

## Resetting protects efficiency of tubuloglomerular feedback

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**Resetting protects efficiency of tubuloglomerular feedback.** Tubuloglomerular feedback (TGF) may effect long-term protection of total body salt and water or may govern minute-to-minute autoregulation of renal function. The task for which TGF is best suited depends on the orientation of ambient tubular flow relative to the inflection point of the TGF curve and on the tendency of TGF to reset in response to prolonged stimulation. Current data suggest that the TGF curve is coupled closely to ambient flow in individual nephrons such that the system is capable of compensating both negative and positive perturbations in tubular flow. This coupling is mediated by events within the juxtaglomerular apparatus that cause the TGF curve to reset laterally in response to sustained shifts in tubular flow. This resetting of TGF occurs within 30 to 60 minutes of an applied stimulus, suggesting that TGF is better suited to mediate dynamic autoregulation than to account for sustained vasoconstriction during proximal tubular injury.

Tubuloglomerular feedback (TGF) is a physiological process conferring an inverse dependence of the single nephron glomerular filtration rate (SNGFR) on the salt content of the tubular fluid flowing past the macula densa. Glomerulotubular balance (GTB) is a term applied to the load dependence of tubular reabsorption, which confers a forward effect of SNGFR on late proximal flow. TGF and GTB form a closed-loop system of negative feedback, which stabilizes both SNGFR and tubular flow against outside disturbances. In life, the system responds rapidly to disturbances of various forms, including changes in tubular reabsorption or blood pressure. Teleological arguments have been advanced for both the long-term protection of total body salt and water content and for minute-to-minute autoregulation of renal blood flow as the main role of TGF. It has been argued, for instance, that TGF serves to prevent catastrophic volume loss when the proximal tubule is injured and unable to reabsorb [1]. Indeed, experiments confirm that TGF is responsible for the reduction in GFR following uranyl nitrate-induced proximal tubular injury [2] or when proximal reabsorption is inhibited by blocking

carbonic anhydrase [3]. Conversely, other data appear to show that TGF may be an important mediator of dynamic renal blood flow autoregulation [4].

