, AlIk was approximately doubled in response to salt restriction (normals: 1,080 ± 108 versus 1,979 ± 318 pg/g of kidney, P < 0.01; STZ: 370 ± 44 versus 699 ± 70 pg/g of kidney, P < 0.01). Sodium restriction reduced AlIk in normal rats (1,080 ± 108 versus 552 ± 86 pg/g of kidney; P < 0.01) and reduced it numerically, although not significantly, in diabetic rats (370 ± 44 versus 256 ± 53 pg/g of kidney; not significant). In both normal and diabetic rats, AlIk was higher in response to salt compared with sodium restriction. The daily treatment with losartan of diabetic rats on a low-sodium diet further reduced AlIk (256 ± 53 versus 72 ± 19 pg/g of kidney; P < 0.01).
All and aldosterone in the plasma were found 1 wk after STZ, although glomerular AII receptors were increased. On the basis of these findings, we speculated that the quality of the renal response to the chronic stimulation of endogenous AII may be related to the duration of diabetes mellitus.

We observed in this study that, with regard to renal hemodynamics and renal hypertrophy, normal nondiabetic rats and rats 6 wk after the onset of diabetes mellitus respond differently to salt and sodium restriction. Although both salt and sodium restriction exerted no effect on renal hemodynamics and KW in normal rats, salt and sodium restriction in established diabetes mellitus further increased GFR and RBF by reducing RVR and also increased KW. This seemingly paradoxical renal response to sodium restriction in rats 6 wk after the onset of diabetes is in contrast to the effect reported in diabetic rats 1 to 2 wk after STZ (9).

The observed decrease in RVR and FF and the increase in RBF and GFR in diabetic rats 6 wk after STZ in response to sodium and salt restriction suggest a reduction in vasoconstrictor influences and/or an increase in vasodilating mechanisms at both preglomerular and postglomerular sites. A lower AIIp, one potential vasoconstrictor system, and a reduced responsiveness to exogenous AII have been described in noninsulin-treated diabetic rats 8 wk after STZ, exhibiting blood glucose levels of about 500 mg/100 mL (10, 11). In this study, on a standard diet, AIIp was not reduced in insulin-treated diabetic rats 6 wk after STZ, exhibiting blood glucose levels of about 350 mg/100 mL, compared with normal rats, probably at least in part reflecting an important influence of glycemic levels on AIIp. We observed that the AII receptor blocker losartan reduced mean systemic arterial blood pressure in diabetic rats on a low-sodium diet, suggesting that AII exerts systemic vasoconstrictor effects under these conditions and that these effects are susceptible to AII receptor blockade. However, losartan did not potentiate the reduction in RVR, implicating a reduced vasoconstrictor influence of the AII system on the preglomerular and postglomerular vasculature that determines RVR under these conditions. A reduced activity of the AII system could be due to a decreased production of AII or changes in the organ AII sensitivity by alterations either of receptors or of signal transduction.

We found that, in diabetic rats on a standard diet, AIIk was reduced to one-third of values in normal rats. In response to sodium and salt restriction, diabetic rats exhibited significantly lower values for both AIIp and AIIk, but the pattern of response was similar compared with normal rats: although sodium and salt restriction both increased AIIp and salt restriction increased AIIk, a low-sodium diet reduced AIIk to 50% in normal rats and tended to reduce AIIk in diabetic rats. In both normal and diabetic rats, AIIk was significantly higher in rats during salt versus sodium restriction. The different response in AIIk to sodium versus salt restriction may reflect the important influ-
ence of Cl⁻ on renin secretion (17). A potential influence of a lower potassium intake in the low-salt group should not be involved in this response because the low-salt diet was not potassium deficient but potassium content was reduced to 40% of the standard and low-sodium diet, respectively; the diet was only given for 1 wk; and potassium serum levels were not different during salt and sodium restriction in both normal and diabetic rats. The mechanism(s) that increases AlIp and concomitantly reduces AlIk in response to sodium restriction is unclear.

