Salt-Sensitivity of Proximal Reabsorption Alters Macula Densa Salt and Explains the Paradoxical Effect of Dietary Salt on Glomerular Filtration Rate in Diabetes Mellitus

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Abstract. GFR varies inversely with dietary NaCl in patients with early type I diabetes and in streptozotocin (STZ)-diabetic rats. To explain this paradox within the laws of physiology, it was hypothesized that it results from heightened sensitivity of the diabetic proximal tubule to dietary salt because changes in proximal reabsorption (Jprox) elicit reciprocal adjustments in GFR through the normal actions of tubuloglomerular feedback (TGF). Micropuncture was done in rats after 5 wk of moderately hyperglycemic STZ-diabetes and 1 wk of different NaCl diets. First, single-nephron GFR (SNGFR) and early distal tubular Na⁺, Cl⁻ and K⁺ concentration (representing the TGF signal) were measured by collecting from early distal nephrons. In nondiabetics, dietary salt did not affect SNGFR or the TGF signal. In diabetics, the TGF signal varied directly with dietary salt while SNGFR varied inversely with dietary salt. Next, Jprox was measured by collecting from late proximal tubules. To control for different SNGFR, SNGFR was manipulated by perfusing Henle’s loop to alter TGF activity. Controlling for SNGFR, dietary salt did not affect Jprox in nondiabetics but exerted a major inverse impact on Jprox in diabetics. In conclusion, normal rats acclimate to dietary NaCl by primarily adjusting transport downstream of the macula densa. In contrast, diabetes renders reabsorption in the proximal tubule sensitive to dietary NaCl with subsequent effects on the TGF signal. This explains the paradoxical effect of dietary NaCl on GFR in early diabetes.

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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 for its onset and progression (1,2). Although no single cause has emerged for early glomerular hyperfiltration, a major portion of it results from a primary increase in proximal tubular reabsorption (3–6), which is due to early tubular hypertrophy (4) and hyperglycemia-induced enhanced load of glucose to the tubular system (3,7). The diabetes-induced increase in proximal tubular transport reduces the luminal signal for tubuloglomerular feedback (TGF), i.e., the sodium, chloride, and potassium concentration in the tubular fluid at the macula densa (3). The resulting TGF-dependent increase in single nephron GFR (SNGFR) tends to restore the fluid and electrolyte delivery to the nephron segments downstream from the macula densa.

Diabetes also alters the renal hemodynamic response to a variety of stimuli, and the basic defect in regulation of renal function may ultimately be revealed by examining the response of the diabetic kidney to different physiologic stimuli. Changes in dietary salt constitute one such stimulus, the renal response to which is abnormal in diabetes. Salt balance requires that salt excretion equal salt intake. It is certainly possible for the kidney to alter urinary salt excretion without affecting renal blood flow or GFR. Nevertheless, when renal blood flow, GFR, and salt excretion all change at the same time, they should do so in parallel. One example of this is the hypertensive African American population, in which a high-salt diet causes GFR to increase (8). It has, however, been shown in both diabetic patients and in rats with experimental diabetes that GFR and renal blood flow vary inversely with dietary salt (9–11). This effect of dietary salt on GFR in diabetes is counterintuitive with regard to salt balance; we, therefore, refer to it as the “salt paradox.” The current dietary recommendation of the American Diabetes Association is for diabetic patients to restrict salt intake (12), and glomerular hyperfiltration is viewed as deleterious; therefore, this nuance of dietary salt and renal function could be clinically relevant.

Neurohumoral systems (e.g., renin-angiotensin system, natriuretic peptides, or renal nerves) that impinge on the glomerulus confer only positive effects of dietary salt on GFR and renal blood flow. Altering their efficiency can strengthen the positive effect or reduce it to zero but cannot make it negative. Hence, the effect of dietary salt on the diabetic kidney presents a paradox that cannot be explained by traditional neurohumoral...
or vascular mechanisms. However, the paradox in diabetes might be explained by a heightened sensitivity of the proximal tubule to changes in dietary salt, because reduced proximal reabsorption in response to high-salt diet will activate TGF, thereby reducing SNGFR; vice versa, enhanced proximal reabsorption in response to low-salt diet will deactivate TGF, thereby increasing SNGFR. If these effects become large enough to outweigh the opposing systemic influences, then GFR and renal blood flow will vary inversely with salt intake. To test this hypothesis in diabetic rats, we studied the impact of salt diet on proximal reabsorption and on the TGF stimulus.

Materials and Methods

Overview

In search of an explanation for the salt paradox in diabetes, the effects of dietary salt on tubular function in the nephron segments upstream from the macula densa, on the actual TGF signal, and on SNGFR were examined by micropuncture in male rats with 5 wk of insulin-treated STZ diabetes. This was accomplished in two series of experiments, henceforth referred to as series A and series B.

