Glomerulotubular balance, dietary protein, and the renal response to glycine in diabetic rats

Larry A. Slomowitz, Aihua Deng, John S. Hammes, Francis Gabbai, and Scott C. Thomson. Glomerulotubular balance, dietary protein, and the renal response to glycine in diabetic rats. Am J Physiol Regulatory Integrative Comp Physiol 282: R1096–R1103, 2002. First published December 21, 2001; 10.1152/ajpregu.00610.2001.—The glomerular filtration rate (GFR) normally increases during glycine infusion, which is a test of “renal reserve.” Renal reserve is absent in diabetes mellitus. GFR increases after protein feeding because of increased tubular reabsorption, which reduces the signal for tubuloglomerular feedback (TGF). Dietary protein restriction normalizes some aspects of glomerular function in diabetes. Renal micropuncture was performed in rats 4–5 wk after diabetes was induced by streptozotocin to determine whether renal reserve is lost as a result of altered tubular function and activation of TGF, whether 10 days of dietary protein restriction could restore renal reserve, and whether this results from effects of glycine on the tubule. TGF activation was determined by locating single-nephron GFR (SNGFR) in the early distal tubule along the TGF curve. The TGF signal was determined from the ionic content of the early distal tubule. In nondiabetic rats, SNGFR in the early distal tubule increased during glycine infusion because of primary vasodilation augmented by increased tubular reabsorption, which stabilized the TGF signal. In diabetic rats, glycine reduced reabsorption, thereby activating TGF, which was largely responsible for the lack of renal reserve. In protein-restricted diabetic rats, the tubular response to glycine remained abnormal, but renal reserve was restored by a vascular mechanism. Glycine affects GFR directly and via the tubule. In diabetes, reduced tubular reabsorption dominates. In low-protein diabetes, the vascular effect is enhanced and overrides the effect of reduced tubular reabsorption.

glomerular filtration; tubuloglomerular feedback; tubular reabsorption; loop of Henle; streptozotocin

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filtration (20, 21). This is similar to the situation described above for nondiabetic rats after protein feeding (13). In other words, nondiabetic rats fed a high-protein diet and diabetic rats fed a normal-protein diet exhibit hyperfiltration, along with increases in reabsorption from the proximal tubule and/or loop of Henle that are too great to be accounted for by GTB.

Considering this background, we performed micropuncture experiments to examine the normal tubular response to glycine infusion, the role of the tubule in diabetic hyperfiltration, the abnormal response of the diabetic kidney to glycine infusion, and the effects of dietary protein on tubular function in diabetes. The hypothesis of this research is that dietary protein and the renal response to glycine infusion are affected by diabetes according to a model depicted in Fig. 1.

**METHODS**

All animal experimentation was conducted in accord with the National Institutes of Health guidelines for the care and use of laboratory animals. In adult male Wistar-Frøenert rats from a breeding colony at San Diego Veterans Affairs Medical Center, diabetes was induced by streptozotocin (65 mg/kg ip; Sigma, St. Louis, MO) dissolved 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/dl were included in further experiments. Diabetic rats were treated daily with propanol insulin (0.5–1.5 IU sc once daily; Anpro Pharmaceutical, Arcadia, CA) or with long-acting insulin pellets placed subcutaneously and supplemented with daily insulin injections as needed to adjust glucose levels to ~19 mmol/l.

The animals were initially allowed free access to a regular rat pellet diet containing 21% protein and tap water. After 4 wk of diabetes, some animals were changed to a diet containing 8% protein (Low Renal Load Diet, ICN Pharmaceuticals, www.icnbiomed.com). Ten days later, nonfasted animals underwent micropuncture. Nondiabetic rats fed the standard diet served as controls.

