Unexpected effect of angiotensin AT₁ receptor blockade on tubuloglomerular feedback in early subtotal nephrectomy

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Singh P, Deng A, Blantz RC, Thomson SC. Unexpected effect of angiotensin AT₁ receptor blocker losartan on tubuloglomerular feedback in early subtotal nephrectomy. Am J Physiol Renal Physiol 296: F1158–F1165, 2009. First published February 11, 2009; doi:10.1152/ajprenal.90722.2008.—After subtotal nephrectomy (STN), the remaining nephrons engage in hyperfiltration, which may be facilitated by a reduced sensitivity of the tubuloglomerular feedback (TGF) response to increased distal delivery. However, a muted TGF response would contradict the notion of remnant kidney as a prototype of angiotensin II (ANG II) excess, since ANG II normally sensitizes the TGF response and stimulates proximal reabsorption. We examined the role of ANG II as a modulator of TGF and proximal reabsorption in 7 days after STN in male rats. Single-nephron glomerular filtration rate (SNGFR) and proximal reabsorption (J_prox) were measured in late proximal collections while perfusing Henle’s loop for minimal and maximal TGF stimulation in rats treated with the angiotensin type 1 (AT₁) receptor blocker losartan or placebo in drinking water for 7 days. Perfusion of Henle’s loop yielded a robust TGF response in sham-operated rats. In STN, the feedback responses were highly variable and nil, on average. Paradoxical TGF responses to augmented late proximal flow were confirmed in SNGFR measurements from the early distal nephron. Chronic losartan treatment normalized the average TGF response without reducing the variabil-
ity. J_prox was subtly affected by chronic losartan in sham surgery or STN, after controlling for differences in SNGFR. However, when administered acutely into the early S1 segment, losartan potently suppressed J_prox in STN and sham-operated rats alike. Chronic losar-
tan stabilizes the TGF system in remnant kidneys. This cannot be explained by currently known actions of AT₁ receptors but is commensurate with a salutary effect of an intact TGF system on dynamic autoregulation of intraglomerular flow and pressure.

proximal tubular reabsorption; glomerulotubular balance; hyperfiltration

REDUCING THE NUMBER OF NEPHRONS evokes a response from those that remain (3). One early and notable feature of this response is an increase in single-nephron glomerular filtration rate (SNGFR). From the body’s standpoint this increase in SNGFR is adaptive, since it compensates for lost filtration. But from the nephron’s standpoint this increase in SNGFR can be maladaptive, since hyperfiltration imposes physical stress on the glomerulus and metabolic stress on the tubule. The latter results from the work required of each remaining nephron to reabsorb most of its increase in filtered sodium, which is necessary for short-term survival. Without this glomerulotubular balance (GTB) the compensatory increase in SNGFR would cause rapidly fatal salt depletion.

The mechanism for sensing a loss of nephrons and evoking compensation from the remainder remains a mystery, but the possible explanations can be classified based on a general understanding of how SNGFR is regulated. When a signal arises outside of the kidney for SNGFR to increase, the influence of that signal is buffered by tubuloglomerular feedback (TGF), which operates within the nephron to prevent SNGFR from changing. To the extent that compensatory hyperfiltration depends on signals from outside the kidney, the decline in overall GFR after loss of nephrons could be mitigated by suppressing TGF and allowing greater leverage to the outside signal. Since TGF operates normally in a single kidney after unilateral nephrectomy, TGF suppression does not appear to be the mechanism of first choice for causing compensatory hyperfiltration. Here, we examined TGF responses 1 wk after subtotal nephrectomy (STN) in the rat, reasoning that TGF suppression might be invoked to facilitate compensatory hyperfiltration when nephron loss is more extreme. We also examined the extent to which chronic GTB in STN mimics that which normally occurs when the filtered load changes from minute to minute.

