Acute saline expansion increases nephron filtration and distal flow rate but maintains tubuloglomerular feedback responsiveness: role of adenosine A1 receptors

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Temporal adaptation of tubuloglomerular feedback (TGF) permits readjustment of the relationship of nephron filtration rate [single nephron glomerular filtration rate (SNGFR)] and early distal tubular flow rate (VED) while maintaining TGF responsiveness. We used closed-loop assessment of TGF in hydropenia and after acute saline volume expansion (SE; 10% body wt over 1 h) to determine whether (1) temporal adaptation of TGF occurs, (2) adenosine A1 receptors (A1R) mediate TGF responsiveness, and (3) inhibition of TGF affects SNGFR, VED, or urinary excretion under these conditions. SNGFR was evaluated in Froment-Wistar rats by micropuncture in (J) early distal tubules (ambient flow at macula densa), (2) recollected from early distal tubules while 12 nl/min isotonic fluid was added to late proximal tubule (increased flow to macula densa), and (3) from proximal tubules of same nephrons (zero flow to macula densa). SE increased both ambient SNGFR and VED compared with hydropenia, whereas TGF responsiveness (proximal-distal difference in SNGFR, distal SNGFR response to adding fluid to proximal tubule) was maintained, demonstrating TGF adaptation. A1R blockade completely inhibited TGF responsiveness during SE and made VED more susceptible to perturbation in proximal tubular flow, but did not alter ambient SNGFR or VED. Greater urinary excretion of fluid and Na+ with A1R blockade may reflect additional effects on the distal nephron in hydropenia and SE. In conclusion, A1R-independent mechanisms adjust SNGFR and VED to higher values after SE, which facilitates fluid and Na+ excretion. Concurrently, TGF adapts and stabilizes early distal delivery at the new setpoint in an A1R-dependent mechanism.

Adaptation of TGF that occurs after SE is accompanied by increases in both SNGFR and early distal delivery increases while SNGFR remains relatively constant. These adjustments allow TGF to maintain normal responsiveness and efficiency while permitting a new relationship between SNGFR and macula densa flow rates that enhance distal delivery and facilitate Na+ excretion under conditions such as (1) reductions in nephron mass (2) and (2) high NaCl diet. Acute saline volume expansion (SE) is another condition in which temporal adaptation of TGF is likely to occur. However, certain conditions impair the ability to undergo TGF temporal adaptation and include (1) nitric oxide synthase (NOS)-I inhibition (29) and (2) cyclooxygenase-2 inhibition (11), and there may be other conditions in which TGF resetting or temporal adaptation is impaired or absent.

Studies in the literature have suggested that acute SE requires modifications in TGF that include both (1) temporal adaptation of the relationship of SNGFR and distal delivery rates and/or (2) diminished sensitivity or responsiveness of TGF (4, 5, 7, 8, 18, 22, 39). These predictions reflect some logic since the combination of both TGF adaptation and suppression of TGF should facilitate NaCl excretion by lifting the TGF-mediated constraints on SNGFR/glomerular filtration rate (GFR), thereby increasing the delivery to the distal tubule. However, the two responses differ with regard to the ability to stabilize early distal delivery at the new set point. We have therefore reexamined this issue to determine whether the temporal adaptation of TGF that occurs after SE is accompanied by suppression of TGF responsiveness and activity or whether TGF remains largely intact under these conditions.