**Micropuncture Protocol**

Micropuncture was performed under anesthesia with thiobutabarbital (100 mg/kg ip; Inactin, Research Biochemicals, Natick, MA) and hypodermic conditions according to protocols previously described (1). Nondiabetic animals received Ringer saline containing [3H]inulin (80 μCi/ml) as a marker of glomerular filtration by continuous intravenous infusion at 2 ml/h. Diabetic animals received Ringer saline at 3 ml/h to compensate for diabetic polyuria. After completion of the preparatory surgery, animals were allowed 60 min to equilibrate before micropuncture was begun. Micropuncture experiments were divided into two periods. During the first period, animals received Ringer saline as described above. During the second period, they received an additional infusion of L-glycine (2.66 M in Ringer saline) at a rate of 1.5 ml/h. Twenty minutes elapsed between initiation of the glycine infusion and resumption of micropuncture. This protocol was established on the basis of past experience with glycine infusion and was intended to maintain isovolemic throughout the second experimental period (4). This protocol has also been demonstrated to raise serum and mid-late proximal tubular glycine concentrations to the same degree in diabetic and nondiabetic rats (3).

SNGFR was measured by [3H]inulin clearance in timed collections of tubular fluid by standard micropuncture (1). For purposes of this study, “natural” values for SNGFR are defined as values that prevail during the normal operation of TGF. To determine the natural SNGFR, tubular fluid was collected from the early distal tubule, which is downstream from the macula densa. SNGFR as measured from the early distal tubule is referred to as SNGFRd. When SNGFR is measured by collecting fluid from the proximal tubule, TGF must be interrupted. This renders the natural state of TGF activation indeterminate. However, when flow past the macula densa is manipulated independent of SNGFR, changes in SNGFR measured by collecting from the proximal tubule characterize the range of possible TGF responses. In the present study, SNGFR was measured from the proximal tubule at both extremes of TGF activation. This was achieved by orthograde perfusion of Henle’s loop at 0 or 40 nl/min with artificial tubular fluid containing 130 mM NaCl, 10 mM NaHCO3, 4 mM KCl, 2 mM CaCl2, 7.25 mmol/l urea, and 0.1% FD & C (pH 7.4). Perfusions were made downstream from a wax block inserted in the late proximal tubule, while collections were made upstream from the wax block. Nephrons were equilibrated for 2 min before each collection, and each collection was made for 3 min. Nephrons were vented during equilibration to avoid a buildup of pressure in the proximal tubule. SNGFR measured during zero micropuffusion is referred to as SNGFRmax. SNGFR measured during micropuffusion at 40 nl/min is referred to as SNGFRmin. The TGF response is traditionally modeled as a symmetric sigmoidal curve (12). SNGFR at the inflection point of this sigmoidal curve is simply the average of SNGFRmax and SNGFRmin and is referred to as SNGFRmid. In some nephrons, SNGFRd was measured before insertion of the wax block for measurements of SNGFRmax and SNGFRmin. In other nephrons, only SNGFRd or SNGFRmax and SNGFRmin were measured. SNGFRmax and SNGFRmin were measured in random order. Before radioactivity was counted, the volume of each early distal collection was determined by transfer to a calibrated constant-bore glass pipette.
From this volume and from SNGFR\(_d\), distal flow rate (\(V_d\)), tubular fluid-to-plasma inulin ratio (\(TF/P_{\text{inulin}}\)), and net tubular reabsorption of water up to the early distal tubule (\(J_{ed}\)) were calculated.

**Assessment of the TGF Signal**

As a surrogate for the TGF signal, using an electrical conductivity microelectrode (19), we measured the ionic conductivity. From this volume and from the fluid-to-plasma inulin ratio (TF/P\(_{\text{inulin}}\)), we measured the ionic conductivity in the early distal nephron (\(T_{ed}\)). Conductivity is the molar concentration of NaCl solution that has the same specific conductance as the given solution. \(T_{ed}\) was determined in free-flowing early distal nephrons and expressed as a fraction of the conductivity of the proximal tubule. Net salt reabsorption up to the early distal tubule (\(J_d\)) was calculated from \(T_{ed}\) and \(V_d\).