Intrarenal angiotensin II (ANG II) is a known modulator of TGF activity that normally reinforces the TGF response (12, 20, 21). We analyzed the effects of continuous angiotensin type 1 receptor (AT₁R) blockade beginning at the time of STN to test the hypothesis that AT₁R blockade would further destabilize SNGFR. We found TGF responses in STN to be diminished, on average, and highly variable. Contrary to expectation, AT₁R blockade normalized the TGF response in STN. Remnant kidney is considered a prototype of ANG II excess (10). Assuming that the kidney benefits from autoregulation normally conferred by TGF, the notion that TGF would be made less responsive by angiotensin blockade is incongruous with the overall salutary effect of angiotensin blockade on the remnant kidney (1, 2, 7, 17).

METHODS

All experimentation was conducted according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Protocols were approved in advance by the Department of Veterans Affairs San Diego Healthcare System Institutional Animal Care and Use Committee. Effects of STN and chronic AT₁R blockade on TGF responses and proximal reabsorption were studied in male Wistar rats (Harlan). The acute effect of AT₁R blocker on proximal reabsorption and the effect of STN on SNGFR as measured from the early distal tubule were tested in Munich-Wistar-Froemter rats, which have surface glomeruli suitable for these purposes. Munich-Wistar-Froemter rats were locally bred in the Veterinary Medical Unit at the Department of Veterans Affairs Medical Center (San Diego, CA). All rats received free access to tap water and standard rat chow. Body weight and water intake were monitored daily from the time of
nephrectomy or sham surgery in all animals and food intake in three of five or six animals in each group. Chronic AT,R blockade was achieved by adding 200 mg/l losartan to the drinking water. Placebo control rats were given a water bottle with no losartan. Acute AT,R blockade was achieved by microperfusing the early S1 segment with 20 mM losartan in artificial tubular fluid (ATF). Losartan was provided as a gift by Merck. Micropuncture experiments were conducted 7 or 8 days after STN or sham surgery.

Subtotal Nephrectomy

This procedure was performed with sterile technique on a temperature-controlled surgical table and under anesthesia with pentobarbital sodium (50 mg/kg ip). A small right flank incision (1.5 cm long) was made. The muscle was clamped for 1 min before cutting to prevent bleeding. The adrenal gland was carefully separated from the right kidney. The exposed right kidney was ligated with 4-0 silk suture tied tightly around the renal artery and vein. The right kidney was then cut from the vasculature and removed. The adrenal gland and attached vascular tissues were returned to the retroperitoneum. The fascia was closed with silk suture and the skin with steel wound clips. A left flank incision was then made and the left kidney maneuvered to expose the renal artery. Two branches of left renal artery were ligated with 4-0 silk suture. The kidney was replaced back into the body, and the incision was closed as above. Sham-operated rats underwent anesthesia and manipulation of the renal pedicles. Rats were kept warm with a heating pad until ambulatory and administered a dose of buprenorphine analgesic.

Surgical Preparation for Renal Micropuncture

Animals were surgically prepared according to previously established protocols (26). Briefly, animals were anesthetized with Inactin (100 mg/kg ip) and body temperature was maintained at 37°C. After tracheostomy (PE-240), catheters (PE-50) were placed in the jugular vein, femoral artery, and urinary bladder. The left kidney was exposed through a 7- to 9-mm-tip pipette connected to a nanoliter perfusion pump (University of Tuebingen). For orthograde perfusion of Henle’s loop from the late proximal tubule, ATF contained (mM) 130 NaCl, 10 NaHCO3, 4 KCl, 2 CaCl2, and 5 glucose, with 30 mg% urea, 1% FD&C, pH 7.4. For perfusion of early proximal tubules, ATF contained (mM) 130 NaCl, 25 NaHCO3, 4 KCl, 2 CaCl2, and 5 glucose, with 30 mg% urea and 0.1% FD&C green, pH 7.4. For perfusion of late proximal tubules, ATF contained (mM) 130 NaCl, 25 NaHCO3, 4 KCl, 2 CaCl2, and 5 glucose, with 30 mg% urea and 0.1% FD&C green, pH 7.4. Tubular localization was carried out with a micropipette (3- to 5-μm tip) containing Ringer saline stained lightly with FD&C green. When necessary to interrupt tubular flow along the nephron, a wax block was injected into the tubule at that point.