We have submitted rats to SE (10% body wt over 1 h) and examined TGF activity with a primarily closed-loop analysis using kidney micropuncture. By closed-loop analysis, we mean assessment of TGF under free-flow conditions and not artificially separating the macula densa flow signal from the SNGFR response, which is termed an open-loop analysis (30). We also examined the consequences of inhibiting adenosine A1 receptor (A1R) with 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a maneuver previously shown by this and other laboratories to inhibit TGF activity under normovolemic conditions (17, 24, 27). Whether A1Rs also mediate the TGF response following acute saline expansion has not been tested. This approach further tested the consequences of TGF inhibition on early distal fluid delivery during saline expansion. The current studies demonstrate that, in spite of massive volume expansion, and increases in both SNGFR and VED, the TGF responsiveness and inhibitory influence on SNGFR is maintained at a new set point. These results suggest that temporal adaptation of TGF after saline expansion permits increases in flow rates to the distal tubule, which facilitates Na+ excretory responses,
flow rates of collections were measured gravimetrically, and Na right kidney was collected from an indwelling bladder catheter. Urine collected via a ureteral catheter from the left kidney. Urine from the hour. Micropuncture was performed on the left kidney, and urine was collections of Nuclear) using scintillation counting (Packard TRICARB2900 liquid infused solution. Doses of DPCPX of 0.1–0.3 mg/kg have been then further diluted one part into 25 parts of normal saline as the (10). DPCPX was suspended at 3 mg/ml in dimethyl sulfoxide and with the administration of inulin and initiation of saline expansion. However, at the same time, the preserved TGF responsiveness prevents any further increases in early distal expansion. Therefore, the doses of DPCPX applied to the systemic circulation were also expected to reach and inhibit the neighboring nephron (24). Therefore, the doses of DPCPX applied to peritubular application as well as when infused in the lumen of a nephron, and 12 nl/min of artificial late proximal tubular fluid (130 m tip pipet containing lightly stained (F D & C green) Ringer/H9262 solution, injections were made in randomly selected proximal tubules after this control collection, a 7- microperfusion pipet was inserted in the last proximal segment on the kidney surface of the same nephron, and 12 nl/min of artificial late proximal tubular fluid (130 mM NaCl, 10 mM NaHCO3, 4 mM KCl, 2 mM CaCl2, and 45 mg/100 mg urea; stained lightly with 0.1 g/100 g F D & C green) was added to normal flow using a highly accurate Hampel microperfusion pump (no. 2 in Fig. 1). We chose 12 nl/min as a rate that encompasses a range beyond the maximum vasoconstrictor capacity of TGF at these values of SNGFR. After 3–5 min of free-flow perfusion, a recollection of the distal tubule was obtained to measure SNGFR and VED (no. 3 in Fig. 1). Upon completion, the microperfusion pipet was removed from the proximal tubule. Three to five minutes later, a third collection was obtained from the last proximal tubule segment on the kidney surface of the same nephron to determine proximal SNGFR (no. 4 in Fig. 1). Although all three collections were obtained in most nephrons, occasionally a single paired observation, either distal SNGFR ± proximal perfusion added or distal SNGFR vs. proximal SNGFR, was performed. All collected samples were counted for [3H]inulin, and the SNGFR as well as the fractional fluid reabsorption (FR) were computed from the TF-to-P inulin ratios (FR = 1 – P/TF). Statistics. Data are means ± SE and were tested for significant differences between 1) proximal vs. distal SNGFR and 2) distal SNGFR vs. distal SNGFR plus adding 12 nl/min to late proximal tubule by paired t-test. Intergroup comparisons were performed using one-way ANOVA followed by Holm-Sidak test for pairwise comparisons. Only results with P < 0.05 were considered statistically significant.

RESULTS

SNGFR and the TGF system. Studies were performed in control hydroptic rats (n = 5, body wt 303 ± 19 g) to assess normal TGF responses in the absence of volume expansion. When 12 nl/min were added to late proximal flow and distal SNGFR reassessed, the SNGFR decreased by 9 ± 1 nl/min (36 ± 3 to 28 ± 3 nl/min, P < 0.0003, n = 14 paired collections; Fig. 2), which constitutes an evaluation of the vasoconstrictor potential of the TGF system (18, 28, 30). The mean difference between control distal SNGFR and proximal SNGFR (zero distal flow) was 8 ± 2 nl/min (42 ± 2 distal and 50 ± 4 nl/min proximal, P < 0.005, n = 15 paired collections), suggesting that the ambient SNGFR at normal distal flow rate sits approximately midway between minimum and maximum TGF activation (zero flow and 12 nl/min added values). During treatment with DPCPX in hydroptic rats (n = 4; body wt of 330 ± 10 g), the difference between proximal and distal SNGFR [49 ± 3 vs. 50 ± 3 nl/min, not significant (NS), n = 20 paired collections] was eliminated, and distal derived SNGFR was significantly higher than during hydroptia without DPCPX (50 ± 3 vs. 39 ± 2 nl/min, all distal values, P =