**Statistical Analysis**

Heterogeneity within groups was excluded by ANOVA. Thereafter, each nephron was entered individually in intergroup comparisons by \(t\)-test or two-way ANOVA. To calculate standard errors for parameters derived from two measured variables where some, but not all, measurements were paired, standard errors were calculated according to the following standard formula

\[
\sigma^2 = \left(\frac{\partial \Psi}{\partial x}\right)^2 + \left(\frac{\partial \Psi}{\partial y}\right)^2 + 2 \left(\frac{\partial \Psi}{\partial x}\right)\left(\frac{\partial \Psi}{\partial y}\right) \sigma_x \sigma_y \rho_{x,y}
\]

where \(x\) and \(y\) are the measured variables, \(\Psi\) is a function of \(x\) and \(y\), \(\sigma^2\) is a variance, and \(\rho_{x,y}\) is the correlation between \(x\) and \(y\).

To test for primary effects of glycine on water reabsorption, an index of reabsorptive efficiency was calculated from the simultaneous effects of glycine on SNGFR\(_d\) and \(J_d\) as previously described (18)

\[
\text{reabsorptive efficiency} = \frac{\Delta J_d}{\Delta \text{SNGFR}_d} \cdot \frac{J_d}{\text{SNGFR}_d}^{-1}
\]

This dimensionless index will equal unity when fractional reabsorption is constant and will equal zero when net reabsorption is constant and independent of SNGFR. This index is less dependent on where along the nephron reabsorption is measured than is the simpler, \(\Delta J_d/\Delta \text{SNGFR}\). Values outside the interval from zero to unity can never be explained by GTB. Values within the interval from zero to unity may or may not be explained by GTB (see DISCUSSION).

**RESULTS**

Data were obtained from 9 nondiabetic control rats and 23 rats that had been diabetic for 4–5 wk. All control rats and 12 diabetic rats were fed 21% protein throughout the study. Eleven diabetic rats were fed 21% protein until 10 days before micropuncture and then fed 8% protein. Body weight at the time of micropuncture was 304 ± 6 g and was not different between groups (\(P = 0.4\)). The diabetic rats continued to grow when given insulin. Hence, weight matching was achieved using rats of similar ages. Blood glucose concentration at the time of micropuncture was slightly higher among the low-protein diabetic rats. Hematocrit was slightly lower in low-protein diabetic rats than in the other two groups. There were no significant intergroup differences in arterial blood pressure before or during glycine infusion (Table 1).

**Control Period**

Before glycine infusion, SNGFR\(_d\) was greater in diabetic than in control nephrons (\(P = 0.028\); Table 2). SNGFR\(_d\) in low-protein diabetic rats was intermediate between control and diabetic rats (not significant). SNGFR\(_{\max}\) was also greatest in diabetes (\(P = 0.024\) vs. low-protein diabetic rats, \(P = 0.12\) vs. control). Perfusing the loop of Henle to activate TGF caused SNGFR to decline in all groups (\(P < 0.001\)). The difference between SNGFR\(_{\max}\) and SNGFR\(_{\min}\) was greatest among diabetic rats and least among control rats (\(P = 0.009\)). SNGFR\(_{\mid d}\) tended to be greatest in diabetes, although intergroup differences were not statistically significant after correction for multiple comparisons. Among control rats, SNGFR\(_d\) was 27 ± 7 nl/min/\(GFR\) less than SNGFR\(_{\mid d}\). In contrast, SNGFR\(_d\) exceeded SNGFR\(_{\mid d}\) for diabetic rats, regardless of diet (\(P < 0.01\)). In other words, although diabetes did not reduce the range of the TGF response, the relationships between glomerular filtration and tubular reabsorption are altered in diabetes, such that diabetic nephrons naturally operate with less TGF activation, regardless of diet.