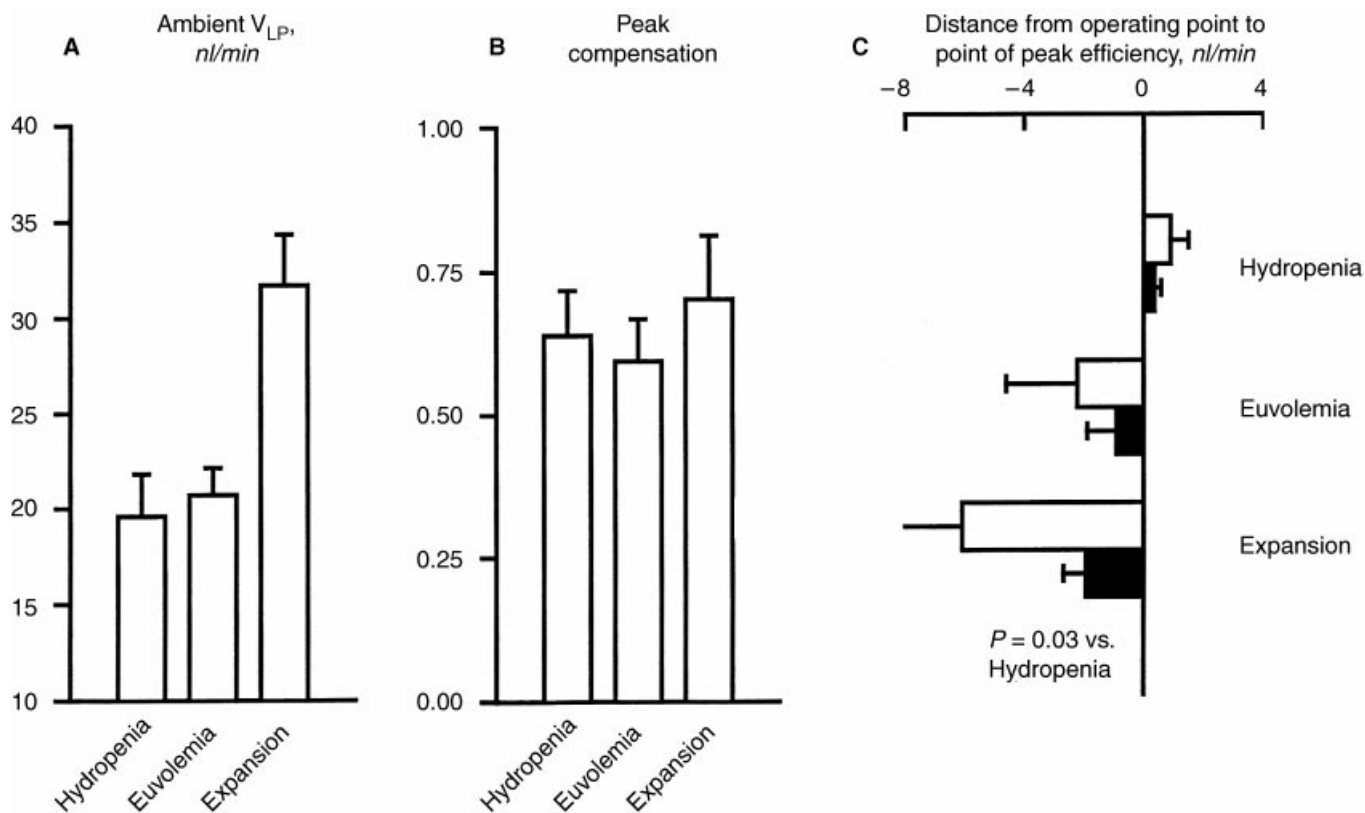
Most TGF studies employ paradigms in which the relationship between TGF stimulus and response is represented by a simple mathematical function. It is generally assumed that during a single experiment in a single nephron, the influence of TGF on SNGFR is described by a single curve in a plane defined by late proximal flow ( $V_{LP}$ , a surrogate for the TGF stimulus) and SNGFR (the TGF response). This curve meets the  $y$ -axis at a point ( $SNGFR_0$ ) representing TGF-independent influences on SNGFR and declines nonlinearly with increasing  $V_{LP}$ , such that the difference between  $SNGFR_0$  and SNGFR resembles a hyperbolic tangent, the steep portion of which is concentrated within a narrow range of  $V_{LP}$ . This TGF curve represents a continuous set of points, along which the nephron can operate. At any instant, the actual operating point (OP) lies at the intersection the TGF curve with a curve representing GTB. When studying TGF behavior, the relationship between  $SNGFR_0$ -SNGFR and  $V_{LP}$  is treated as constant, whereas  $SNGFR_0$  and GTB are permitted or forced to change. A change in  $SNGFR_0$  may be imposed, for instance, by changing blood pressure, a change in GTB may be imposed by changing proximal reabsorption or by artificially manipulating tubular flow with a microperfusion apparatus.<sup>1</sup>

The tendency for the TGF-GTB system to stabilize nephron function (TGF's "homeostatic efficiency") is greatest when the OP resides at the steepest point on the TGF curve and is negligible when the OP lies on a flat part of the TGF curve. Because the steep part of the TGF curve in an individual nephron is concentrated over a range of flows no wider than the variance in ambient flow among nephrons, the physiological relevance of TGF-GTB depends on close coordination between TGF and GTB in each nephron. The relative orientation of TGF and GTB

**Key words:** nephron glomerular filtration rate, glomerulotubular balance, operating point, late proximal flow, fractional compensation

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<sup>1</sup> In viewing this as a closed-loop feedback system that stabilizes its internal variables against outside disturbance, the definition of "outside disturbance" here encompasses any TGF-independent change in the physiological milieu that would affect SNGFR or  $V_{LP}$ . This includes GTB, even though GTB is an intrinsic component of the feedback system.