Micropuncture Protocols

SNGFR, TGF responsiveness, and proximal GTB. Micropuncture experiments were performed to assess the impact of STN with or without chronic AT,R blockade on SNGFR and TGF responsiveness and on GTB in the proximal tubule. Animals were prepared for micropuncture as described above. After 1 h for equilibration, late proximal nephrons 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 ATF was inserted downstream from the wax block to perfuse the loop of Henle. Controlled perfusion of the loop of Henle was performed to activate TGF and thereby cause SNGFR to change. During perfusion, timed collections of tubular fluid were made upstream from the wax block in order to measure SNGFR and late proximal flow (VLP). Collections were made from each nephron during minimal TGF activation (loop of Henle microperfusion at 0 nl/min) and maximal TGF activation (loop of Henle microperfusion at 35 nl/min in sham-operated rats and ≥50 nl/min in STN). Several nephrons were studied in each animal, and the two perfusions per nephron were done in alternating order. Nephrons were vented upstream from the wax block before each collection to prevent pressure from building up in the proximal tubule. Two minutes was allowed for equilibration before each collection, and each collection was for 3 min or more. Tubular fluid samples were assayed for volume by transfer to a constant-bore glass capillary and then counted for radioactivity to determine SNGFR. Data from these paired collections were exploited to 1) determine SNGFR at both extremes of TGF activation, which gives the range for the TGF response, and 2) characterize proximal reabsorption as a function of SNGFR as a method of testing the efficiency of GTB and for revealing primary effects of STN with and without losartan on proximal reabsorption. Justification for this method of analyzing proximal reabsorption has been developed in previous publications (11, 26, 29).

Distal SNGFR and TGF operating point. Micropuncture experiments were performed to establish the orientation of ambient distal SNGFR (SNGFRd) relative to the TGF curve. SNGFRd was measured twice in each nephron, once to establish ambient SNGFRd and once to establish SNGFR at the elbow of the TGF curve. The latter was accomplished by using a nanoliter pump with ATF to augment flow in the late proximal tubule (VLP) by 10–15 nl/min. These experiments were done in Munich-Wistar-Froemter rats, in which early distal nephrons are readily identified by proximity to their parent glomerulus, into which a small pulse of dye-stained ATF was injected to delineate the anatomy.

Acute AT,R blockade and proximal reabsorption. The tonic influence of AT,R on proximal reabsorption was tested by perfusing early proximal tubules with ATF containing losartan. This was undertaken after the results of the experiments described above failed to confirm a primary diuretic effect of chronic losartan on the proximal tubule. A small bolus of FD&C green-stained ATF was delivered into Bowman’s space of a surface glomerulus and allowed to flow downstream to identify early and late proximal tubule. A wax block was then placed in Bowman’s space to interrupt the flow of glomerular filtrate into the proximal tubule. ATF containing 6 cpm/nl of [3H]inulin was delivered by nanoliter pump into the early S1 segment at 30 nl/min in STN rats and 20 nl/min in sham-operated rats, reasoning that STN nephrons were acclimated to 50% higher SNGFR than sham nephrons. After 3–5 min for equilibration, a small amount of fluid was sampled from the late proximal tubule. Thereafter, a second perfusion-collection was made with the same ATF or ATF containing 20 mM losartan. Samples were measured for volume and counted for radioactivity. Fractional delivery to the late proximal tubule (FD) was computed as the perfusate-to-collectate inulin concentration ratio. Fractional reabsorption is 1 – FD.

Statistical Analysis

Micropuncture data were analyzed by two-way analysis of variance (ANOVA) with design for repeated measures, analysis of covariance (ANCOVA), or Student’s t-test done with commercial software (Systat, Evanston, IL). Adjustment for multiple group comparisons was by Tukey test as appropriate. Unless stated otherwise, results are presented as group means ± SE as generated by least-squares ANOVA, which pools the variance and redistributes according to sample size. This form of presentation conceals intergroup differences in raw variance, which were computed by the Fmax test (11) and are discussed separately. In addition to ANCOVA a nonlinear logistical model was used to parse the role of GTB from data on proximal reabsorption. ANCOVA assumes that the effect of the covariate, SNGFR, on proximal reabsorption (Jprox) is linear and has the same

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slope in all groups. But there are several faults with this assumption. For example, from the stochastic nature of proximal reabsorption one can deduce that a GTB curve should be concave downward, not linear (23). Also, a GTB curve cannot have a positive y-intercept as do the linear regression formulas obtained from our data. Hence, we applied a nonlinear logistical model for estimating $p_{\text{max}}^*_\text{prox} = A^*\text{SNGFR}^*k$. This equation complies with common sense about GTB, assuming $A$ and $k$ between zero and unity, and it has the same number of parameters (2) as the linear regression, which allows direct comparison with ANCOVA for goodness of fit.

