Ornithine decarboxylase inhibitor eliminates hyperresponsiveness of the early diabetic proximal tubule to dietary salt

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Miracle CM, Rieg T, Mansoury H, Vallon V, Thomson SC. Ornithine decarboxylase inhibitor eliminates hyperresponsiveness of the early diabetic proximal tubule to dietary salt. Am J Physiol Renal Physiol 295: F995–F1002, 2008. First published June 18, 2008; doi:10.1152/ajprenal.00491.2007.—Heightened sensitivity of the diabetic proximal tubule to dietary salt leads to a paradoxical effect of salt on glomerular filtration rate (GFR) via tubuloglomerular feedback. Diabetic hyperfiltration is a feedback response to growth and hyperreabsorption by the proximal tubule. The present studies were performed to determine whether growth and hyperfunction of the proximal tubule are essential for its hyperresponsiveness to dietary salt and, hence, to the paradoxical effect of dietary salt on GFR. Micropuncture was performed in four groups of inactin-anesthetized Wistar rats after 10 days of streptozotocin diabetes drinking tap water or 1% NaCl. Kidney growth was suppressed with ornithine decarboxylase (ODC) inhibitor, DFMO (200 mg·kg⁻¹·day⁻¹), or placebo. Single nephron GFR (SNGFR) was manipulated by perfusing Henle’s loop so that proximal reabsorption (J_prox) could be expressed as a function of SNGFR in each nephron, dissociating primary effects on the tubule from the effects of glomerulotubular balance. Alone, DFMO or high salt reduced SNGFR and suppressed J_prox independent of SNGFR. Suppression of J_prox was eliminated and SNGFR increased when high salt was given to rats receiving DFMO. ODC is necessary for hyperresponsiveness of the proximal tubule to dietary salt and for the paradoxical effect of dietary salt on GFR in early diabetes. This coupling of effects adds to the body of evidence that feedback from the proximal tubule is the principal governor of glomerular filtration in early diabetes.

METHODS

Overview

The effect of dietary salt on proximal reabsorption was determined by micropuncture in early streptozotocin diabetic rats and in diabetic rats administered the ornithine decarboxylase (ODC) inhibitor, difluoromethyl ornithine (DFMO), which is known to mitigate kidney enlargement in early streptozotocin diabetes (7, 8, 15). A micropuncture protocol was employed in which the TGF signal is artificially manipulated as a means for changing SNGFR so that proximal tubular reabsorption can be characterized over a range of SNGFR in individual nephrons. The effects of dietary salt and DFMO on this relationship between kidney size, proximal reabsorption, and SNGFR were analyzed as a direct test for primary differences in proximal reabsorption. All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals with an IACUC-registered protocol.

Diabetes

Adult male Wistar rats (Simonson Labs, Gilroy, CA) were made diabetic with streptozotocin (Sigma, 65 mg/kg ip × 1 dose). Thereafter, blood glucose was measured daily in late morning by glucometer and titrated in the range of 19–25 mM with daily subcutaneous injection of long-acting insulin (PZI, Blue Ridge Pharmaceuticals, Memphis, TN). Rats were housed in pairs with one rat in each cage also receiving daily subcutaneous injection of DFMO (200 mg·kg⁻¹·day⁻¹) and the other receiving saline placebo. Half the cages were free-fed standard rat chow (NaCl content 0.4% wt/wt) and were given ad libitum access to tap water. The other half were fed standard rat chow and 1% saline was added to the drinking water. DFMO and saltwater were begun on the same day that streptozotocin was given.

Surgical Preparation for Micropuncture

Micropuncture experiments were performed after 10–11 days of diabetes and ~24 h after the final doses of DFMO and insulin. Rats that were housed together were studied on consecutive days with the order (DFMO vs. placebo) alternated. Animals were surgically prepared according to previously established protocols (15). Briefly, animals were anesthetized with inactin (100 mg/kg ip; Research Biochemicals, Natick, MA) and body temperature was maintained on servo-controlled heating table. Airway was maintained with trache-
Table 1. Systemic data obtained during micropuncture