MATERIALS AND METHODS

All experimentation was conducted according to the National Institutes of Health 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. Studies utilized Frømter-Wistar rats (280–350 g body wt) housed in a colony at the San Diego Veterans Affairs Veterinary Medical Unit. Rats were maintained on a normal NaCl diet before the day of experiments. After thiobutabarbital anesthesia was induced (100 mg/kg ip; Sigma), rats were surgically prepared for micropuncture as previously described (2, 29–31, 33–35). After micropuncture surgery and during inulin equilibration, rats were studied either in hydropenia (1.2 ml/h of isotonic NaCl-NaHCO3) or submitted to 10% body weight infusion of isotonic saline solutions over 1 h, and the infusion was then adjusted to equal total urine flow rates from both right and left kidneys to maintain a relatively stable volume-expanded condition (38). Total time of micropuncture measurements was limited to 2 h after equilibration or completion of saline expansion. In certain studies, animals in hydropenia and those submitted to saline expansion were concurrently treated with an A1R blocker, DPCPX, infused as a bolus of 1 mg/kg followed by continuous infusion at 0.6 mg kg−1 h−1 beginning with the administration of inulin and initiation of saline expansion (10). DPCPX was suspended at 3 mg/ml in dimethyl sulfoxide and then further diluted one part into 25 parts of normal saline as the infused solution. Doses of DPCPX of 0.1–0.3 mg/kg have been proposed to inhibit renal reabsorption in the rat via specific blockade of A1R (6, 12). DPCPX has been shown to inhibit TGF with luminal and peritubular application as well as when infused in the lumen of a neighboring nephron (24). Therefore, the doses of DPCPX applied to the systemic circulation were also expected to reach and inhibit the A1Rs involved in the TGF response.

GFR was assessed from the clearance of [3H]inulin (New England Nuclear) using scintillation counting (Packard TRICARB2900 liquid scintillation counter). Urine was collected from two consecutive collections of ~1 h duration, and plasma samples were obtained each hour. Micropuncture was performed on the left kidney, and urine was collected via a ureteral catheter from the left kidney. Urine from the right kidney was collected from an indwelling bladder catheter. Urine flow rates of collections were measured gravimetrically, and Na+ and K+ concentrations were measured in urine samples by flame photometry (Cole-Parmer Instruments). Evaluation of TGF activity was performed primarily using closed-loop approaches (Fig. 1). Using a 3-μm tip pipet containing lightly stained (F D & C green) Ringer/H9262 solution, injections were made in randomly selected proximal tubules on the kidney surface to identify late proximal and the earliest distal tubular sites. After injection of a short oil block, a timed 2.5–3 min collection was obtained from the early distal tubular site to determine SNGFR and VED (no. 1 in Fig. 1). We ensured that the distal tubular site was well vented with a pipet tip to prevent tubular obstruction. After this control collection, a 7-μm microperfusion pipet was inserted in the last proximal segment on the kidney surface of the same nephron, and 12 nl/min of artificial late proximal tubular fluid (130 mM NaCl, 10 mM NaHCO3, 4 mM KCl, 2 mM CaCl2, and 45 mg/100 mg urea; stained lightly with 0.1 g/100 g F D & C green) was added to normal flow using a highly accurate Hampel microperfusion pump (no. 2 in Fig. 1). We chose 12 nl/min as a rate that encompasses a range beyond the maximum vasoconstrictor capacity of TGF at these values of SNGFR. After 3–5 min of free-flow perfusion, a recollection of the distal tubule was obtained to measure SNGFR and VED (no. 3 in Fig. 1).
TUBULOGLOMERULAR FEEDBACK AFTER ACUTE SALINE EXPANSION

Fig. 2. Assessment of the TGF status by examining SNGFR at various levels of early distal tubular flow rate using closed-loop analysis in hydropenia, saline expansion, and during treatment with the A1R antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). SNGFR in “Proximal” collections reflects zero flow, “Distal” is SNGFR at ambient flow rates, and “Distal plus 12 nl/min” during addition of fluid to freely flowing late proximal tubule of the same nephron. These data examine the vasodilatory and vasoconstrictor elements of TGF and find the system intact in hydropenia and during saline expansion and inhibited by adenosine A1 receptor (A1R) blockade; the inhibition by A1R blockade of the vasoconstrictor arm of TGF to adding tubular fluid was blunted in hydropenia. NS, not significant. *P < 0.05 vs. distal.