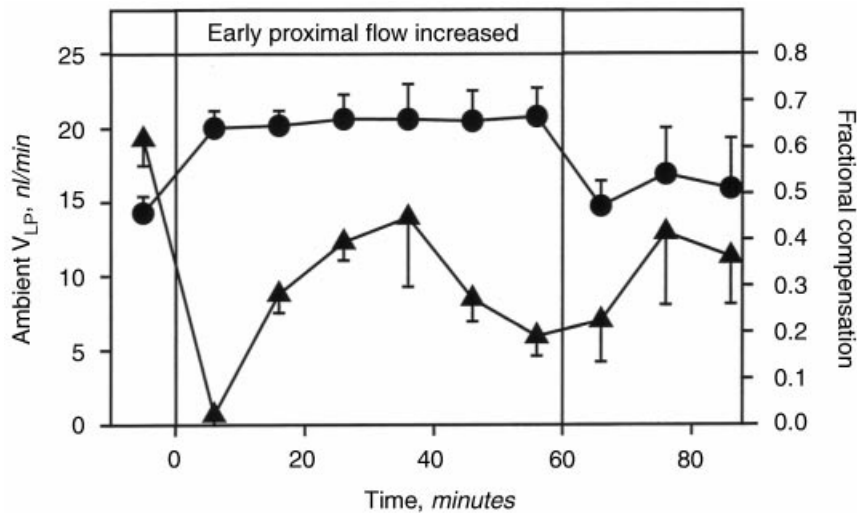


**Fig. 1. Ambient flow rate (A), peak fractional compensation (FC; B), and distance from the operating point (OP) to the point of peak tubuloglomerular feedback (TGF) efficiency (C).** Parameters are expressed both in units of late proximal flow rate ( $V_{LP}$ ) and the associated perturbation required to evoke that change in  $V_{LP}$ . Note that the point of peak efficiency moves leftward relative to the OP after acute volume expansion and that the variance of  $\Delta V_{LP}$  is less than the variance of ambient  $V_{LP}$  in each group. Symbols in C are: (□) perturbation; (■)  $\Delta V_{LP}$ . Adapted from data in [6].

will also determine whether the system is actively engaged in the minute-to-minute control of nephron function or whether it is more suited as a backup system operating only when the tubule is injured. This is because TGF reacts asymmetrically to negative or positive perturbations, depending on the location of the OP relative to the TGF curve's inflection point. If the initial OP is located left of the inflection point, TGF will compensate more efficiently for positive perturbations, whereas if the initial OP is located right of the inflection point, negative perturbations will be compensated more efficiently. The system may thus be more capable of vasoconstriction or of vasodilation depending on where along the TGF and GTB curves intersect. A system with more vasoconstrictive than vasodilatory reserve would be more suited to guard against volume loss, and a system equally capable of vasoconstriction and vasodilation would be more suited to buffering random perturbations, without sign preference.

Addressing the actual location of the OP on the TGF curve, Moore and Mason have summarized the results from studies comparing proximal-distal SNGFR differences with the reduction in SNGFR seen when TGF is saturated by microperfusion. In general, in volume repletion or expansion, the proximal-distal difference constituted a larger

fraction of the maximum TGF response than during volume contraction [5]. We addressed this issue in a closed-loop analysis of TGF under conditions of hydropenia, euvoemia, and acute, isoncotic plasma volume expansion [6]. These experiments were designed to detect differences in the TGF responses to relatively small positive or negative perturbations of  $V_{LP}$  in free-flowing nephrons. Nephron flow was perturbed by using a microperfusion apparatus to add or subtract fluid, and tubular flow was monitored continuously immediately upstream from the perturbation site by videometric flow velocitometry (VMFV), an optical technique for measuring flow in a tubule without interrupting it. The fractional compensation (FC), a convenient index of homeostatic efficiency, was calculated for each perturbation from the change in flow effected by TGF-GTB in response to the perturbation. Because FC is greatest when ambient flow and inflection point of the TGF curve coincide, a nephron operating to the right of the inflection point should compensate more efficiently for negative perturbations and vice versa. Indeed, profiles of FC as a function of the applied perturbation for animals in each group yield single peaks of similar height that shift progressively leftward with increments in volume state (Fig. 1). In hydropenia, compensation was



**Fig. 2.** Ambient late proximal flow rate ( $V_{LP}$ ) and fractional compensation (FC) for  $\square$  5 nl/min perturbations applied repeatedly to  $V_{LP}$  before, during, and after continuous addition of 20 nl/min to the early proximal tubule. Symbols are: ( $\blacktriangle$ ) compensation; ( $\bullet$ )  $V_{LP}$ . Derived from data in [7].