<table>
<thead>
<tr>
<th>Arterial BP, mmHg</th>
<th>Blood Glucose, mM</th>
<th>Urine Flow, μl/min</th>
<th>Glucose Reabsorption, μmol/min</th>
<th>FEGlu, %</th>
<th>GFR, ml/min</th>
<th>Kidney Wt. g</th>
<th>GTB Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n = 7)</td>
<td>118 ± 5</td>
<td>18 ± 2</td>
<td>11 ± 6</td>
<td>43 ± 6</td>
<td>4 ± 2</td>
<td>2.5 ± 0.2</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>Placebo + HS (n = 7)</td>
<td>122 ± 5</td>
<td>17 ± 2</td>
<td>13 ± 5</td>
<td>27 ± 7</td>
<td>0.8 ± 0.5</td>
<td>2.0 ± 0.2</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>DFMO (n = 6)</td>
<td>111 ± 5</td>
<td>24 ± 2</td>
<td>18 ± 7</td>
<td>43 ± 6</td>
<td>6 ± 3</td>
<td>1.9 ± 0.2</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>DFMO + HS (n = 5)</td>
<td>118 ± 6</td>
<td>20 ± 2</td>
<td>23 ± 8</td>
<td>46 ± 7</td>
<td>3 ± 1</td>
<td>2.5 ± 0.2</td>
<td>3.6 ± 0.1</td>
</tr>
</tbody>
</table>

ANOVA, P values

- DFMO: 0.07
- Salt: 0.11
- DFMO*Salt: 0.02
- Placebo: 0.03
- Placebo + HS: 0.01
- DFMO + HS: 0.03

Values ± SE from least squares ANCOVA. BP, arterial blood pressure; FE, fractional excretion; GTB, glomerulotubular balance; GFR, glomerular filtration rate; HS, high salt. *With blood glucose as covariate. †With single nephron GFR (SNGFR) as covariate. Statistics for FEGlu based on log-transformed data. Cells left blank for $P > 0.15$. 

Statistics

Statistical analysis of micropuncture data was by two-way ANOVA with design for repeated measures, analysis of covariance (ANCOVA), or Student’s t-test. Analyses were done with commercial software (Systat, Evanston, IL), except for certain Student’s t-tests, which were done by hand. Outliers were identified by Studentized residual $>2.5$ from the AN(C)OVA and analyses were repeated. Adjustment for multiple group comparisons was by Tukey or Bonferroni tests as appropriate.

RESULTS

General Data Leading up to Micropuncture

Animals lost weight during the first 2–3 days of diabetes and then begun to gain weight. Rats on high salt experienced less initial weight loss (7 ± 1 vs. 12 ± 2 g, $P = 0.05$). There was no significant effect of DFMO on initial weight loss (11 ± 2 vs. 8 ± 2 g) and the effect of dietary salt was independent of DFMO. Beyond day 3 of diabetes, animals gained weight and there were no significant differences in weight gain between groups.

Hyperglycemia was moderated by daily injection of long-acting insulin, which was dose adjusted each day based on glycemic history. By design, there were no differences between groups in the course of blood glucose concentrations. Animals receiving DFMO wound up receiving 50% more insulin per day than those receiving placebo (1.07 ± 0.06 vs. 0.72 ± 0.06 U/day, $P < 0.01$). Insulin requirement was not significantly influenced by dietary salt.

Animals were polydipsic and polyuric as is typical of diabetic rats. It was not possible to determine the effect of DFMO on water drinking, since DFMO- and placebo-treated animals were housed together in pairs. Based on per-cage water consumption, rats on tap water drank 140 ± 17 ml/day and those on high salt drank 160 ± 22 ml/day.

General Data During Micropuncture

Blood pressure. Mean arterial pressure ranged from 88 to 139 mmHg with a minor, nonsignificant tendency to be higher in those fed high salt (120 ± 4 vs. 114 ± 4 mmHg; Table 1). DFMO did not appear to alter the influence of dietary salt on blood pressure. The combined blood pressure and body weight data suggest that the efficiency of salt balance is not grossly affected by DFMO, notwithstanding major effects of DFMO.
on how the responsibility for salt balance is parsed along the nephron (vide infra).