In series A, measurements were taken of the volume, ionic composition, and inulin clearance of fluid reaching the early distal tubule to test for differential effects of dietary salt on the ambient TGF signal and SNGFR in diabetic and nondiabetic Sprague Dawley rats. This series was performed at the University of Tübingen, Germany.

In series B, differential effects were sought for the effects of dietary salt on behavior of the proximal tubule in diabetic and nondiabetic Wistar rats. To test for primary differences in tubular function, one must control for differences in delivered load. Therefore, TGF was used as a tool for manipulating SNGFR in series B so that net proximal reabsorption could be defined as a function of SNGFR in each nephron. This series was performed at the University of California San Diego and San Diego Veterans Administration Medical Center.

Experimental Diabetes and Salt Diets

All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Adult male Wistar or Sprague-Dawley rats were made diabetic by STZ (65 mg/kg intraperitoneally; Sigma, St. Louis, MO) dissolved in sodium citrate buffer (pH 4.2). Diabetic rats were treated daily with long-acting insulin (0.5 to 1.5 IU subcutaneously four times daily; Anpro Pharmaceutical, Arcadia, CA) or with long-acting insulin pellets placed subcutaneously and supplemented with daily insulin injections as needed to adjust blood glucose levels at approximately 350 mg/dl. This insulin regimen permits the diabetic rats to maintain an outward appearance of good health and to grow. Nondiabetic rats receiving STZ-vehicle served as controls. Normal blood glucose levels in nonfasted nondiabetic Sprague-Dawley or Wistar rats are in the range of 100 to 150 mg/dl. The animals were allowed free access to a regular rat pellet diet and tap water. After 4 wk, the following changes in diet were performed.

Series A. Animals were switched to low-NaCl diet (0.015% Na+ and 0.03% Cl−) or high-NaCl diet by maintaining regular pellet diet (0.25% Na+ and 0.35% Cl−) and adding 0.25% or 1% NaCl to drinking water to account for different fluid intake in diabetic and control rats, respectively.

Series B. Animals were maintained on regular pellet diet (a normal NaCl diet) or switched to high-NaCl diet as described above. Initial experiments revealed that this maneuver achieved an approximately fourfold increase in NaCl intake compared with normal NaCl diet in control and diabetic rats. One week later, nonfasted rats were prepared for micropuncture studies.

Preparation for Renal Micropuncture

Rats were prepared for micropuncture under Inactin anesthesia (100 mg/kg intraperitoneally; Research Biochemicals, Natick, MA) according to protocols previously described (3,4). Nondiabetic animals received Ringer solution containing 80 to 100 μCl/ml [3H]-inulin as a marker of glomerular filtration by continuous intravenous infusion at 2 ml/h. Diabetic animals received Ringer solution at 3 ml/h to compensate for diabetic polyuria. After completing the preparatory surgery, animals were allowed 60 min to equilibrate before beginning micropuncture.

Micropuncture Experiments in Series A

SNGFR, fluid and electrolyte transport up to the early distal tubule, and TGF signal were studied. For purposes of these studies, “natural” values for SNGFR are defined as those 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 nephron is referred to as SNGFRd. The first distal tubular segment on the kidney surface was identified, and a timed collection of tubular fluid was performed by using a short mineral oil block. Early distal tubular fluid collections were analyzed for tubular flow rate (VED) and sodium, potassium, and chloride ion concentration ([Na+]ED, [K+]ED, [Cl−]ED) as well as 3H-inulin concentration to determine SNGFRd as described previously (3). These data and the respective ion concentrations in plasma were used to calculate absolute and fractional reabsorption of fluid, Na+, K+, or Cl− between the glomerulus and the early distal tubule (3).

Micropuncture Experiments in Series B

SNGFRd and fluid transport up to the early distal tubule were studied. As performed in series A, tubular fluid was collected from the early distal tubule. Tubular fluid collections were analyzed for flow rate and 3H-inulin concentration to determine SNGFRd. These data were used to calculate reabsorption of fluid up to the early distal tubule.