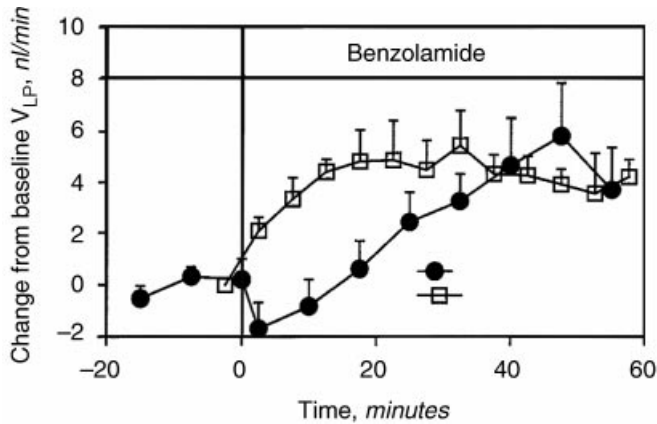
maximum when changes in the applied perturbation were centered about +1 nl/min. After acute volume expansion, compensation was maximum for perturbations centered about -6 nl/min. Acute increases in extracellular volume thus shift the OP rightward along the TGF curve. These findings suggest that meaningful adaptation of the TGF system to changes in volume status needs not involve major shifts in the overall capacity of the system to react but may be accomplished by minor resetting of the TGF curve relative to the OP.

The position of the OP along the TGF curve creates a compromise between the ability to increase GFR at the expense of volume and the ability to conserve volume at the expense of GFR. The particular way in which the OP moves down the TGF curve with acute volume expansion has teleological appeal, inasmuch as the permission to increase GFR is favored over the protection against volume loss as volume is expanded. However, acute volume expansion is associated with shifts or changes in all three determinants of the OP:  $SNGFR_0$ , GTB, and TGF. Teleological appeal notwithstanding, it is thus imaginable that movement of the OP along the TGF curve is a passive or coincidental outcome of independent processes, rather than an ordered response to a physiological requirement. On the other hand, the variance in ambient flow within each group was considerably greater than the variance in the difference between ambient flow and the TGF inflection point for that group. Furthermore, acute volume expansion caused ambient  $V_{LP}$  to increase by 12 nl/min while the SD for the distance from ambient flow to the TGF inflection point after volume expansion was only 3 nl/min. This suggests that within individual nephrons the coupling of the increase in  $V_{LP}$  and the rightward resetting of TGF are too close to arise by chance.

Merely noting that changes in  $V_{LP}$  and resetting of TGF are coupled, or even causally related, does not answer the

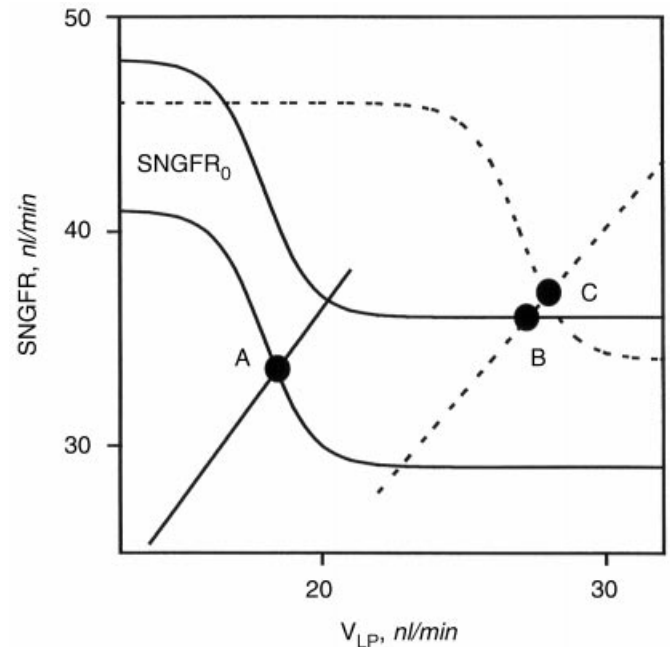
question of cause and effect, a problem applying to most studies testing the role of TGF in causing SNGFR and tubular flow to differ in different physiological states. In models of increased SNGFR, the TGF curve generally resets rightward or upward; in models of decreased SNGFR, it resets downward or leftward. However, experimental data mostly do not permit one to distinguish whether a shift in the TGF curve is the primary cause or a later consequence of a change in  $SNGFR_0$  or tubular function. An attempt to make this distinction might be aided by knowledge of the order of events during transition from one state to the other, in contrast to the usual approach of comparing the system in two steady states.