**GFR.** In placebo-treated diabetic rats, adding NaCl to the drinking water reduced two-kidney GFR by 20%, consistent with past observations on the diabetic salt paradox (20, 21, 23). DFMO lessened GFR by 25% in diabetic rats on standard diet, which also reproduced a prior finding (15). In contrast, adding NaCl to the drinking water of DFMO-treated rats caused GFR to increase by 26%. In other words, DFMO reversed the paradoxical effect of dietary salt on GFR (P = 0.03). GFR and total kidney weight tended to correlate (r = 0.46, P = 0.1). GFR data are not normalized for kidney weight because absolute differences in GFR are of bottom-line interest, with kidney weight afforded status as a potential determinant in our paradigm.

**Urine flow rate.** Urine flow rate ranged from 4 to 63 μl/min. Although rats on high salt or DFMO produced more urine on average, these effects were not significant by straightforward ANOVA. A multivariate analysis showed no effect of blood pressure or GFR on urine flow rate, but urine flow rate did vary directly with blood glucose (P = 0.001). Using ANCOVA to adjust for blood glucose lessened the variability in urine flow by nearly half, which rendered a 67% greater urine flow rate among high-salt rats nearly significant (P = 0.09). Controlling for glucose excluded DFMO as a determinant of urine flow rate in the same analysis.

**Blood glucose and glucose transport.** Although there was no effect of dietary salt or DFMO on the course of blood glucose leading up to micropuncture (vide supra), DFMO-treated animals wound up with higher blood glucose concentrations during micropuncture (22 ± 2 vs. 17 ± 2 mM, P < 0.03). Fractional excretion of glucose (FEglu) was measured in 21 animals and ranged 150-fold from 0.002 to 0.298. FEglu appeared log normal by inspection of normal probability plots (Systat) so AN(C)OVA for FEglu was done on log-transformed data after exclusion of a single outlier (Studentized residual 2.75). FEglu correlated with the filtered load of glucose (r = 0.64, P = 0.001). By two-way ANOVA, high salt diet appeared to lower FEglu by about half (P = 0.02) and this effect of dietary salt persisted when the test was repeated with filtered glucose as a covariate. DFMO tended to increase FEglu (Table 1), but the effect was nonsignificant and disappeared altogether after controlling for filtered glucose. Therefore, the present data are best viewed as underpowered and inconclusive regarding the role of ODC-mediated kidney growth in glucose reabsorption by the early diabetic kidney.

**Kidney weight.** Kidneys were removed and weighed after micropuncture. There was one heavy outlier in the high salt group excluded from the analysis (Studentized residual = 4). Kidneys from placebo-treated rats on standard diet averaged ~10% smaller than what we typically observe at this stage of diabetes in male Wistar rats of this size (15, 20). Consistent with past experience in early streptozotocin diabetes (15, 20), DFMO tended to reduce kidney size in rats fed standard diet, and high salt tended to reduce kidney size in placebo-treated rats. The ANOVA confirmed opposite effects of dietary salt on kidney weight in DFMO- vs. placebo-treated rats (P < 0.01).

**Micropuncture Results**

**SNGFR.** SNGFR was measured twice from the late proximal tubule in each nephron, once during maximal TGF activation (SNGFR_{min}), and once with no TGF activation (SNGFR_{max}). In all, this included 272 individual micropuncture collections from 136 nephrons. By two-way ANOVA applied to SNGFR_{max}, there was no independent effect of dietary salt or DFMO. However, the cross term (dietary salt × DFMO) was highly significant (P < 0.001), which reflects the following: in diabetic rats on a standard salt intake, DFMO reduced SNGFR_{max} (P < 0.04 by ANOVA with Tukey adjustment for multiple comparisons), reproducing a prior result (15). Meanwhile, increasing dietary salt reduced SNGFR_{max} (P < 0.03 by ANOVA with Tukey), reproducing the diabetic salt paradox (20). But in DFMO-treated diabetic rats, dietary salt had an effect normally seen in nondiabetic rats (16), which was to increase SNGFR_{max}. In other words, the diabetic salt paradox was reversed by DFMO (Fig. 1). This interaction between dietary salt and DFMO was also noted for SNGFR_{min} (P < 0.02 for Salt × DFMO cross-term in ANOVA).