0.002). However, when 12 nl/min was added to proximal flow, the distal SNGFR decreased by 9 ± 2 nl/min (from 52 ± 2 to 44 ± 3 nl/min, P = 0.001, n = 17 paired collections). These results indicated complete TGF inhibition in response to A1R blockade when ambient delivery to the macula densa was reduced. In comparison, the TGF response to an increase in the delivery to the macula densa markedly above ambient levels was not inhibited by DPCPX during hydropenia.

After saline expansion, we observed persistence of TGF activity when evaluated by this closed-loop assessment (n = 9 rats; body wt 316 ± 11 g). When 12 nl/min were added to late proximal flow rates, distal SNGFR decreased by 11 ± 3 nl/min (63 ± 3 to 52 ± 3 nl/min, P < 0.002, n = 17 paired collections), demonstrating a vigorous vasoconstrictor capacity, and the proximal/distal SNGFR difference was 6 ± 2 nl/min (65 ± 3 nl/min proximal vs. 59 ± 2 distal, P < 0.01, n = 35 paired collections), suggesting normal vasodilatory potential, similar to values obtained during hydropenia. This implies that even after saline expansion TGF-mediated increases and decreases in SNGFR relative to the ambient SNGFR or operating point were preserved, consistent with TGF adaptation. During administration of DPCPX, the A1R blocker, we found quite different results during saline expansion (n = 9 rats; body wt 309 ± 9 g). The reduction in SNGFR when 12 nl/min were added to free-flowing proximal tubules was blunted (55 ± 3 to 54 ± 4 nl/min, NS, n = 12 paired collections). Also, there was no difference between proximal and distal derived SNGFR (56 ± 4 nl/min proximal and 54 ± 4 distal, NS, n = 20 paired collections). Thus, inhibition of A1Rs completely eliminated TGF activity, indicating that A1R activity mediates the TGF response after saline expansion. All measurements of SNGFR were obtained during a 2-h period in all groups. There was no tendency for ambient distal SNGFR to increase or decrease during this 2-h period, suggesting stability of nephron function.

DPCPX treatment increased ambient distal SNGFR in hydropenia (50 ± 3 vs. 39 ± 2 nl/min, P = 0.002) but did not change it during saline expansion (55 ± 3 vs. 59 ± 2 nl/min, NS). As noted above, DPCPX eliminated the proximal/distal differences in both hydropenia and SE. It is of interest that the values for distal SNGFR after DPCPX approximated the values for distal rather than proximal SNGFR in the saline expansion group. In comparison, DPCPX increased distal SNGFR to the values observed for proximal SNGFR in hydropenia. Blood pressure did not differ among groups (Table 1).

Early distal flow rates. In hydropenia, the basal VED was 10 ± 1 nl/min, and the FR of fluid up to the early distal tubule (FR) was 0.76 ± 0.02. When TGF was activated, by adding 12 nl/min to the proximal flow, VED tended to increase by 5 ± 2 nl/min (NS) and FR fell to 0.50 ± 0.06 (P < 0.03). DPCPX significantly increased VED in hydropenia from 10 ± 1 to 15 ± 1 nl/min (P < 0.001), and, when 12 nl/min was added to freely flowing proximal tubules, VED further increased (by 14 ± 3 nl/min) to 29 ± 4 nl/min, a significantly greater increase than during untreated hydropenia (P < 0.05). The results are consistent with an inhibitory influence of A1R tone on ambient VED during hydropenia as well as when fluid is added to the proximal tubule, in spite of the fact that DPCPX did not prevent SNGFR from decreasing in response to the added fluid.

During SE, VED rose significantly to 22 ± 1 nl/min with a FR of 0.62 ± 0.02. When 12 nl/min were added to proximal flow to activate TGF, VED tended to increase (4 ± 2 nl/min, NS) and FR fell to 0.50 ± 0.03 (P < 0.03), suggesting that TGF was equally efficient in regulating early distal flow rates after saline expansion (Fig. 3). After DPCPX, VED and FR were at 23 ± 2 nl/min and 0.60 ± 0.02, values not different from the SE group, indicating that DPCPX did not alter upstream fluid reabsorption with saline expansion. However, when 12 nl/min were added to proximal flow, the blunted fall in SNGFR (see above) was associated with an increase in VED by 13 ± 3 nl/min, which was significantly greater than with SE.