RESULTS

Systemic and Whole Kidney Data

Micropuncture was performed in 21 rats 8 days after STN ($n = 11$) or sham surgery ($n = 10$). Six STN rats and five sham-operated rats received losartan from the time of initial surgery. STN animals drank more water than sham-operated animals. STN animals not treated with losartan tended to lose weight after surgery, while the other three groups all tended to gain weight. Body weight at the time of micropuncture did not differ significantly between groups (Table 1). In losartan-treated animals, persistence of AT1R blockade was confirmed by a lack of blood pressure response to ANG II bolus (100 ng iv) at the end of micropuncture. A 10-fold-lower dose (10 ng) of ANG II increased arterial blood pressure by 10–15 mmHg in placebo-treated rats.

Blood pressure was monitored throughout each micropuncture experiment and recorded at the start of each tubular fluid collection (Table 1). The values obtained for each animal were averaged to give the mean blood pressure for that animal. STN animals not receiving losartan were hypertensive relative to all other groups. Among all STN animals, those chronically receiving losartan had blood pressure that was lower by 50 mmHg ($P < 0.0004$). Losartan also reduced blood pressure in sham-operated rats ($P < 0.002$), although the hypotensive effect of losartan in sham-operated rats was only half of that observed in STN rats. Total GFR was reduced more than two-thirds in STN rats than sham-operated rats ($P < 0.0005$), consistent with a reduction in nephron number of at least two-thirds. GFR tended to be higher in animals receiving losartan, although the effect of losartan was not statistically significant ($P = 0.09$ from 2-way ANOVA for effects of losartan and STN) and was not affected by STN ($P = 0.3$ for STN × losartan cross-term from the same ANOVA). None of these impressions was altered when ANOVA was repeated with body weight as a covariate, although body weight was a weak determinant of GFR ($P = 0.09$).

**Table 1.** Systemic data during micropuncture

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>GFR, ml/min</th>
<th>Urine Flow, ml/min</th>
<th>Urinary Na Excretion, neq/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham ($n = 5$)</td>
<td>311±11</td>
<td>131±3</td>
<td>2.89±0.23</td>
<td>14.26±4.0</td>
<td>552±114</td>
</tr>
<tr>
<td>Sham+Los ($n = 5$)</td>
<td>298±16</td>
<td>112±3</td>
<td>3.5±0.23</td>
<td>18.53±4.0</td>
<td>672±114</td>
</tr>
<tr>
<td>STN ($n = 5$)</td>
<td>275±19</td>
<td>158±10</td>
<td>0.85±0.23</td>
<td>19.27±3.6</td>
<td>472±114</td>
</tr>
<tr>
<td>STN+Los ($n = 6$)</td>
<td>300±9</td>
<td>105±3</td>
<td>1±0.21</td>
<td>19.27±3.6</td>
<td>417+104</td>
</tr>
<tr>
<td>ANOVA table $P$ values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STN</td>
<td></td>
<td></td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Los</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STN×Los</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE for $n$ rats generated from least-squares ANOVA. Sham, sham surgery; STN, subtotal nephrectomy; Los, losartan; GFR, glomerular filtration rate.

**Micropuncture Data**

**SNGFR.** Micropuncture data were derived from 250 collections from 125 nephrons in 21 rats. SNGFR was measured twice from the late proximal tubule in each nephron, once at each extreme of TGF activation. Values obtained in the absence of a TGF signal are denoted SNGFR$\text{max}$. Values obtained during maximum stimulation of TGF are denoted SNGFR$\text{min}$. The maximum TGF stimulus was applied during the first collection in 69 nephrons and during the second collection in 56 nephrons. The order of perfusion was not a significant covariate for SNGFR$\text{max}$ ($P = 0.85$), SNGFR$\text{min}$ ($P = 0.26$), tubular fluid/plasma inulin (TF/Pinulin) during inactive TGF ($P = 0.64$), or TF/Pinulin during maximal TGF activation ($P = 0.68$).