Because AlIp was lower by about one-third in response to both sodium and salt restriction and AlIk was reduced by approximately one-half during sodium restriction and to one-third by salt restriction, comparing diabetic with normal rats and considering the unresponsiveness of the RVR to AII receptor blockade during sodium restriction in diabetic rats, it is possible that the described hemodynamic changes in diabetic rats are somehow based on reduced AII activity. However, salt restriction reduced RVR, augmenting glomerular hyperfiltration in diabetic rats, whereas AlIp and AlIk were increased compared with a standard diet. These findings may implicate the possibility that kidney AII sensitivity is altered either on a receptor or a signal transduction level or that a reduced activity of another vasoconstrictor influence and/or an increased activity of vasodilator mechanisms may play a critical role under these conditions. In this study, losartan increased GFR in response to a low-sodium diet in diabetic rats. These results may implicate that an AII receptor–mediated glomerular mechanism, presumably a modulation of glomerular capillary ultrafiltration coefficient (18), is limiting the increase in GFR under these conditions, which could indicate an AII receptor–mediated response in glomerular cells, but not in renal vascular smooth muscle. The afferent arteriolar resistance, one component of the total RVR, is, at least in part, regulated by the tubuloglomerular feedback (TGF), i.e., an afferent arteriolar vasoconstriction as a negative feedback response elicited by an increased NaCl concentration in the tubular fluid at the macula densa (19). Evidence for a reduced TGF activity has been provided during modest hyperglycemia in nondiabetic rats (20) and in diabetic rats 3 (21) and 8 wk (22) after STZ. This alteration of TGF activity could be based on increased glucose levels sufficient to exceed the transport maximum for proximal tubular glucose reabsorption exerting an osmotic effect in tubular fluid, which may reduce either the concentration of NaCl in the tubular fluid or the net NaCl transport at the macula densa. Assuming that sodium and salt restriction could increase proximal tubular sodium reabsorption in diabetic rats, and thereby reduce further the NaCl concentration at the macula densa, the resulting alteration of the TGF activity may play a role in the findings presented here. Because AII is known to influence TGF activity (23,24), our finding that AlIk is reduced in diabetic compared with normal rats under standard, low-sodium, and low-salt diets could be related to a diminished TGF activity in diabetic rats during a standard but also a low-sodium and a low-salt diet.

In this study, diabetic rats on a low-sodium and a low-salt diet were able to reduce UNaV in spite of an increase in tubular sodium load due to hyperfiltration. This may indicate that renal sodium reabsorption mechanisms and tubular Na,K-ATPase activity have been activated. An increase in Na,K-ATPase activity has been associated with renal hypertrophy in a variety of experimental systems (25). Ku et al. (26) have suggested that the development of renal hypertrophy is associated in parallel with an increase in renal Na,K-ATPase activity in the first 8 wk after STZ-induced diabetes mellitus. Cellular mechanisms triggering or signaling an increase in renal tubular Na,K-ATPase and renal hypertrophy in diabetes are not well understood, although a potential role for hormones such as the renin-angiotensin system has been suggested (27). However, the mechanism(s) generating further hypertrophy in response to salt and sodium restriction in established diabetes but not in normal rats remains unclear. One potential difference between normal and diabetic rats in response to a sodium and salt restriction was the increase in GFR and, therefore, the filtered sodium load in diabetic compared with normal rats. This quantitative difference in sodium load and, therefore, in the tubular sodium reabsorptive rate could have played a stimulatory role. Further studies are necessary to study the role of vasodilator and vasoconstrictor mechanisms during renal hyperfiltration and hypertrophy in diabetes mellitus on standard diets and with sodium and salt restriction.

In summary, although sodium restriction reduced and salt restriction increased AlIk, both diets increased AlIp without affecting renal hemodynamics and KW in normal rats. In diabetic rats 6 wk after STZ, salt and sodium restriction further increased GFR and RBF by reducing RVR, increased KW, and changed AlIp and AlIk in a similar pattern, but at significantly lower values compared with normal rats. Chronic AII receptor blockade did not affect the reduction in RVR and the increase in KW, reduced RBF as the result of a decrease in blood pressure, and further increased GFR in response to sodium restriction in diabetic rats. We conclude that (1) AlIk but not AlIp is affected differently by sodium compared with salt restriction in both normal and diabetic rats; (2) sodium and salt restriction change AlIp and AlIk in a similar pattern but at significant lower values in diabetic compared with normal rats; and (3) with regard to renal hemodynamics and renal hypertrophy, normal rats and rats 6 wk after the onset of diabetes mellitus respond differently to salt and sodium restriction. Salt and sodium restriction augmented renal hyperfiltration and hypertrophy in rats 6 wk after STZ by a mechanism that is not directly AII receptor dependent.
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