Table 1. Summary of GFR, SNGFR, urinary excretion rates, and mean arterial pressure

<table>
<thead>
<tr>
<th>Condition</th>
<th>GFR, ml/min</th>
<th>SNGFR, ml/min</th>
<th>UV, μl/min</th>
<th>UNaV, μeq/min</th>
<th>UKV, μeq/min</th>
<th>MAP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydropenia</td>
<td>1.4 ± 0.2</td>
<td>39 ± 2</td>
<td>4 ± 1</td>
<td>0.3 ± 0.06</td>
<td>0.3 ± 0.05</td>
<td>136 ± 3</td>
</tr>
<tr>
<td>Saline expansion</td>
<td>1.6 ± 0.2</td>
<td>59 ± 2*</td>
<td>80 ± 10*</td>
<td>8 ± 1</td>
<td>1.5 ± 0.2*</td>
<td>139 ± 4</td>
</tr>
<tr>
<td>Saline expansion + DPCPX</td>
<td>2.1 ± 0.2</td>
<td>55 ± 3*</td>
<td>132 ± 12*†</td>
<td>13 ± 1*†</td>
<td>2.2 ± 0.4*</td>
<td>141 ± 5</td>
</tr>
<tr>
<td>Hydropenia + DPCPX</td>
<td>1.3 ± 0.2</td>
<td>50 ± 2*</td>
<td>9 ± 2*</td>
<td>1.8 ± 0.3*</td>
<td>2.7 ± 0.3*</td>
<td>130 ± 2</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 5–9 rats/group. GFR, glomerular filtration rate; SNGFR, single nephron GFR; UV, UNaV, and UKV, urinary excretion of fluid, sodium, and potassium, respectively; MAP, mean arterial pressure; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine. SNGFR is derived from collection from the distal tubule. †From the left micropuncture kidney only. *P < 0.01 vs. hydropenia. ‡P < 0.01 vs. saline expansion.
alone (P < 0.01). The increases in $V_{ED}$ to adding 12 nl/min to proximal flow during A1R blockade were strikingly similar during hydropenia and SE. Part of this response in $V_{ED}$ was also due to a fall in FR. During saline expansion, this fall probably reflected the increased fluid load to the proximal tubule as a consequence of adding 12 nl/min in the absence of a reduction in SNGFR. The results indicate that an intact TGF system regulates and limits early distal flow rates during saline expansion.

Figure 4 shows the positive linear relationship between SNGFR and $V_{ED}$ observed in all groups except during DPCPX in hydropenia; A1R tone appeared to enhance tubular fluid reabsorption upstream of the early distal tubule, in particular, in nephrons with low tubular loads (SNGFR) in hydropenia.

![Figure 4](http://ajprenal.physiology.org/)

**Temporal adaptation of TGF.** SE significantly increased both distal SNGFR (39 to 59 nl/min, $P < 0.001$) and $V_{ED}$ (10 to 22 nl/min, $P < 0.05$) compared with hydropenia while TGF responsiveness was maintained, demonstrating TGF adaptation. Whereas A1R blockade inhibited TGF responsiveness, the observed values for ambient SNGFR (55 nl/min) and $V_{ED}$ (23 nl/min) were nearly identical to untreated SE. In comparison, during hydropenia, DPCPX increased both distal SNGFR and $V_{ED}$, indicating a stronger inhibitory tone of A1R/TGF on these parameters during hydropenia compared with SE.

**Whole kidney values for GFR and urinary excretion.** As described in prior studies, whole kidney GFR responses do not always parallel the changes in superficial SNGFR (13, 14, 19). This was also the observation in this study. While distal SNGFR increased from 39 to 59 nl/min after saline expansion (+50%), the GFR of the left kidney (used for micropuncture) did not change significantly (1.4 to 1.6 ml/min), indicating a greater SNGFR than GFR response (Table 1). The right kidney GFR values changed more or less in parallel (1.7 ± 0.3 in hydropenia and 1.8 ± 0.1 ml/min during SE). When treated with DPCPX, the values for distal SNGFR did not appear to change significantly with SE. When rats were treated with DPCPX, GFR was 2.1 ± 0.2 ml/min for the left kidney and 2.2 ± 0.3 ml/min for the right kidney during SE and 1.3 ± 0.2 for the left and 1.4 ± 0.2 ml/min for the right kidney during hydropenia. By ANOVA, the values for GFR were not significantly different among hydropenia vs. saline expansion and between DPCPX-treated vs. untreated groups. The greater effects of SE on SNGFR vs. GFR suggest some “redistribution of GFR” (13, 14, 19, 25, 38).