As a surrogate for the natural TGF stimulus, we measured the conductivity of fluid in the early distal tubule of free-flowing nephrons (Table 2). Results are expressed as a fraction of the conductivity of the early proximal tubule and are, hence, unitless. The conductivity of early distal tubular fluid was 30% less in diabetic than in control nephrons (\(P < 0.00003\)), indicating that the ambient TGF signal is unequivocally reduced in diabetes. Reducing dietary protein normalized the TGF signal in diabetes, increasing conductivity to control values (\(P = 0.758\), low-protein diabetic vs. nondiabetic rats).

**Response to Glycine Infusion**

**Control rats.** During glycine infusion in control rats, SNGFR\(_d\) increased by 41% (\(P < 0.03\)), SNGFR\(_{\max}\) by 35% (\(P = 0.03\)), SNGFR\(_{\min}\) by 48% (\(P = 0.04\)), and SNGFR\(_{\mid d}\) by 46% (\(P = 0.02\); Table 2). Early distal flow rate and net distal delivery of salt increased by 60% during glycine infusion (\(P < 0.01\)), although early dis-

<table>
<thead>
<tr>
<th>Group</th>
<th>Before glycine</th>
<th>During glycine</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Blood Glucose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAP, mmHg</td>
<td>Hct, %</td>
</tr>
<tr>
<td>Control</td>
<td>Before</td>
<td>During</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>128 ± 8</td>
<td>128 ± 4</td>
</tr>
<tr>
<td>Low-protein</td>
<td>120 ± 2</td>
<td>115 ± 3</td>
</tr>
<tr>
<td>diabetes</td>
<td></td>
<td></td>
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</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; Hct, hematocrit. *\(P < 0.05\) vs. low-protein diabetes; †\(P < 0.05\) vs. before glycine (by paired \(t\)-test).
Dietary Protein and Tubular Reabsorption in Diabetes

Table 2. Renal variables in control and low- and normal-protein diabetic rats infused with glycine

<table>
<thead>
<tr>
<th></th>
<th>SNGFRmax, nl/min</th>
<th>SNGFRmin, nl/min</th>
<th>SNGFRd, nl/min</th>
<th>SNGFRmid, nl/min</th>
<th>SNGFRd - SNGFRmid, nl/min</th>
<th>V̇, nl/min</th>
<th>J̇v</th>
<th>J̇s/Filtrate</th>
<th>J̇/Filtrate Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.0 ± 3.0</td>
<td>21.9 ± 2.6</td>
<td>26.8 ± 2.3</td>
<td>29.5 ± 2.5</td>
<td>2.7 ± 0.7</td>
<td>5.6 ± 1.2</td>
<td>0.22</td>
<td>0.010</td>
<td>25.6 ± 2.3</td>
</tr>
<tr>
<td>+Glycine</td>
<td>50.1 ± 4.9*</td>
<td>36.0 ± 5.1*</td>
<td>37.8 ± 3.5*</td>
<td>43.1 ± 4.9*</td>
<td>5.3 ± 2.9</td>
<td>8.9 ± 1.6*</td>
<td>0.21</td>
<td>0.009</td>
<td>32.9 ± 2.7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>46.1 ± 3.3</td>
<td>21.2 ± 2.9</td>
<td>39.2 ± 3.6</td>
<td>37.3 ± 3.0</td>
<td>5.5 ± 1.7</td>
<td>6.5 ± 0.7</td>
<td>0.10</td>
<td>0.009</td>
<td>32.7 ± 3.3</td>
</tr>
<tr>
<td>Low-protein</td>
<td>44.9 ± 2.9</td>
<td>27.6 ± 3.5</td>
<td>35.6 ± 2.8</td>
<td>36.2 ± 3.0</td>
<td>0.6 ± 1.7*</td>
<td>8.2 ± 1.2*</td>
<td>0.22</td>
<td>0.007*</td>
<td>27.4 ± 2.0</td>
</tr>
</tbody>
</table>