The same result for SNGFR was obtained when ANOVA was performed with repeated measures to incorporate both SNGFR_{max} and SNGFR_{min} into the model; the cross-term (dietary salt × DFMO) was significant (P < 0.001) while the opposing influence of dietary salt in placebo- vs. DFMO-treated animals ensured no primary effect of either salt or DFMO. The within-subjects portion of the repeated-measures ANOVA tests for the effect of salt and DFMO on the range of the TGF response. The means ± SD for the range of the TGF response in 136 nephrons were 16 ± 11 μl/min and there was no significant effect of dietary salt or DFMO. In the occasional nephron the recorded range of the TGF response was an underestimate of the true range because the effect of full-activation was so strong that flow ceased altogether. To pro-

**Fig. 1.** Effects of dietary salt (DS) and difluromethyl ornithine (DFMO) on single nephron glomerular filtration rate (SNGFR) in early diabetic rats. HS, high-salt diet. Circles, least squares means ± SE for SNGFR_{max} from ANOVA. Top and bottom of boxes indicate group mean SNGFR during zero (SNGFR_{max}) and full (SNGFR_{min}) stimulation of tubuloglomerular feedback (TGF), respectively. *P < 0.001 for the cross term from 2-way ANOVA applied to SNGFR_{min}. Data represent 272 tubular fluid collections from 136 nephrons.
duce data from those nephrons for studying proximal reabsorption, the LOH perfusion rate was reduced to allow measurable flow in the proximal tubule. So what can be legitimately claimed about TGF responsiveness is that it remained robust in all four groups.

**Proximal Reabsorption**

The effects of dietary salt and DFMO on proximal reabsorption are displayed in Figs. 2 and 3. Figure 2 makes pairwise comparisons among the experimental groups of net proximal reabsorption (Jprox) against SNGFR. After controlling for differences in SNGFR, either DFMO or dietary salt significantly reduced proximal reabsorption. However, dietary salt had no effect on Jprox if rats were also receiving DFMO. The significance of these findings was confirmed by several independent statistical methods. Multiple methods were used to mitigate the consequences should the data violate a core assumption of one or another of them. It turned out that all of the methods unambiguously supported the same conclusions: first, ANCOVA was performed for data with SNGFR as covariate. This test confirmed an inhibitory effect of dietary salt on proximal reabsorption:equation 1

\[
\text{GTB efficiency} = \frac{\Delta J_{\text{prox}}}{\Delta \text{SNGFR}} \left( \frac{J_{\text{prox}}}{\text{SNGFR}} \right)^{-1}
\]

where \( \Delta J_{\text{prox}} \) and \( \Delta \text{SNGFR} \) are corresponding differences between the two levels of TGF activation in a nephron and \( J_{\text{prox}}/\text{SNGFR} \) is the fractional reabsorption at the midpoint of this range. This index of GTB efficiency will equal unity if fractional reabsorption is constant over the physiologic range of SNGFR and will be zero if \( J_{\text{prox}} \) is independent of SNGFR. As flow in the proximal tubule approaches zero, the residence time of a sodium ion in the proximal tubule becomes infinite and its probability of being reabsorbed approaches unity. Conversely, as flow in the tubule approaches infinity, the residence time of a sodium ion in the proximal tubule approaches zero and so must its probability of being reabsorbed. Hence, GTB efficiency is expected to reside somewhere between zero and unity. Measurement error for GTB efficiency in a given nephron is amplified when \( \Delta \text{SNGFR} \) is small, thus contributing outliers that may unduly influence the statistics. As a demonstration of this, kurtosis (a measure of the degree to which outliers cause a distribution to be non-Gaussian) for the overall distribution of GTB efficiency was reduced by 90% when 10 out of 136 nephrons were dropped from the analysis based on squared-Studentized residual exceeding unity. The median range of the TGF response for those nephrons was only 4 nl/min, whereas the median range of the TGF response for the remaining nephrons was 17 nl/min. Results are shown in Table 1. Overall, GTB was ~70% efficient at sustaining the fractional reabsorption over the physiologic range of SNGFR.
as represented by the extremes of TGF activity. Commensurate with prior observations in diabetic rats, high salt diet (20) or DFMO (15) tended to lessen GTB efficiency, although no pairwise difference was statistically significant after adjustment for multiple comparisons. DFMO reversed the normal effect of dietary salt on GTB efficiency ($P = 0.06$ for the ANOVA cross term). Differences in GTB efficiency were not explained by differences in SNGFR. In fact, the strength of the DFMO*Salt interaction was augmented ($P = 0.03$) when SNGFR$_{mid}$ was made a covariate. This implies that GTB efficiency was not merely improved by raising SNGFR.