Saline expansion increased urine flow rate of the left micropuncture kidney (4 ± 1 to 80 ± 10 μl/min, $P < 0.002$), Na⁺ excretion (0.3 ± 0.06 to 8 ± 1 μeq/min, $P < 0.003$), and to a lesser degree K⁺ excretion (0.3 ± 0.1 to 1.5 ± 0.2 μeq/min, $P < 0.03$) compared with hydropenia (Table 1). In hydropenic rats treated with DPCPX, both Na⁺ (0.3 ± 0.1 to 1.8 ± 0.3 μeq/min, $P < 0.001$) and K⁺ (0.3 ± 0.1 to 2.7 ± 0.3 μeq/min, $P < 0.001$) were suppressed compared with untreated hydropenia.
In an earlier study, we utilized videometric flow velocimetry with the induced efferent, glomerular filtration response. A greater K\textsuperscript{+} excretion (132 ± 1 µeq/min) and urine volume (132 ± 12 µl/min) compared with untreated SE (P < 0.01 vs. SE for both), there was a tendency for greater K\textsuperscript{+} excretion (2.2 ± 0.4 µeq/min, NS). The right kidney behaved nearly identically as the left micropuncture kidney for all groups (data not shown).

**DISCUSSION**

Temporal adaptation or resetting of TGF has been established, but the involved mechanisms and the physiological purposes for this phenomenon are less clear (2–4, 9, 29–31, 33–35). The results of this study have provided insights into this issue pertinent to the renal response to acute SE. First, we have established the magnitude of temporal adaptation of TGF and the changes in the relationship of SNGFR with V\textsubscript{ED}, both of which increase significantly with SE. Second, we have demonstrated that TGF responses, both the vasoconstrictor and vasodilatory limbs, remain substantial after SE. Third, A\textsubscript{1R} blockade completely inhibits TGF following SE but does not affect SNGFR and V\textsubscript{ED} at the new operating point. Fourth, in practical terms, our data suggest that temporal adaptation during acute SE permits higher values for SNGFR and V\textsubscript{ED} that should facilitate Na\textsuperscript{+} excretion, whereas the maintenance of TGF responsiveness made V\textsubscript{ED} less susceptible to perturbation in proximal tubular flow.

Several prior studies have examined TGF characteristics during acute volume loading, but with somewhat different techniques and goals in mind (4, 5, 7, 8, 18, 22, 39). For the most part, the earlier studies utilized open-loop techniques by perfusing the macula densa segments from the late proximal tubule and assessing SNGFR or stop-flow pressure responses. The prior studies have not addressed the net physiological impact of TGF adaptation or an intact TGF system on V\textsubscript{ED} or whether A\textsubscript{1R} mediate TGF after TGF adaptation. Some of the studies have suggested partial suppression of TGF activity using open-loop techniques that focus primarily on the vasoconstrictor responses of TGF (5, 23). Such “suppression of TGF” was judged primarily by observing that the range of vasoconstrictor responses and SNGFR reduction in response to Loop of Henle perfusion was diminished when expressed as a percentage of SNGFR at zero flow values. This suppression of TGF was corrected by angiotensin II infusion or inhibition of nitric oxide generation (5, 23). These studies at least suggested that decreased sensitivity of TGF may contribute to the efficient off-loading or excretion of Na\textsuperscript{+} and water following SE. However, other studies concluded that TGF persisted after more modest SE, actually acting to limit the increase in GFR (7, 8).