Effect of diabetes on response to glycine in rats on normal diet

| P          | <0.02 | NS | <0.01 | 0.05 | <0.02 | NS | <0.005 | <0.01 | NS | <0.05 | 0.13 |

Effect of dietary protein on response to glycine in rats with diabetes

| P          | <0.02 | NS | <0.05 | <0.06 | NS | <0.02 | NS | 0.10 | 0.18 | 0.18 | 0.09 |

Values are means ± SE. SNGFRmax and SNGFRmin, single-nephron glomerular filtration rate at 0 and 40 ml/min microperfusion; SNGFRmid, average of SNGFRmax and SNGFRmin; SNGFRmid, single-nephron glomerular filtration rate in early distal tubule; V̇, distal flow rate; J̇v and J̇s, net water and salt reabsorption; DM, diabetes mellitus; NS, not significant. *P < 0.05 vs. before glycine. †Volume of glomerular filtrate with salt content (J̇s) or volume of filtrate cleared of salt by reabsorption.

Glycine infusion in diabetic rats had no significant effect on SNGFRd, SNGFRmax, SNGFRmin, or SNGFRmid (Table 2). Two-way testing for the effect of diabetes on the response to glycine confirmed that the effects of glycine on SNGFRmax (P < 0.02) and SNGFRd (P < 0.01) were blunted by diabetes. In diabetic rats before glycine infusion, SNGFRd significantly exceeded SNGFRmid, as mentioned above. Glycine caused SNGFRd to move farther from SNGFRmax and closer to SNGFRmin. Because of this shift of the operating point along the TGF curve, SNGFRd and SNGFRmid coincided during glycine infusion. In diabetic rats, early distal flow increased by 25% during glycine infusion (P = 0.05), while early distal conductivity increased by 50% (P < 0.0000001). The net delivery of ions to the early distal tubule was increased by glycine in diabetes, despite a tendency toward decreased delivered load. Because a paradoxical relationship between filtered load and distal delivery can never be explained by GTB (see above), these results can only be explained by a primary decrease in tubular reabsorption during glycine infusion.

Low-protein diabetic rats. Glycine infusion in low-protein diabetic rats increased SNGFRmax by 48% (P = 0.005), SNGFRmin by 76% (P = 0.028), SNGFRmid by 50% (P = 0.011), and SNGFRd by 18% (P = 0.07; Table 2). Low protein enhanced or restored the effects of glycine on SNGFRmax (P = 0.015), SNGFRmid (P = 0.06), and SNGFRd (P < 0.05) in low-protein diabetic compared with diabetic rats. In contrast, low protein did not alter the impact of glycine on SNGFRmin or early distal conductivity. As noted above, SNGFR and tubular reabsorption were paradoxically related in diabetic rats fed the standard diet. In low-protein diabetic rats, this paradoxical relationship disappeared. However, there appeared to be less reabsorptive efficiency among low-protein diabetic than nondiabetic control rats (P = 0.06).

Relative effects of glycine on SNGFR and tubular reabsorption. To test the hypothesis that glycine infusion influences proximal tubular reabsorption independent of its effect on the load delivered to the tubule, efficiency indexes were calculated for changes in net reabsorption of fluid up to the early distal tubule during glycine infusion (see METHODS). In control rats, the combined effects of glycine on SNGFRd and water reabsorption yielded a reabsorptive efficiency of 0.90 ± 0.13. This value is consistent with GTB alone or with a small primary increase in reabsorption superimposed on GTB (see DISCUSSION). In normal-protein diabetic rats, glycine was associated with a numerical decline in SNGFRd and a reabsorptive efficiency index greater than unity, implying a direct inhibitory effect of glycine on reabsorption in the proximal tubule and/or descending limb of Henle’s loop. In low-protein diabetic rats, glycine caused an increase in SNGFRd that was largely unmatched by increased water reabsorption, yielding a reabsorptive efficiency of 0.21 ± 0.34 (P = 0.06 vs. control).