Given that the rightward shift in the TGF inflection point is slightly, but significantly, less than the increase in ambient  $V_{LP}$  after acute volume expansion, we speculated that a sustained increase in  $V_{LP}$  *per se* might drive the resetting of TGF and that the residual lag between ambient  $V_{LP}$  and the TGF inflection point is a form of error signal that results from finite gain in a feedback loop, which governs the resetting of TGF. We tested this hypothesis in free-flowing nephrons by monitoring the ability of TGF-GTB to compensate for transient perturbations in  $V_{LP}$  while imposing an increase in early proximal flow [7]. Rather than the usual paradigm, which sees the TGF curve as invariant with time during an experiment, these experiments examined time-dependent changes in TGF. Again, we used VMFV and perturbation analysis in free-flowing late proximal nephrons with measurements made before and during the continuous addition of 20 nl/min to the early proximal tubule. The FC for small perturbations in ambient  $V_{LP}$  was severely diminished immediately after imposing the large increase on early proximal flow, consistent with the system having been forced onto a flat portion of the TGF curve. However, within 20 to 40 minutes, and without further change in ambient flow, FC returned to 70% of its original



**Fig. 3.** Changes in ambient late proximal flow rate ( $V_{LP}$ ) from baseline during systemic infusion of benzolamide. Measurements were made in free-flowing nephrons [tubuloglomerular feedback (TGF) intact];  $\square$  and in nephrons in which TGF was interrupted ( $\bullet$ ) by a wax block downstream from the site of measurement.  $V_{LP}$  ultimately increased similarly in both sets of nephrons, but nephrons with TGF intact lagged behind due to the time required for resetting of TGF. Adapted from data in [11].

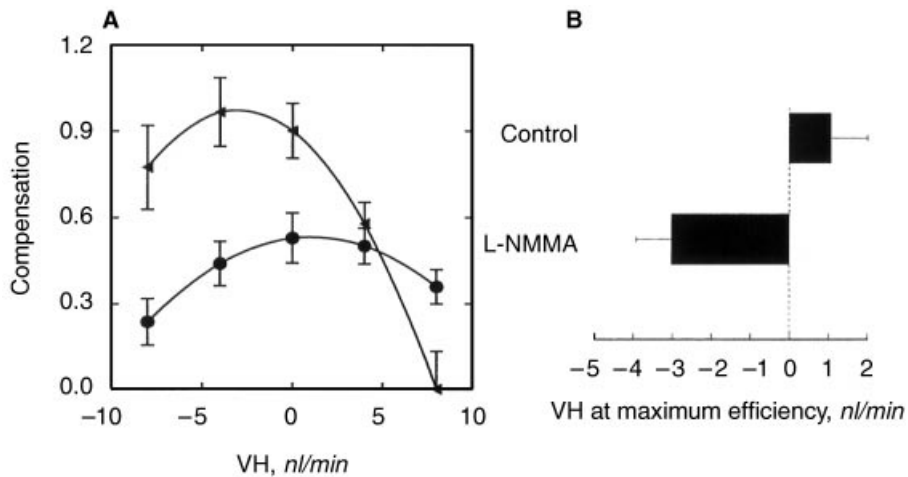
value (Fig. 2), presumably reflecting rightward resetting of the TGF curve to coincide with the new artificial OP. Subsequently, FC began to decline due to a generalized desensitization of TGF. This interpretation was verified in further experiments testing for time-dependent changes in the symmetry of the FC with respect to ambient flow. These experiments revealed a gradual rightward shift in the peak of the compensation profile such that after 30 to 40 minutes TGF realigns with the new OP. These findings confirm the prior impression that a major increase imposed on proximal tubular flow is sufficient to induce TGF resetting and that this resetting occurs on a time scale amenable to explaining the previously noted effects of acute volume expansion. However, this interpretation cannot account completely for the data because the rightward resetting of TGF was not accompanied by any further increase in ambient  $V_{LP}$  during resetting as should have occurred as the OP moved back along the TGF curve. The absence of an increase in  $V_{LP}$  during resetting of TGF may be due to increased proximal reabsorption, or more likely, the sustained major supplementation of early proximal flow causes TGF resetting downward and rightward such that  $SNGFR_0$  decreased along with the rightward resetting. This reduction of  $SNGFR_0$  over 20 to 40 minutes is, by definition, distinct from “TGF,” which operates with a time constant of 30 to 60 seconds [8]. Two other groups have reported analogous observations. Once TGF is engaged, early proximal flow (a surrogate for SNGFR) is stable during prolonged perfusion of Henle’s loop at 40 nl/min, but on stopping perfusion, 30 minutes elapses before full recovery to its preperfusion level [9]. Although interpreted differently at the time, this is most consistent with a downward and rightward shift of the TGF curve during perfusion, then upward and leftward during the 30-minute recovery period.