The current study provides information on SNGFR and V\textsubscript{ED} sufficient to evaluate the characteristics of TGF adaptation following SE. The closed-loop approach provides data on both the vasodilatory and vasoconstrictor arms of the TGF system around the natural operating point. The closed-loop analysis recognizes that changes in V\textsubscript{ED} are in continuity and conjunction with the induced efferent, glomerular filtration response. In an earlier study, we utilized videometric flow velocimetry wherein flow in the late proximal tubule was varied by addition or withdrawal of fluid in freely flowing tubules while the upstream flow response was monitored on-line during euvolemia and plasma volume expansion, compared with hydropenia, to assess TGF activity (30). We observed that TGF efficiency, or the “gain” of TGF around the turning or operating point of the system, was largely intact in volume-expanded conditions. In the current study, we assessed SNGFR directly at ambient, spontaneous flow rates and elevated flow rates from distal tubular collections and at zero flow to the distal tubule using proximal tubular collections from the same nephron. The values for SNGFR in hydropenia and saline expansion were quite different. However, the absolute changes in SNGFR from distal collections when 12 nl/min were added, the vasoconstrictor limb, and between proximal and distal SNGFR, the vasodilatory arm, were nearly identical between hydropenia and acute saline expansion, indicating similar TGF activities around the two quite different operating points. Moreover, in hydropenia and acute SE, the operating point was located close to the midpoint halfway between SNGFRs at zero distal delivery and at maximum flows to the distal tubule/macula densa, consistent with TGF adaptation.

The current studies also aimed to assess the physiological impact of persistence of TGF during acute SE. The research literature provides strong support for the concept that adenosine, acting via the A\textsubscript{1R}, mediates the TGF. Pharmacological inhibition and/or clamping adenosine levels clearly eliminate TGF activity (27). Genetic deletion of A\textsubscript{1R} also eliminates TGF (26, 37). Mice lacking the extracellular adenosine-forming enzyme endo-5'-nucleotidase also exhibit major reductions in TGF, with the remaining activity attributable to intracellular sources of adenosine (15). Therefore, we chose A\textsubscript{1R} blockade as a mechanism to inhibit TGF. Under conditions of SE, we observed that A\textsubscript{1R} blockade completely eliminated TGF, indicating that A\textsubscript{1R} also mediates TGF under these conditions. During hydropenia, the effects were somewhat more complex. Adding 12 nl/min to late proximal flow rate activated TGF in untreated hydropenia and resulted in insignificant and modest increases in V\textsubscript{ED} of ~5 nl/min, suggesting highly efficient TGF activity. DPCPX eliminated the difference between proximal and distal tubular collections for SNGFR in hydropenia and increased ambient values of both distal SNGFR and V\textsubscript{ED}, indicating a stronger inhibitory tone of A\textsubscript{1R}/TGF on these parameters under hydropenia compared with SE; however, when 12 nl/min were added to proximal flow, SNGFR decreased significantly in spite of DPCPX treatment, yet V\textsubscript{ED} increased by 14 nl/min, in part due to a marked fall in FR (0.70 ± 0.02 to 0.41 ± 0.05, P < 0.001). These results indicated a prominent influence of A\textsubscript{1R} tone on distal delivery, independent of an SNGFR effect, and that A\textsubscript{1R} blockade appears to disrupt glomerulotubular balance when fluid is added.

During acute saline expansion, the increase for early distal flow rate with fluid added was not significant (~4 nl/min), findings again in support of the persistence and overall efficacy of TGF in this volume-expanded condition (Fig. 3). However, when TGF was inhibited by application of the A\textsubscript{1R} blocker during saline expansion, we observed the importance of an intact TGF system. During this condition, V\textsubscript{ED} increased to a much greater degree when fluid was added to the proximal tubule; in fact, the increase in distal flow was approximately
equal to the volume of fluid added upstream (12–13 nl/min) (nearly identical to the changes observed with DPCPX in hydropenia). These results demonstrate that preserved TGF activity after acute SE made VED less susceptible to further increases in proximal tubular flow.

Notably, we observed in superficial nephrons that inhibition of TGF by A1R blockade during SE did not alter ambient SNGFR or VED compared with untreated rats, findings somewhat different from those observed with DPCPX during hydropenia. These findings indicate that A1R-independent mechanisms are important for adjusting ambient SNGFR and distal fluid delivery following SE. These mechanisms may include the coordinated action of circulating atrial natriuretic peptide on the preglomerular vascular resistance (7) and changes in NOS-1 activity in the macula densa (9, 29, 32). Although treatment with DPCPX eliminated the proximal/distal SNGFR difference in both hydropenia and during acute SE, the mechanisms appeared to differ in that DPCPX increased ambient SNGFR to values equal to proximal SNGFR in hydropenia, whereas DPCPX decreased proximal SNGFR to distal values during SE.