**DISCUSSION**

Glomerular hemodynamic abnormalities are pathogenic in diabetic renal disease, and dietary protein restriction is advocated as a means to normalize glomerular function and delay the progression of diabetic renal disease (2, 7, 22). However, the mechanisms that underlie the normal glomerular response to protein, diabetic hyperfiltration, and the protection afforded by protein restriction in diabetes are not fully understood. These experiments provide information regarding the normal response to amino acid infusion, the role of the tubule in diabetic hyperfiltration, the abnormal re
response of the diabetic kidney to amino acid infusion, and the effects of dietary protein on tubular function in diabetes. We will discuss these aspects individually.

Normal Response to Glycine

In normal rats, glycine caused SNGFR to increase as expected. This was accompanied by increases in reabsorption of water and salt up to the early distal tubule. One goal of these experiments was to determine whether the effects of glycine on tubular reabsorption merely reflect the normal actions of GTB or result from direct effects of glycine on the tubule that change the actual behavior of GTB. This is important, because it has been proposed that the renal hemodynamic response to glycine is absent in diabetes because of a primary reduction in tubular reabsorption, which leads to activation of TGF (5). However, it may be difficult to distinguish a primary change in tubular reabsorption from a change in reabsorption that is due to GTB. GTB causes net reabsorption to vary directly with the delivered load. Therefore, SNGFR and net reabsorption cannot change in opposite directions unless there is a primary change in tubular reabsorption. On the other hand, when SNGFR and net reabsorption change in parallel, it still may be possible to recognize a primary change in tubular reabsorption. This occurs most obviously whenever a change in reabsorption exceeds the associated change in SNGFR, since such a change in reabsorption could never be load dependent. Furthermore, it might be possible to invoke a primary change in tubular reabsorption during glycine infusion, not only on qualitative grounds, but by a quantitative comparison of the response to glycine with normal GTB. To quantify normal GTB, one must have a technique for making SNGFR an independent variable without impinging on the tubule. Unfortunately, there is no way to do this for SNGFRd. However, with TGF as a tool for manipulating SNGFR, it is possible to characterize GTB up to the late proximal tubule and to calculate an index of GTB efficiency that is relatively independent of the location along the proximal tubule where measurements are made. During physiological GTB, the index must lie between zero and unity. When net fluid reabsorption and SNGFR change in opposite directions, the index will be negative. When a change in reabsorption exceeds a parallel change in SNGFR, the index will exceed unity. We recently measured the efficiency of proximal GTB in two sets of normal hydropenic rats: 0.64 ± 0.02 (18) and 0.72 ± 0.06 (unpublished observations). These values are comparable to, or slightly less than, the efficiency index calculated for Jw during glycine infusion (0.90 ± 0.13). Making the reasonable assumption that the autoregulatory index for GTB does not increase significantly along the pars recta and descending limb of Henle’s loop, where reabsorption is passive, we conclude that direct effects of glycine on the tubule in normal rats are neutral or stimulatory for water reabsorption.

By measuring the ionic content of the early distal tubule, we also assessed the impact of glycine on solute transport. In any given nephron, early distal condosity varies linearly with late proximal flow throughout the physiological range. According to previously published microperfusion data in hydropenic rats (20)

\[
\frac{\Delta \text{condosity}}{\Delta \text{late proximal flow}} = 0.009 \pm 0.001
\]

Comparing this value to the present glycine response provides insight into the effects of glycine on ascending limb transport. On the basis of the data in Table 2 and with the assumption that TFPmaxin ≈ 2 in the late proximal tubule, glycine would have increased the ambient late proximal flow by ~4.8 nl/min. This is almost identical to the normal effect of proximal tubular GTB as assessed in prior studies from this (10) and other (6) laboratories and is consistent with the foregoing assessment of the effects of glycine on water reabsorption. However, had there not been an additional primary increase in ascending limb reabsorption superimposed on the increase due to GTB, infusing glycine in control rats would have increased the normalized condosity from 0.22 to ~0.26. It is apparent that the present study had sufficient power to detect an increase in condosity much smaller than this and that no such increase occurred. Therefore, the normal response to glycine must include a primary increase in solute transport by the loop of Henle (Fig. 2).