VLP

In open-loop micropuncture, the natural VLP cannot be known precisely because normal TGF is interrupted in the course of collecting the proximal tubular fluid. The dual collection method presently employed provides a broad range of possible VLP in each nephron as depicted by the boxes in Fig. 4. But it has been shown that an average diabetic nephron operates near its TGF midpoint (19). In previous studies, we used a noninvasive optical technique to localize VLP relative to the TGF midpoint under various conditions (13). Even in conditions as radical as acute 3% BW isoncotic plasma volume expansion (13), acute 50% increase imposed on early proximal flow (14), and acute suppression of proximal reabsorption by 50% (18), TGF reset within 60 min so that ambient VLP returned to within 1–2 nl/min of the TGF midpoint. Hence, changes in VLP$_{mid}$ likely reflect similar changes in natural VLP, where we define VLP$_{mid}$ as the average of the late proximal collections during minimal and maximal TGF activation. In placebo- or DFMO-treated rats, high salt diet caused VLP$_{mid}$ to increase by 2–3 nl/min ($P = 0.02$). A 1 nl/min average increase due to DFMO was not significant.

In DFMO-treated diabetic rats, high salt caused SNGFR$_{mid}$ and VLP$_{mid}$ to increase in parallel in a ratio of 0.68, which is almost exactly the ratio (0.65) predicted for 70% GTB efficiency and quite close to the ratio (0.60) calculated from data recently published to describe effects of high salt diet in nondiabetic Wistar rats (16). Hence, the impact of dietary salt on VLP in the DFMO-treated diabetic rat appears to be commensurate with the impact of dietary salt on VLP in normal rats without diabetes. In contrast, a similar increase in VLP among placebo-treated rats on high salt occurred as SNGFR declined, reflecting a primary decrease in proximal reabsorption.

DISCUSSION

The current study reveals that blocking the ODC-polyamine pathway prevents those previously described strange effects of dietary salt on kidney function in early diabetes referred to as the salt paradox. This is the main new finding. In the following paragraphs, we will delve into the history and the logic of this.
A reciprocal, or paradoxical, relationship between salt intake and GFR has been repeatedly demonstrated in both humans (1, 3) and rats (20, 21, 23) with early diabetes. In seeking to identify the mechanism for this, we used a theoretical construct for the control of GFR. The construct parses all inputs to GFR into two categories: tubular or vascular. By deduction, we then isolated the source of the salt paradox to the tubule segments upstream from the macula densa and confirmed this experimentally (reviewed in Ref. 17). The construct accommodates all known (or yet-to-be-described) feedback mechanisms for connecting GFR to the total body salt and its only assumptions are that there are no hormonal or biochemical pathways unique to diabetes and that diabetes does not alter the fundamental action of any nerve or hormone (for example, diabetes does not convert angiotensin II to a vasodilator). A version of the construct is included in the APPENDIX, wherein is derived a single equation for GFR as a function of dietary salt. This expression defines the conditions that are necessary and sufficient for a salt paradox to occur. The model also explains how GFR, proximal reabsorption, and total body salt become conspicuous nondeterminants of VLP in early diabetes, if distal GFR is constrained by osmotic diuresis.

A distinguishing feature of the salt paradox is that it implies a positive feedback relationship between GFR and the total body salt; to wit, more salt begets lower GFR which begets more salt. But positive feedback is inherently destabilizing and uncommon in body fluid physiology. While there are many parallel and interconnected feedback loops that include both GFR and the total body salt, there is only one of these that can provide the positive feedback necessary to drive the salt paradox. That particular feedback loop combines the inhibitory effect of total body salt on proximal reabsorption with the ensuing TGF response to increased distal delivery.