STN essentially doubled both SNGFR$\text{max}$ and SNGFR$\text{min}$ ($P < 0.0005$ for primary effect of STN in 2-way ANOVA). Losartan had no primary effect on SNGFR$\text{max}$ or SNGFR$\text{min}$, nor did it alter the effect of STN on SNGFR$\text{max}$. However, losartan tended to reduce SNGFR$\text{min}$ in STN rats and to increase SNGFR$\text{min}$ in sham-operated rats such that the effect of losartan on SNGFR$\text{min}$ was clearly different between STN and sham-operated rats ($P = 0.002$ for the STN × losartan cross-term from 2-way ANOVA; Table 2).

**TGF responses.** To make sure that the high flow delivered to Henle’s loop was adequate to elicit any physiologically relevant TGF response nephrons expected of hyperfiltration were perfused at a higher rate. Adequacy of perfusion was confirmed after the fact by the difference between the perfusion rate and the resultant late proximal flow. On average, the TGF stimulus exceeded the resultant late proximal flow by 27 nl/min, and this did not differ between groups. In only 6 of 125 nephrons was the excess perfusion <6 nl/min and only 1 of those came from the STN group, which is important for reasons that will become apparent (vide infra).

Activating TGF reduced SNGFR by 50% in sham-operated rats ($P < 0.00005$). TGF responses tended to be less in losartan-treated sham-operated rats, but this difference between losartan- and placebo-treated sham-operated rats was not significant. In STN rats, the mean TGF response was reduced to almost nil ($P < 0.0005$ vs. sham-operated rats by Tukey test), while in STN rats receiving losartan the TGF response was essentially normalized ($P = 0.003$ vs. STN by Tukey test and $P = 0.001$ for the STN × losartan cross-term in 2-way ANOVA). In other words, 1) STN reduced the average TGF response nearly to zero and 2) the TGF response in STN was paradoxically increased to normal by chronic losartan (Table 2).
The effect of STN on the TGF response was not fully characterized by its effect on the average response. STN also caused the TGF response to be highly variable from one nephron to the next. Since the average response was nil, this required SNGFR in some nephrons to respond paradoxically by increasing in response to loop of Henle perfusion. This is made apparent in Fig. 1, which depicts SNGFRmax and SNGFRmin in each nephron and shows them to be reversed in several cases. The mean square error for the TGF response in each group is shown in Fig. 2. The apparent difference in variability was confirmed to be statistically significant (P < 0.05) by the Fmax-test, which assigns a P value based on the ratio of variances and the degrees of freedom (8). TGF responses were almost evenly split between “normal” and “paradoxical” within each STN animal. Losartan also tended to increase TGF variability, although this effect was not significant.

**Distal SNGFR and TGF response.** SNGFRd was measured with and without augmentation of VLP in each of 15 nephrons from STN and 7 nephrons from sham-operated Wistar-Froe-meter rats. Ambient SNGFRd tended to be greater in STN than sham-operated rats [41 ± 4 vs. 31 ± 5, P = not significant (NS)]. Ambient early distal flow rate was greater in STN rats (11.2 ± 1.3 vs. 6.6 ± 2.0, P = 0.06). VLP was augmented by adding 15 nl/min to the free-flowing late proximal tubule in STN rats and 10 nl/min in sham-operated rats. The intent was to determine the orientation of ambient SNGFR relative to the elbow of the TGF curve and to confirm the paradoxical relationship between SNGFR and TGF stimulus noted above for late proximal collections. Among sham-operated nephrons, augmenting VLP caused SNGFRd to decline by 8.2 ± 2.8 nl/min, indicating that the typical sham-operated nephron operates ~8 nl/min above the elbow of its TGF curve. Among STN nephrons the average effect was nil (0.6 ± 2.0 nl/min decline during augmented VLP), with half the nephrons responding paradoxically (P < 0.04 for the difference in TGF response between STN and sham-operated rats). The variance of the SNGFR difference was greater in STN rats [96 vs. 48 (nl/min)2], consistent with observations on the overall range of the TGF response (Fig. 3).