Proximal Reabsorption

Because of glomerulotubular balance, reabsorption will vary with SNGFR and hyperfiltration will confound other effects of diabetes on proximal reabsorption. To correct for this, we manipulated the TGF signal to determine the relationship of proximal reabsorption to SNGFR in individual nephrons as described (4). A primary change in proximal reabsorption was defined as a change in this relationship. Briefly, late proximal tubules were localized on the kidney surface, and an obstructing wax block was inserted immediately upstream from the most downstream accessible segment. A microperfusion pipette containing artificial tubular fluid (ATF; 130 mM NaCl, 10 mM NaHCO3, 4 mM KCl, 2 mM CaCl2, 45% urea, 0.1% FD&C, pH 7.4) was inserted downstream from the wax block to perfuse the loop of Henle, thereby activating TGF and causing SNGFR to change. Timed collections of tubular fluid were made upstream from the wax block to measure SNGFR and late proximal tubular fluid flow rate in each nephron during minimal TGF activation (loop of Henle microperfusion at 0 nl/min [SNGFRmax]) and maximal TGF activation (loop of Henle microperfusion at 40 nl/min [SNGFRmin]). Perfusions were in random order. Nephrons were vented upstream from the wax block.
before each collection to prevent pressure from building up in the proximal tubule. Tubular fluid samples were assayed for volume and 

$\text{H}^{-}\text{inulin}$ to calculate SNGFR and reabsorption of fluid in the proximal tubule. Data from these paired collections were employed to characterize the dependence of proximal reabsorption on SNGFR. The TGF response is traditionally modeled as a symmetric sigmoid. SNGFR at the inflection point of this sigmoid curve is simply the average of SNGFRmax and SNGFRmin; it is referred to here as SNGFRmid. As an index of TGF activation, SNGFRd was compared with SNGFRmid.

Table 1. Series A: Systemic and whole kidney data in control and diabetic Sprague-Dawley rats on low- versus high-NaCl diet. In control rats, dietary salt did not affect SNGFRd or net transport of water or electrolytes up to the early distal tubule (Table 2 and Figure 2). In contrast, dietary NaCl was a major determinant of both SNGFRd and NaCl transport up to the early distal tubule in rats with diabetes; diabetic rats fed high-NaCl diet manifest lesser SNGFRd but greater early distal electrolyte concentrations than diabetic rats fed low-NaCl diet. Accordingly, absolute reabsorption of NaCl up to the early distal tubule was diminished in diabetic rats on high- versus low-NaCl diet.

Statistical Methods

Heterogeneity of animals 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 standard formula (4). $P < 0.05$ was the cutoff for statistical significance.

Results

Series A: Effect of Low- versus High-NaCl Diet on SNGFRd. Transport of Fluid and Electrolyte up to the Early Distal Tubule, and the TGF Stimulus

Low-NaCl diet significantly reduced urinary Na$^+$ and Cl$^-$ excretion compared with high-NaCl diet in control and diabetic rats. This was evident in awake rats placed in metabolic cages as well as those under anesthesia in micropuncture experiments (Table 1). Dietary NaCl did not affect body weight, mean arterial pressure, blood glucose concentration, or hematocrit in control or diabetic rats (Table 1 and Figure 1). Among diabetic rats that were fed low-NaCl diet, the kidneys weighed more and GFR was greater than in diabetic rats fed high-NaCl (Figure 1). In contrast, dietary NaCl did not affect kidney weight or GFR in control rats.

Series B: Effect of Normal- versus High-NaCl Diet on Transport in the Proximal Tubule

Body weights, blood glucose concentrations, BP, or kidney wet weights were not significantly altered by high- versus normal-NaCl diet in control or diabetic rats (Table 3). Increasing flow through Henle’s loop from 0 to 40 nl/min by microperfusion caused SNGFR to decline in all groups ($P < 0.00005$). The range of the TGF response was slightly greater in diabetes ($P = 0.0003$) and unaffected by dietary salt ($P = 0.7$). High-salt tended to increase SNGFR in controls and significantly reduced SNGFR in diabetics. The paradoxical response of the diabetic kidney to dietary salt was significant by 2-way ANOVA for SNGFRmax, SNGFRmin, SNGFRmid, and SNGFRd (Figures 3 and 4).

To test for primary differences in tubular function, TGF was used as a tool for manipulating SNGFR so that absolute proximal reabsorption could be defined as a function of SNGFR and SNGFRd (Figures 3 and 4). Among rats fed normal-salt diet, diabetics manifest lesser SNGFRd but greater early distal electrolyte concentrations than diabetic rats fed high-NaCl diet. In controls, high-NaCl diet did not reduce proximal reabsorption or alter distal fluid delivery. In fact, controlling for differences in SNGFR, absolute proximal reab-

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$^a$ Data are mean ± SE. UV, UNaV, or UCIV, urinary excretion of fluid, Na, or Cl, respectively; BGL, blood glucose level; Hct, hematocrit. ND, not determined.

$^b$ $P < 0.05$ vs. high-NaCl.
Absorption in control rats tended to increase on a high-NaCl diet. In contrast, feeding high-NaCl diet to diabetic rats caused a decline in absolute proximal reabsorption, which persisted after controlling for differences in SNGFR.