**Fig. 4.** Idealized transition of tubuloglomerular feedback (TGF) and glomerulotubular balance (GTB) in a hypothetical nephron responding to acute plasma volume expansion. Sigmoid curves represent TGF, straight lines GTB, and the dotted curve and line the equilibrated state of the nephron after volume expansion and resetting. A, B, and C represent serial operating points (OPs), the y-intercept, nephron glomerular filtration rate ( $SNGFR_0$ ), the sum of TGF-independent inputs to SNGFR. TGF efficiency is dependent on the slope of the TGF curve at the OP. The initial effect of volume expansion is to increase  $SNGFR_0$  and to shift GTB. This moves the OP from A to B and reduces the efficiency of TGF. Over the next 20 to 40 minutes, GTB is fixed as TGF resets rightward,  $SNGFR_0$  decreases slightly, and the OP moves from B to C. Note that as the system moves from B to C, TGF efficiency is restored, although slight asymmetry persists with respect to the OP. The slight decrease in  $SNGFR_0$  during resetting offsets further increases in  $V_{LP}$  and SNGFR, which would have resulted from a pure rightward resetting. The pattern of resetting that takes the OP from B to C is analogous to that occurring with supplementation of flow in individual nephrons or when proximal reabsorption is reduced with benzolamide. Resetting is driven by the initial increase in  $V_{LP}$ . The initial increase in  $SNGFR_0$  is not required.

Also consistent is the secondary decline in tubular stop-flow pressure seen during prolonged loop perfusion, providing the perfusion is allowed to reach the distal nephron beyond the macula densa [10].

In each of the three cited studies, which appear to show a secondary or slow wave of negative feedback from the distal tubule to glomerulus, sustaining tubular flow at supraphysiological levels has evoked the effect. We have since examined TGF resetting in a more “physiological” model, stimulating TGF tonically by reducing proximal reabsorption with benzolamide, a carbonic anhydrase inhibitor, rather than by microperfusion [11]. Ambient  $V_{LP}$  and FC for small perturbations in  $V_{LP}$  were monitored in free-flowing nephrons before and during continuous systemic infusion of benzolamide. Initially, benzolamide seemed to reduce TGF efficiency, consistent with TGF saturation. Over the next 45 to 60 minutes, FC recovered to



**Fig. 5. Closed-loop perturbation of late proximal flow before, during and after addition of L-NMMA to the late proximal fluid.** Symbols are: (●) control; (▲) L-NMMA. Inhibition of nitric oxide synthase with L-NMMA increases peak tubuloglomerular feedback (TGF) efficiency and shifts the compensation profile leftward relative to the operating point (OP). VH refers to the applied perturbation. Reprinted with permission from [16].