**Proximal reabsorption extracted from GTB.** For the combined 250 proximal tubular fluid collections, Jprox and SNGFR were highly correlated (Pearson correlation coefficient r = 0.88), confirming SNGFR as the principal determinant of Jprox. However, the point of studying Jprox is to test for so-called primary effects on Jprox, which are, by definition, independent of differences in SNGFR. This amounts to

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**Table 2. SNGFR from late proximal collections**

<table>
<thead>
<tr>
<th>Group</th>
<th>SNGFRmax, nl/min</th>
<th>SNGFRmin, nl/min</th>
<th>TGF Response, nl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>STN (n = 31)</td>
<td>58±4</td>
<td>58±4</td>
<td>0.1±2.9</td>
</tr>
<tr>
<td>STN + Los (n = 37)</td>
<td>60±2</td>
<td>45±3</td>
<td>14.9±2.9</td>
</tr>
<tr>
<td>Sham (n = 29)</td>
<td>34±2</td>
<td>17±2</td>
<td>17.1±1.6</td>
</tr>
<tr>
<td>Sham + Los (n = 28)</td>
<td>38±3</td>
<td>24±3</td>
<td>13.2±2.4</td>
</tr>
</tbody>
</table>

ANOVA table P values:

- STN: <0.0005
- Los: 0.004
- STN×Los: 0.002

Values are means ± SE for n rats from raw data. SNGFRmax, single-nephron GFR (SNGFR) in absence of tubuloglomerular feedback (TGF) signal; SNGFRmin, SNGFR during maximum stimulation of TGF.
expressing $J_{\text{prox}}$ as a function of SNGFR for each group to obtain its GTB curve and comparing the GTB curves between groups. This is shown graphically in Fig. 4, where $J_{\text{prox}}$ is plotted as a function of SNGFR with separate plots for each of the three relevant intergroup comparisons, sham surgery vs. STN (Fig. 4A), sham surgery vs. sham surgery + losartan (Fig. 4B), and STN vs. STN + losartan (Fig. 4C). The statistics of $J_{\text{prox}}$ were analyzed both by ANCOVA and by a nonlinear regression, which revealed some minor, but statistically significant, primary differences in proximal reabsorption.

First, we performed ANCOVA on all 250 tubular fluid collections with STN and losartan as categorical variables and SNGFR as covariate. Denoting $J_{\text{prox}}^*$ as the adjusted value of proximal reabsorption after correcting for SNGFR by ANCOVA, STN reduced $J_{\text{prox}}^*$ by 10% ($P < 0.01$), while chronic treatment with losartan caused $J_{\text{prox}}^*$ to change by $<$2% (NS). Repeating the ANCOVA with TGF stimulus as a third categorical variable revealed a 10% primary increase $J_{\text{prox}}^*$ during TGF activation ($P < 0.01$) and did not alter the impression regarding STN and losartan. There was no hint of any significant interactions among STN, losartan, and the applied TGF stimulus with respect to their influence on $J_{\text{prox}}^*$.

Fitting the nonlinear model, $J_{\text{prox}}^* = A*\text{SNGFR}^k$, to the 250-point data set yielded $A = 0.73 \pm 0.11$ and $k = 0.86 \pm 0.04$, with a goodness of fit that was equivalent to the linear regression ($r = 0.8$), observed vs. predicted for either method. Notably, $k$ was significantly less than unity ($P < 0.001$), as it should be for realistic, concave downward, GTB. Comparing the measured values of $J_{\text{prox}}$ to those predicted by this nonlinear model upheld the impressions of the original ANCOVA, namely, that STN leads to a slight reduction in $J_{\text{prox}}^*$ ($P = 0.03$), while losartan has no effect. We also applied the nonlinear regression formula to the individual groups (Fig. 4). The exponents $k$ were significantly less than unity in all groups except for STN ($P < 0.01$ vs. all other groups). This means that those STN nephrons with the lowest SNGFRs contributed more to the average $J_{\text{prox}}^*$ being lower.