Consistent with the changes in proximal reabsorption, it was observed in this series that high-NaCl diet elicited a significant reduction in absolute and fractional reabsorption of fluid up to the early distal tubule in diabetic rats, whereas no significant effect of dietary salt was observed in control rats (Figure 5).

In controls, SNGFRd, which reflects the operating point, nearly coincided with SNGFRmid, which reflects the TGF midpoint or TGF inflection point. In diabetics on normal NaCl diet, SNGFRd exceeded SNGFRmid by 8.2 ± 1.9 nl/min (Figure 3). In control rats fed high-NaCl diet the operating point tended to drift up from the TGF midpoint, whereas in diabetics fed high-NaCl diet, the operating point tended to drift down toward the TGF midpoint (Figure 3). A shift downward along the TGF curve implies greater relative activation of TGF. The effects of dietary salt on the orientation of the operating point relative to the TGF midpoint did not achieve statistical significance. However, the tendencies were all consistent with the changes expected on the basis of the significant primary changes in proximal reabsorption, which were documented by late proximal collections (Figure 4).

**Discussion**

The present findings permit us to explain the salt paradox in diabetes (9–11) by a simple shift in the balance of forces between TGF and the other neurohumoral regulators of glomerular filtration. This occurs because diabetes causes proximal tubular reabsorption to become more sensitive to changes in dietary salt. This results in a strong influence of dietary salt.
over the TGF signal such that eating more salt leads to greater activation of TGF. This explanation is appealing because it can explain the salt paradox on the basis of a quantitative difference in a normal physiologic process rather than by invoking some physiologic pathway unique to diabetes or uniquely missing in diabetes.

TGF could become the dominant intermediary between dietary salt and GFR in either of two ways. First, there could be a greater TGF response to a given change in delivery of salt to the macula densa. Second, there could be a stronger inverse influence of dietary salt on reabsorption upstream from the macula densa. The latter mechanism appears to apply, and this was predictable on the basis of the prior observation that the TGF effector response to an incremental or decremental stimulus around the operating point is blunted, not enhanced, in diabetes (13). Indeed, the present data reveal that feeding a high-salt diet to diabetic rats causes a major decline in proximal reabsorption that cannot be explained by a change in the load delivered to the tubule. In other words, feeding salt to a diabetic rat causes a primary decrease in proximal reabsorption. Furthermore, by measuring distal delivery of salt, we also confirm that this primary effect of dietary salt on tubular reabsorption strongly links the TGF signal to dietary salt in diabetes. An observed tendency for the TGF operating point to drift down toward the TGF midpoint among high-salt diabetics but up from the TGF midpoint in high-salt controls is also consistent with the notion that dietary salt selectively leads to greater TGF activation in diabetes. Normal rats manage salt balance with minimal primary effect on proximal reabsorption; therefore, an inverse effect of dietary salt on GFR is not normally seen.

This hypothesis invokes TGF to explain the salt paradox in diabetes. 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. One 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. SNGFRmax was reduced by high-salt diet in diabetics (Figure 4), suggesting that high-salt diet reduced SNGFR independently of the macula densa. However, the TGF relationship itself is capable of resetting such that events within the juxtaglomerular apparatus may cause SNGFRmax to change (re-
viewed in reference 14). The gist of these reports is that the nephron normally operates along the steep portion of its TGF curve and that a sustained alteration in salt delivery to the macula densa, which initially causes a change in TGF activity, ultimately causes TGF to reset. Therefore, a sustained alteration in proximal and/or loop of Henle reabsorption can be the cause of a parallel change in SNGFRmax.

We have confirmed the salt paradox in moderately hyperglycemic diabetic rats of different strains and both genders by micropuncture and whole kidney clearance experiments performed in Germany and in the United States over the past 6 yr. Most importantly, the phenomenon has also been observed in diabetic patients (9). Given the limits of TGF, however, the capacity to increase GFR by reducing distal salt delivery is clearly less than the capacity to reduce GFR through the systemic influences of salt depletion on GFR, which, in the extreme case, will result in zero GFR. Hence, one can predict that in conditions under which dietary salt restriction elicits significant salt and volume depletion, which may occur, e.g., under concomitant osmotic natriuresis due to severe hyperglycemia, the systemic influences on GFR can attenuate or even prevent the salt paradox.

In conclusion, normal rats depend mostly on the distal nephron for salt balance. On the other hand, the proximal tubular reabsorption is sensitive to dietary salt in diabetic rats. This renders the TGF signal sensitive to dietary salt and leads to a paradoxical effect of dietary salt on GFR, which, in the extreme case, will result in zero GFR. Hence, one can predict that in conditions under which dietary salt restriction elicits significant salt and volume depletion, which may occur.

Acknowledgments

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References


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