prebenzamide levels as  $V_{LP}$  increased gradually by 5 nl/min. The ultimate magnitude of the gradual increase in  $V_{LP}$  during TGF resetting matched that of the immediate increase in  $V_{LP}$  caused by benzamide when TGF was interrupted by a wax block in the late proximal tubule (Fig. 3). These data, showing rightward TGF resetting to accommodate reduced proximal reabsorption, differ from the former microperfusion data in that the gradual increase in  $V_{LP}$  throughout the course of TGF resetting obviates the need to invoke any decrease in  $SNGFR_0$ . However, when the TGF curve is steep at the OP, FC is less sensitive to changes in  $SNGFR_0$  than to shifts in ambient  $V_{LP}$ . Thus, although a decrease in  $SNGFR_0$  is not implied by the response to benzamide, it is not precluded. It is even possible that increased tubular flow mediates a reduction in  $SNGFR_0$  after acute volume expansion, which is superimposed on a greater tendency for other neurohumoral events surrounding acute volume expansion to increase  $SNGFR_0$  (Fig. 4). In any event, the increase in tubular flow imposed in the microperfusion studies is greater than that achievable by reducing proximal reabsorption with benzamide, suggesting that a greater stimulus may be required to elicit a reduction in  $SNGFR_0$  than to elicit rightward resetting of TGF.

A main advantage of studying TGF by the closed-loop perturbation approach is that the calculated efficiency of TGF does not depend on where along the tubule the perturbations are made. However, when the perturbations are made in the proximal tubule and the system reduced to its components, TGF and GTB, events in the loop of Henle are ascribed necessarily to the TGF component. We have begun to examine the possibility that the rightward resetting of TGF during benzamide is due to increases in loop reabsorption. We administered benzamide for 24 hours prior to testing for increases in the capacity for loop reabsorption. Benzamide withdrawal causes GFR to rise to supranormal levels, consistent with leftward movement of the OP along the TGF curve. However, benzamide has

no effect on the capacity for Na-K-2Cl transport in the loop of Henle, although it does cause a small increase in bicarbonate-dependent transport that is measurable only when the loop is perfused with high bicarbonate concentrations (unpublished observations). It thus appears likely that TGF resetting during increased tubular flow is mediated by events within the juxtaglomerular apparatus (JGA) rather than in the loop of Henle.

Although the chain of events whereby increased tubular flow causes TGF resetting may be localized to the JGA, few data suggest which, among the many paracrine substances operating within the JGA, may mediate this type of resetting. However, both renin and macula densa nitric oxide (NO) are relevant in this context. Perfusion of the macula densa with increasing [NaCl] progressively inhibits renin release with a time course similar to that required for a change in flow to cause TGF resetting [12]. Because the renin-angiotensin system is a recognized modulator of TGF activity [13], sustained increases in tubular flow could reset TGF by suppressing local renin activity. There is also circumstantial evidence that tubular flow could modulate TGF activity via effects on macula densa NO synthase (NOS). Given that functional NOS activity is enhanced [14] and TGF activity suppressed [15] by a high-salt diet, we addressed the functional role of NO in the TGF system. We studied the homeostatic efficiency of TGF, constructing compensation profiles from closed-loop perturbation studies in which measurements were made in each nephron before and during addition of the NOS inhibitor L-NMMA to the late proximal tubule. L-NMMA increased both ambient and maximum FC and shifted the point of maximum compensation leftward relative to the OP (Fig. 5), thus implying the existence of an NO pool that is accessible from the tubular lumen and that exerts, at baseline, a tonic rightward force on the TGF curve. This pool of NO modulates, but cannot mediate, the TGF effector response because the efficiency of TGF is actually enhanced by NOS inhibition [16]. The possibility that TGF resets during

volume expansion due to changes in macula densa NO awaits testing.

In summary, renal vasoconstriction accompanying many forms of primary tubular injury has been attributed to TGF [17]. However, sustained stimulation of TGF, whether by acute volume expansion, direct supplementation of proximal tubular flow, or pharmacological inhibition of proximal reabsorption, rapidly resets TGF rightward. This challenges the idea that TGF can mediate prolonged vasoconstriction. The TGF-GTB system seems willing to exchange long-term control over distal delivery for the ongoing ability to respond efficiently to shorter term disturbances. Nonetheless, the TGF system, as traditionally defined, may not be the only route of communication from the tubule to glomerulus. There appears to be another system, linked to tubular flow, that acts more slowly than TGF and reduces SNGFR independently of TGF. This system may be activated more readily by microperfusion than by benzolamide. Whether the glomerular response to tubular injury differs from the response to benzolamide, because injury lowers the threshold for activating this slow feedback or because tubular injury interferes with the normal rightward resetting of TGF, remains to be studied.