Effects of STN and losartan on late proximal flow (VLP) are shown in Fig. 5. In proximal tubular micropuncture, the natural VLP is indeterminate because TGF is interrupted in the course of collecting the tubular fluid. But using other methods, we have shown that TGF tends to operate near its inflection point (24) and VLP at the inflection point is obtained by averaging VLP at the two extremes of TGF activity. This was done by applying ANOVA to the entire data set of VLP, in which both extremes were represented for each nephron. STN had the expected unequivocal effect of increasing midpoint VLP. Losartan tended to increase VLP in sham-operated rats and to decrease VLP in STN rats, with the ANOVA cross-term being statistically significant ($P < 0.03$).

Acute effects of losartan on $J_{\text{prox}}$. The weak effect of chronic losartan on $J_{\text{prox}}$ is at variance with our prior experience with acute losartan as a potent proximal diuretic (27). To sort out the nature of this discrepancy, we measured the tonic influence of proximal tubular ANG II in STN by acutely delivering losartan to early S1 segments (Fig. 6). Each nephron was its own control. Comparison of the placebo-losartan sequence was made to placebo-placebo time controls. Losartan administered directly into the early proximal tubule reduced fractional reabsorption by $\sim 30\%$ ($P < 0.01$ vs. time controls). The effect of losartan tended to be greater in sham-operated nephrons, but the difference was not significant. These effects of locally administered losartan on $J_{\text{prox}}$ are commensurate with the acute response to systemic losartan previously observed in hydropenic rats (27).

**DISCUSSION**

We examined the remnant kidney during its early compensatory stage, before glomerular and tubular damage, and obtained four unexpected results. First, the maximal impact on SNGFR of TGF signaling from the macula densa (MD) was highly variable and diminished overall. Second, the average TGF response was normalized by chronic exposure to losartan. Third, the tonic influence of ANG II over proximal reabsorption was unaffected by STN. Fourth, irrespective of nephron number, the inhibitory effect of AT1R blockade on proximal reabsorption disappeared with prolonged exposure. In the following paragraphs we discuss these findings one at a time.

**STN and Diminished Net TGF Response**

Others have studied TGF responsiveness in early STN by using tubular stop-flow pressure ($P_{SF}$) as an index of glo-
merular capillary hydrostatic pressure (P_CT) and reported PSF responses that are right-shifted but otherwise intact (18, 30). Our direct measurements of SNGFR appear not to align with the literature on PSF responses inasmuch as the average SNGFR response to a maximum TGF stimulus was reduced to zero. This may be the result of efferent arteriole TGF as described in the literature, whereby the TGF mediator adenosine dilates the efferent arteriole after it constricts the afferent arteriole (15, 16). Dilating the efferent arteriole in isolation will always reduce PSF but may cause SNGFR to increase, decrease, or stay the same, depending on the prior ratio of afferent/efferent resistances (6).

Fig. 5. VLP as function of SNGFR. Values obtained are means(SE) generated by 2-way ANOVA applied to 250 late proximal collections and represent VLP at the TGF midpoint. The effect of losartan differed between sham-operated and STN rats (*P < 0.03 for cross-term from ANOVA).

In the present studies, the TGF response was not simply reduced in STN, it also became significantly more variable (P < 0.05). Heightened variability has been seen before in the remnant kidney, though not quantified (18, 30). Increased variability in the TGF response could reflect one or more of the following processes: 1) TGF that is relatively stable in a given nephron but heterogeneous between nephrons; 2) time-varying TGF responsiveness within individual nephrons, with different nephrons caught at different phases of the cycle; and 3) time-varying SNGFR over periods of 3–8 min that is independent of TGF and causes TGF to be measured against a wandering background.

The appearance of a more variable TGF response might result from time-varying SNGFR over periods of 3–8 min (about the time it takes from the end of the first to the end of the second collection in a nephron) that is independent of TGF such that SNGFR_{max} and SNGFR_{min} were measured against a wandering background.