A further requirement for the salt paradox to occur is that the positive gain of this particular feedback loop must outweigh the sum of all those negative feedback loops with which it competes for control of GFR. In other words, salt signaling through the macula densa must become the overriding influence on GFR. There are only two ways this could occur: 1) the TGF response to a given change in macula densa salt must become more vigorous relative to the sum of all other controlling inputs to GFR or 2) a given change in salt intake must have a greater impact on NaCl reabsorption upstream from the macula densa. In previous studies, we quantified the incremental gain of the TGF response to a unit disturbance in tubular flow and found it to be dampened, rather than increased, by diabetes (19). This doesn’t fully eliminate the first option, since TGF could become dominant if all other mechanisms for constricting the afferent arteriole were paralyzed while TGF is spared, but this seems unlikely. We subsequently discovered that diabetes confers a strong inverse dependence of proximal reabsorption on the dietary salt intake. This leads to a large error signal in the macula densa delivery consistent with the second option, which is that the salt paradox results from heightened sensitivity of the proximal tubule to changes in salt intake (20).

The current task is to explain something of the mechanism whereby diabetes causes the proximal tubule to become more sensitive to dietary salt. The present data reveal that ODC is essential for this. Should one have suspected this in the first place? One basis for suspicion is that early diabetic kidney growth, hyperfiltration, and hyperreabsorption all happen to depend on ODC (2, 8, 15), so perhaps the salt paradox does too? A stronger argument for tying ODC to the salt paradox begins by considering the salt balance. Under all conditions, a given increment in salt intake will eventually lead to an equivalent increase in salt excretion, which can only come about through increased GFR, decreased reabsorption upstream from the macula densa, and/or decreased reabsorption downstream from the macula densa. If the GFR fails to increase (or actually decreases as in the salt paradox), then it is left to the nephron segments to generate the exact decrease in overall reabsorption required for the inevitable salt balance. All else remaining equal, the net contribution of a given nephron segment to achieving the salt balance will vary along with the fraction of the overall reabsptive machinery allocated to that segment. In early diabetes, there is disproportionately high reabsorption upstream from the macula densa (9, 15, 22) and reabsorption upstream from the macula densa assumes a disproportionate role in salt balance (20). Hence, it was reasonable to suspect that the salt paradox, which arises due to the disproportionate role of the diabetic proximal tubule in salt balance, would disappear if the proximal tubule were prevented from assuming that role, and it was previously shown that blocking ODC slows growth of the diabetic tubule and diminishes its influence (15). The alternative explanation, that DFMO might impact the proximal tubule independent of diabetes, is discounted based on prior experiments in nondiabetic rats in which DFMO had no discernable effect (15).

Since hyperglycemia promotes diabetic kidney hypertrophy by an unknown mechanism (12) and stimulates proximal reabsorption via sodium-glucose cotransport (22, 24), efforts were made to equalize blood glucose concentrations among the four experimental groups from the onset of diabetes. To accomplish this, DFMO-treated animals wound up receiving more insulin along the way, in spite of which they had higher blood glucose levels at the time of micropuncture. This raises concern that the salt paradox could have been eliminated in these studies due to confounding effects of insulin and glucose. It is clear that DFMO did not cause the diabetes to be milder, so DFMO could not have eliminated the salt paradox by causing milder diabetes. Furthermore, there was no correlation between dietary salt and insulin dosing, which reduces potential for a confounding influence of insulin on the response to dietary salt. Finally, confounding by blood glucose proved not to account for the apparent effects of DFMO on glomerular filtration or proximal tubular function according to ANCOVA.

The tubular hypothesis of early diabetes invokes negative feedback to make SNGFR subservient to proximal reabsorption. Hence, when proximal reabsorption is high, as it is on a standard diet, there is hyperfiltration; when proximal reabsorption plummets, as it does on a high salt diet, SNGFR declines; and when DFMO prevents the tubule from growing, hyperfiltration fails to emerge. It is assumed, without proof, that this negative feedback from tubule to glomerulus is routed through the macula densa. However, this cannot be explained by the same TGF process that operates from minute-to-minute. For example, a minute-to-minute TGF-mediated increase in SNGFR will shift the TGF operating point leftward toward the shoulder of the TGF curve, whereas TGF actually operates near its inflection point in diabetes (19). Furthermore, the TGF signal is disrupted whenever SNGFR is measured from the
proximal tubule, yet proximal tubular micropuncture has been used many times to demonstrate diabetic hyperfiltration. Finally, diabetic hyperfiltration was reported in the adenosine A1 receptor knockout mouse, which lacks a normal TGF system (11). Still, the present data add to a remarkable list of documented situations in which spontaneous or manipulated changes in proximal reabsorption are too great to be caused by associated changes in SNGFR, but could cause those changes in SNGFR.