With saline expansion, SNGFR increased to a greater extent than whole kidney GFR. Such “redistribution” of nephron GFR has been observed previously during saline expansion and chronic NaCl loading, analogous to observations of Goodyer and Jaeger (13) on the redistribution of GFR (14, 19, 38). A1R blockade did not affect SNGFR and VED after SE in superficial nephrons accessible to micropuncture but tended to increase whole kidney GFR and significantly increased urinary excretion of fluid and Na+ (Table 1). Blockade of A1R can inhibit reabsorption in the proximal tubule and collecting duct (36), which may have contributed to the overall results with A1R blockade on urinary excretion during SE. During hydropenia, DPCPX increased urinary Na+ and K+ excretion, which may in part be due to the increase in VED from 10 to 15 nl/min. The fact that VED was unchanged during SE with A1R blockade argues against a major effect on proximal tubular reabsorption of A1R in this condition. In accordance, earlier studies have demonstrated that the impact of A1R blockade on tubular reabsorption is diminished by higher salt intake and volume status (16). However, it remains possible that blockade of adenosine effects via A1R within the more distal tubule and collecting duct could have further augmented and explained the greater urinary fluid and Na+ excretion while VED, at least in superficial nephrons, was not affected. Alternatively, A1R blockade may have increased SNGFR in deeper nephrons, which explains the greater diuresis and natriuresis and the tendency of whole kidney GFR to increase. Although this explanation is highly speculative, it is compatible with the findings of an increase in whole kidney GFR while superficial SNGFR remained constant after A1R blockade and inhibition of TGF.

Why should TGF activity persist after temporal adaptation in acute saline expansion, and what benefit does this provide to the organism? It is counterintuitive that TGF should be fully maintained after NaCl loading, since TGF acts to limit the capacity to off-load or excrete Na+ in a prompt fashion. Our previous study examining the effects of chronic high NaCl diet may provide some valuable insights (33). We found that late proximal flow rate and GFR/SNGFR were only modestly increased with high NaCl intake, and TGF was fully operational and intact. Angiotensin II levels in proximal tubular fluid were elevated, whereas plasma and whole kidney angiotensin II levels were suppressed as expected. When angiotensin AT1R blockers were supplied to these high NaCl intake rats, TGF was partially suppressed, SNGFR and GFR increased, proximal reabsorption decreased, and, consequently, late proximal tubular flow was markedly increased, indicating that the antinatriuretic tone of angiotensin II via TGF/GFR and the proximal tubule was preserved during high NaCl intake. Similarly, maintaining TGF activity during volume expansion means preserving antinatriuretic tone. Elimination of TGF during saline loads could facilitate Na+ excretion, but excessive increases in distal tubular delivery rates could overload distal tubular capacities for reabsorption and magnify ion secretion, including K+ wasting (1, 4, 20, 21). We speculate that maintaining TGF activity during acute Na+ loading is more important to the organism in some manner, possibly to preserve a capacity to separate Na+ and K+ homeostasis, than a highly efficient and prompt excretion of Na+.

In summary, temporal adaptation of TGF does occur during and after acute saline expansion whereby the relationship between SNGFR and VED changes markedly, but TGF activity remains essentially intact, capable of full regulation of the stability of tubular fluid flow rates and SNGFR during volume expansion. A1R blockade eliminates TGF activity following acute saline expansion but did not alter the SNGFR and VED in superficial nephrons, indicating that A1R mediate TGF under these conditions but are not critical for adapting SNGFR and VED to the new operating point. Maintaining TGF activity in nephrons may constrain the rise in GFR and limit the magnitude of early distal delivery during acute saline expansion, which may benefit the organism by limiting secondary, potentially adverse consequences of excessive distal delivery.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: R.C.B., P.S., A.D., S.C.T., and V.V. analyzed data; R.C.B., P.S., S.C.T., and V.V. interpreted results of experiments; R.C.B., P.S., S.C.T., and V.V. drafted manuscript; R.C.B. and V.V. edited and revised manuscript; R.C.B. and V.V. approved final version of manuscript; R.C.B., P.S., A.D., and S.C.T. performed experiments; A.D. and V.V. prepared figures.

REFERENCES

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