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#### APPENDIX

Abbreviations used in this article are: FC, fractional compensation; GTB, glomerulotubular balance; JGA, juxtaglomerular apparatus; NO, nitric oxide; NOS, nitric oxide synthase; OP, operating point; SNGFR, nephron GFR; TGF, tubuloglomerular feedback;  $V_{LP}$ , late proximal flow; VMFV, videometric flow velocitometry.

#### REFERENCES

1. THURAU K, BOYLAN JW: Acute renal success: The unexpected logic of oliguria in acute renal failure. *Am J Med* 61:308–315, 1976
2. BLANTZ RC: The mechanism of acute renal failure after uranyl nitrate. *J Clin Invest* 55:621–635, 1975
3. TUCKER BJ, STEINER RW, GUSHWA LC, BLANTZ RC: Studies on the tubuloglomerular feedback system in the rat: The mechanism of reduction in filtration rate with benzolamide. *J Clin Invest* 62:993–1004, 1978
4. HOLSTEIN-RATHLOU NH, WAGNER AJ, MARSH DJ: Tubuloglomerular feedback dynamics and renal blood flow autoregulation in rats. *Am J Physiol* 260:F53–F68, 1991
5. MOORE LC, MASON J: Tubuloglomerular feedback control of distal fluid delivery: Effect of extracellular volume. *Am J Physiol* 250:F1024–F1032, 1986
6. THOMSON SC, BLANTZ RC: Homeostatic efficiency of tubuloglomerular feedback in hydropenia, euvoemia, and acute volume expansion. *Am J Physiol* 264:F930–F936, 1993
7. THOMSON SC, BLANTZ RC, VALLON V: Increased tubular flow induces resetting of tubuloglomerular feedback in euvoemic rats. *Am J Physiol* 270:F461–F468, 1996
8. HOLSTEIN-RATHLOU NH, MARSH DJ: Oscillations of tubular pressure, flow, and distal chloride concentrations in rats. *Am J Physiol* 256:F1007–F1014, 1989
9. BRIGGS JP, SCHUBERT G, SCHNERMANN J: Quantitative characterization of the tubuloglomerular feedback response: Effect of growth. *Am J Physiol* 247:F808–F815, 1984
10. MORSING P, VELAZQUEZ H, ELLISON D, WRIGHT FS: Resetting of tubuloglomerular feedback by interrupting early distal flow. *Acta Physiol Scand* 148:63–68, 1993
11. THOMSON SC, VALLON V, BLANTZ RC: Reduced proximal reabsorption resets tubuloglomerular feedback in euvoemic rats. *Am J Physiol* (in press)
12. LORENZ JN, WEIHPRECHT H, SCHNERMANN J, SKOTT O, BRIGGS JP: Characterization of the macula densa stimulus for renin secretion. *Am J Physiol* 259:F186–F193, 1990
13. PLOTH D, ROY R: Renin-angiotensin influences in tubuloglomerular feedback activity in the rat. *Kidney Int* 22(Suppl 12):S114–S121, 1982
14. SHULTZ PJ, TOLINS JP: Adaptation to increased dietary salt intake in the rat: Role of endogenous nitric oxide. *J Clin Invest* 91:642–650, 1993
15. DAVIS JM, HÄBERLE DA, KAWATA T: The control of glomerular filtration rate and renal blood flow in chronically volume-expanded rats. *J Physiol (Lond)* 402:473–495, 1988
16. VALLON V, THOMSON S: Inhibition of local nitric oxide synthase increases homeostatic efficiency of tubuloglomerular feedback. *Am J Physiol* 269:F892–F899, 1995
17. BRAAM B, MITCHELL KD, KOOMANS HA, NAVAR LG: Relevance of the tubuloglomerular feedback mechanism in pathophysiology. *J Am Soc Nephrol* 4:1257–1274, 1993