All else remaining equal, the heightened sensitivity of diabetic proximal tubule to changes in salt intake will shorten the lag time required to restore salt balance, thereby conferring more efficient salt homeostasis. But to leverage this opportunity, the diabetic kidney must reset its TGF response rightward to accommodate increases in distal delivery in response to a greater salt intake. In fact, rightward resetting of TGF is the normal response to a sustained increase in late proximal flow (14, 18). In addition, the high salt- and placebo-treated diabetic kidney transduces a major reduction in proximal reabsorption into a TGF-mediated decline in SNGFR. In the end, this kidney manifests roughly the same increase in VLP when placed on a high salt diet as does the DFMO-treated kidney, or the nondiabetic kidney (16) in which the proximal tubule is insensitive to dietary salt.

It is notable that the high salt diet increased VLP to a nearly identical extent in placebo- and DFMO-treated diabetic animals, but by entirely different mechanisms. This suggests an efficient internal control over VLP that succeeds to the same degree regardless of how the proximal tubule performs. But one can prove, by syllogism, that no special “design” or degree regardless of how the proximal tubule performs. The solution for TBS is a decaying exponential with time constant, \( k \), that approaches a new steady state where

\[
\frac{dTBS}{dt} + k \cdot TBS = DS
\]

\[
k = \frac{\gamma(\alpha G + \beta)}{1 + GT}
\]

DS is a change in dietary salt, \( \alpha \) and \( \beta \) are direct effects of TBS on GFR and proximal reabsorption, G is GTB in the proximal tubule, T is the TGF response, and \( \gamma \) is GTB downstream from the late proximal nephron. Notably absent are any assumptions about the glomerular and proximal tubule. Details are provided in the Appendix.

The present data don’t provide much detail as to how lessening growth of the diabetic proximal tubule eliminates its hyperresponsiveness to dietary salt. Growth of the diabetic proximal tubule involves hyperplasia, then hypertrophy (10). ODC is mainly elevated during the hyperplastic phase (2, 7), so it may be that DFMO eliminates salt sensitivity because the salt sensitivity resides in new cells. But it is hard to imagine how the nerves and hormones that communicate changes in total body salt to the proximal tubule could be more tightly connected to a newly divided cell than to one that is established and merely hypertrophic. Moreover, we previously showed that the salt paradox cannot be explained by differences in renal nerve activity (1) and we observed that the tonic influence of angiotensin II over proximal reabsorption is already diminished in early diabetes (S. C. Thomson, unpublished studies), such that further suppression by high salt diet is not a viable explanation for the tubular sensitivity to salt. The present findings indicate that kidney hypertrophy is necessary for the salt paradox, but they don’t address whether kidney hypertrophy is sufficient to cause a salt paradox. In fact, we recently confirmed that the salt paradox does not occur in compensatory hypertrophy, where changes in proximal reabsorption were fully explained by GTB (4).

In summary, we showed that ODC-mediated growth is necessary for hyperresponsiveness of the proximal tubule to dietary salt and for the paradoxical effect of dietary salt on GFR in early diabetes. This coupling of ODC to tubular salt sensitivity and to the salt paradox adds to the body of evidence that feedback from the proximal tubule is the principal governor of glomerular filtration in early diabetes.

APPENDIX

Figure 5 contains a linear systems model for salt balance. The model implies a linear first-order differential equation for changes in total body salt (TBS)

\[
\frac{dTBS}{dt} + k \cdot TBS = DS
\]

\[
k = \frac{\gamma(\alpha G + \beta)}{1 + GT}
\]

TBS is a change in dietary salt, \( \alpha \) and \( \beta \) are direct effects of TBS on GFR and proximal reabsorption, G is GTB in the proximal tubule, T is the TGF response, and \( \gamma \) is GTB downstream from the late proximal nephron.