Does this increase in reabsorption mediate the increase in SNGFR during glycine infusion? In theory, one could determine when a change in SNGFR is mediated through TGF by eliminating TGF and determining whether the change in SNGFR persists. The obvious way to remove the influence of TGF is to measure SNGFR from the proximal tubule when there is no flow past the macula densa. This is how we measured SNGFRmax in the present experiments. On the basis of a static model of TGF in which the relationship between SNGFRd and macula densa salt remains constant over time, SNGFRmax is strictly independent of TGF. However, the TGF relationship itself is capable of resetting within the time frame of a micropuncture experiment, such that events within the juxtaglomerular apparatus may cause SNGFRmax to change. A primitive understanding of the factors involved in TGF resetting has emerged from recent studies (reviewed in Ref. 19). The gist of these reports is that the nephron normally operates near the inflection point of its TGF curve, where TGF is most efficient, and that a sustained alteration in salt delivery to the macula densa, which initially alters TGF activity, ultimately causes TGF to reset to accommodate the new operating point. Therefore, determining whether a change in SNGFR is the result of a prior change in tubular reabsorption is not as simple as measuring SNGFRmax.

The present data confirm the prior observation that nephrons naturally operate with SNGFRd near the TGF inflection point (SNGFRmid) (17). However, during glycine infusion, SNGFRd tended to shift closer to the new SNGFRmin. This implies that, even though...
Abnormal diabetic response to glycine. Diabetes is one of several conditions in which the kidney fails to vasodilate and in which proximal reabsorption declines during glycine infusion (5). However, to complete the argument that activation of TGF suffices to explain the absence of vasodilation, one must confirm that proximal reabsorption would not also decline in normal animals during glycine infusion if the load delivered to the tubule were prevented from increasing by some other external force. Such data do not exist apart from the independent effect of glycine on ascending limb transport revealed by the present data and discussed above.

The present data confirm prior observations that SNGFR is not increased by glycine in diabetic rats fed a standard commercial rat chow (3, 16). At the same time, salt and water reabsorption up to the early distal tubule were reduced during glycine infusion. This effect was more pronounced for electrolytes than for water, suggesting a selective reduction in ascending limb transport. These data violate the basic laws of mass action, which dictate GTB. Therefore, we conclude that glycine enhances reabsorption in nondiabetic rats while inhibiting reabsorption in the proximal tubule and loop of Henle in rats with diabetes.

Could this decrease in reabsorption account for the failure of SNGFR to increase during glycine infusion? There are two ways to look at this. First, does glycine cause more TGF activation in diabetic than in control rats? Second, if the tubular response to glycine in diabetic rats were normalized, would this normalize the SNGFR response? As indicated by the downward shift of SNGFR relative to the TGF inflection point, TGF was more activated during glycine infusion (5). However, to complete the argument that activation of TGF suffices to explain the absence of vasodilation, one must confirm that proximal reabsorption would not also decline in normal animals during glycine infusion if the load delivered to the tubule were prevented from increasing by some other external force. Such data do not exist apart from the independent effect of glycine on ascending limb transport revealed by the present data and discussed above.

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Feeding low protein to diabetic rats increases fractional water reabsorption up to the early distal tubule and tends to reduce SNGFRd. These combined effects are consistent with normal GTB in the water-permeable nephron segments. However, the increase in early distal condosity brought about by reducing dietary protein can only be explained by a primary decrease in salt reabsorption from the ascending loop of Henle, because GTB can only explain an increase in distal delivery if there is an increase in SNGFR. The effects of dietary protein on tubular function in these diabetic rats are analogous to the effects reported for normal rats, in which increased salt reabsorption from the loop of Henle reduces the TGF signal and accounts for the increase in GFR after protein feeding (13).