As for internephron heterogeneity of TGF, when faced with a prolonged stimulus juxtaglomerular apparatus normally recalibrates TGF so that nephrons resume operating where TGF is steep. This process, which lessens the heterogeneity of TGF responses, is referred to as TGF resetting (22, 25, 28). There is some evidence of impaired resetting of TGF in STN taking form as a high proximal-distal (P-D) difference in SNGFR (14, 30). Since the P-D difference will increase as the TGF operating point moves out along the TGF curve, a failure of normal rightward TGF resetting is one cause of an elevated P-D difference.

There is precedent for time-varying renal autoregulatory mechanisms being detected at the whole kidney level and for these properties to differ between hypertensive and normotensive rat strains (4, 31). It is possible that our findings of increased TGF variability are the result of this and that we caught different nephrons at different phases of their cycle.

The appearance of a more variable TGF response might result from time-varying SNGFR over periods of 3–8 min (about the time it takes from the end of the first to the end of the second collection in a nephron) that is independent of TGF such that SNGFR_{max} and SNGFR_{min} were measured against a wandering background.

In addition to being more variable, the TGF response in several STN nephrons was frankly anomalous. This could be explained by opposing effects of MD salt that operate in parallel. For example, MD salt initiates both adenone A_{1} receptor-mediated vasoconstriction via apical Na^{+}-K^{+}-2Cl^{-} cotransporter (NKCC)2 (19) and nitric oxide-mediated vasodilation via apical Na^{+}/H^{+} exchanger (NHE)2 (9). While the latter never appears to dominate in normal physiology, this may be intrinsically possible if the relative strengths of these opposing effects are somehow altered in remnant kidneys. Also, the aforementioned TGF-mediated vasodilation of efferent arteriole by adenosine A_{2} receptors can be invoked to explain the paradoxical response since the SNGFR can increase or decrease depending on the relative afferent and efferent resistances (6).
TGF Normalized by Chronic AT\(_1\) Blockade

The average TGF response remained normal in STN rats treated chronically with losartan. This was unexpected, since ANG II normally operates in the background to enhance the TGF response and blocking AT\(_1\)R normally subdues the TGF response (12, 20, 21). We can think of no way to explain this based on known immediate actions of ANG II on the juxtaglomerular apparatus or vascular function. Hence, it is likely that a "trophic" effect of ANG II is involved. While losartan normalized the mean TGF response, it did not reduce the variability of that response. In fact, losartan tended to increase variability of the TGF response in both STN and sham-operated rats, consistent with the main role of intrarenal ANG II being to stabilize nephron function while systemic ANG II stabilizes the total body salt (27).

AT\(_1\) Receptors, Proximal Reabsorption, and Long-Term GTB

We have previously shown (27) systemic losartan to be a potent proximal diuretic when administered acutely. Others have reported the same result (5). The present results suggest that this effect wears down over time. This may simply reflect the inevitability of long-term salt balance, which is an added boundary condition for all chronic experiments. The kidney has latitude to accomplish long-term salt balance through any combination of changes in GFR and segmental reabsorption as long as the net result matches salt excretion to salt intake. Countermeasures that happen to impinge on the proximal tubule could obliterate any evidence that losartan continues to act as a proximal diuretic without violating the generic principles of negative feedback.

The acute effect of losartan on \(J_{\text{prox}}\) indicates that the tonic influence of ANG II over proximal reabsorption is unaffected by STN. This is a different view of ANG II than one gets from studying glomerular hemodynamics in the remnant kidney where tonic influence of ANG II is increased (13). The tendency for losartan to increase VLP in sham-operated rats was significantly different from its tendency to reduce VLP in STN rats (Fig. 5). Nonetheless, the primary differences in \(J_{\text{prox}}\) were subtle. In other words, whether a challenge to long-term salt balance comes from STN or chronic losartan, the kidney invokes changes in proximal reabsorption that ultimately render GTB minimally affected.

In summary, many nephrons in the early remnant kidney manifest anomalous TGF responses, where increased flow to the MD causes SNGFR to increase. While this cannot be explained by known immediate actions of ANG II on TGF signaling, it is abrogated by chronic exposure to AT\(_1\)R blockade.

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