The solution for TBS is a decaying exponential with time constant, \( k \), that approaches a new steady state where

\[
TBS = DS \frac{1 + GT}{\gamma(\alpha G + \beta)}
\]

\[
VLP = DS \frac{1}{\gamma}
\]

\[
GFR = DS \frac{\alpha - \beta T}{\gamma(\alpha G + \beta)}
\]

There are several features of this that are noteworthy. First, the salt paradox will occur if and only if \( \beta \) > \( \alpha \). In diabetes, the salt paradox occurs due to large \( \beta \), which corresponds to the 6 nl/min downward shift in the high salt curve (Fig. 2, middle). When \( \beta \) is reduced to zero by DFMO, as indicated by near superposition of GTB curves in Fig. 2, right, the salt paradox disappears.

Second, TGF is “anti-homeostatic” for TBS, which becomes more sensitive to DS as the strength of the TGF response (T) increases. It is the function of TGF to stabilize GFR and VLP and to the degree

Fig. 5. System model for response to a change in DS. DS initiates a change in total body salt (TBS), which affects GFR (\( \alpha \)) and \( J_{\text{prox}} \) (\( \beta \)). Change in VLP is sum of inputs from proximal glomerulotubular balance (G) and \( \beta \). GFR is stabilized by TGF (-\( T \)). VLP affects salt excretion via glomerulotubular balance downstream from the late proximal tubule (\( \gamma \)). The overdot signifies a time derivative. The model parameters \( \alpha \), \( \beta \), \( \gamma \), G, and T are all positive. The variables TBS, GFR, and VLP represent changes from the prior equilibrium state.
that VLP is stabilized by TGF, it is prevented from facilitating the salt balance. Finally, the new steady-state VLP following a given change in DS depends solely on $\gamma$. In other words, VLP is independent of GFR and TBS. It is this relationship that allows us to explain why DFMO does not alter the effect of DS on VLP despite disparate effects on SNGFR and proximal reabsorption. At first, it seems counterintuitive that a deterministic expression for VLP would not contain $\alpha, \beta, G$, and $T$ since these parameters, along with the variables GFR and TBS, are the physical determinants of VLP. But there is only one value for VLP, $VLP = DS/\gamma$, at which the salt excretion matches the salt intake. This makes DS and $\gamma$ the sole determinants of steady-state VLP. Therefore, without knowing anything about the physiological parameters $\alpha, \beta, G$, or $T$, one can predict that a given DS will cause TBS and GFR to change in some combination that yields the identical VLP, which is determined by $\gamma$. Conversely, if a given DS yields the same VLP in two animals, one may conclude that $\gamma$ is the same in those animals. Hence, it is not an unlikely coincidence that DFMO markedly alters $\gamma$ without changing the ultimate effect of DS on VLP. In fact, the conserved effect of salt on VLP is the only possible outcome if DFMO does not alter GTB in the distal nephron. Furthermore, the fact that DFMO did not alter the effect of DS on VLP implies that it does not affect GTB in the distal nephron. It is likely that $\gamma$ is fixed at a high value in diabetes due to osmotic effects of glucose in the collecting duct. A typical hyperphagic diabetic rat on standard rat chow will eat (and excrete) 5 mmol Na and 150 ml water per day (S. C. Thomson, unpublished studies). The same diabetic rat on standard rat chow will eat (and excrete) 5 mmol Na and 5 mmol K per day to remain in balance. The rat has 60,000 nephrons. If the late proximal flow increases from 10 to 12 ml/min per nephron (as it presently did with high salt diet, irrespective of DFMO), then the total amount of Na delivered beyond the proximal tubule will increase from 130 to 156 mmol/day. This increase of 26 mmol/day delivered out of the proximal tubule is nearly identical to the 25 mmol increase in Na excretion required for sodium balance. In other words, there appears to be no net effect of DS or DFMO on sodium reabsorption down-stream from the late proximal tubule in diabetes. It is likely that the high flux of water and glucose osmoles renders the distal nephron unable to compensate for changes in DS, thus relegating inevitable salt balance to the glomerulus and proximal tubule.

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REFERENCES