By reducing loop of Henle transport, a low-protein diet normalizes the concentration of salt in tubular fluid reaching the macula densa in diabetes. However, a low-protein diet does not normalize the tubular response to glycine (Fig. 2). The increase in water reabsorption during glycine infusion in low-protein diabetic rats is less than in control rats and less than expected for normal GTB. Also, early distal condosity during glycine infusion increases by twice the amount predicted for GTB on the basis of the efficiency of volume reabsorption, late proximal TFPinsulin = 2, and the normal dependence of condosity on late proximal flow in diabetes (20). This implies that a primary reduction in ascending limb transport occurs during glycine infusion in the low-protein diabetic rats. In other words, diabetes causes a paradoxical reduction in ascending limb of Henle transport during glycine infusion, and this is not prevented by dietary protein restriction.

Although reduced tubular reabsorption accounts for much of the failed SNGFR response to glycine in normal-protein diabetic rats and reducing dietary protein does not reverse these effects of glycine on the diabetic tubule, reducing dietary protein did partially restore the effect of glycine on SNGFRd. With application of Eq. 1 to estimate what would have been the effect of glycine on SNGFRd in low-protein diabetic rats had condosity remained constant during glycine infusion, values are obtained for ∆SNGFRd that range from 9.7 to 21.7 nl/min, depending on whether estimates for the TGF slope and late proximal flow-condosity relationship are taken from diabetic or normal rats (20). Addition of only the lowest estimate, 9.7 nl/min, to SNGFRd in the low-protein diabetic rats during glycine infusion would cause the effect of glycine in this group to exceed its effect in control animals. Hence, protein restriction of the diabetic rat must sensitize the glomerular microvasculature to vasodilation by glycine, an effect that is concealed by the simultaneous reduction in loop of Henle reabsorption, which causes activation of TGF.

**Perspectives**

The body’s internal environment is regulated in large part by processes occurring in the juxtaglomeru-
lar apparatus of nephrons, and TGF can mediate, facilitate, or mitigate changes in GFR. The normal kidney vasodilates in response to protein feeding, a phenomenon that enhances nitrogen homeostasis. It is overly simplistic to view glycine infusion as the equivalent of protein feeding. Nonetheless, the two maneuvers have some things in common. For example, glycine infusion and protein feeding cause GFR to increase by vasodilating the kidney while simultaneously acting to prevent TGF from overriding that vasodilation. Furthermore, both maneuvers circumvent TGF by increasing tubular reabsorption, which reduces the TGF signal. This contrasts with the response to other maneuvers such as acute plasma volume expansion, where the TGF signal is increased but vasodilation is facilitated by resetting the TGF response within the juxtaglomerular apparatus (17). Selectively targeting the TGF signal or the TGF response to facilitate increases in GFR enables the kidney to optimize homeostasis of nitrogen or extracellular volume, respectively.

The pathogenesis of diabetic nephropathy is poorly understood, but intrarenal hemodynamic abnormalities such as glomerular hyperfiltration are thought to be among the foremost factors responsible (2). The present data complement prior evidence favoring a tubular hypothesis of glomerular hyperfiltration (18, 21) according to which hyperfiltration originates with a reduced amount of salt reaching the macula densa. Furthermore, these data suggest that the tubular hypothesis might be extended to explain the salutary effects of dietary protein restriction on the diabetic kidney (6, 22). In the present experiments, reducing dietary protein caused a primary decrease in tubular reabsorption and normalized the TGF signal. A primary increase in tubular reabsorption drives glomerular hyperfiltration in the first place, and these data suggest that the glomerular hemodynamic response to a low-protein diet in diabetes is also mediated through the effects on the tubule.

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REFERENCES