Effect of sustained flow perturbations on stability and compensation of tubuloglomerular feedback

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Oldson, Darren R., Leon C. Moore, and Harold E. Layton. Effect of sustained flow perturbations on stability and compensation of tubuloglomerular feedback. Am J Physiol Renal Physiol 285: F972–F989, 2003.—A mathematical model previously formulated by us predicts that limit-cycle oscillations (LCO) in nephron flow are mediated by tubuloglomerular feedback (TGF) and that the LCO arise from a bifurcation that depends heavily on the feedback gain magnitude, γ, and on its relationship to a theoretically determined critical value of gain, γc. In this study, we used that model to show how sustained perturbations in proximal tubule flow, a common experimental maneuver, can initiate or terminate LCO by changing the values of γ and γc, thus changing the sign of γ − γc. This result may help explain experiments in which intratubular pressure oscillations were initiated by the sustained introduction or removal of fluid from the proximal tubule (Leyssac PP and Baumbach L. Acta Physiol Scand 117: 415–419, 1983). In addition, our model predicts that, for a range of TGF sensitivities, sustained perturbations that initiate or terminate LCO can yield substantial and abrupt changes in both distal NaCl delivery and NaCl delivery compensation, changes that may play an important role in the response to physiological challenge.

THE TUBULOGLOMERULAR FEEDBACK (TGF) SYSTEM is a key moment-to-moment regulator of the single-nephron glomerular filtration rate (SNGFR). A large body of experimental and theoretical evidence indicates that TGF can mediate sustained, regular oscillations of 20–47 mHz in tubular flow, pressure, and intratubular thick ascending limb (TAL) NaCl concentration (4, 6, 7, 12, 18). These regular oscillations are called limit-cycle oscillations (LCO) because, after initiation, they tend to more and more closely approximate a fixed cycle, provided that system parameters do not change. Spontaneous LCO appear to occur in large numbers of nephrons, as they have been detected in recordings of renal blood flow and pressure taken in conscious, chronically instrumented dogs (8).

A common and useful method of investigating TGF is to impose sustained perturbations of proximal tubule (PT) fluid flow in a freely flowing nephron where the TGF feedback loop is closed and functional (3, 5, 6, 20, 21, 26). Using this technique, Leyssac and collaborators (5, 17, 18) have demonstrated that LCO can be initiated or extinguished in halothane-anesthetized rats by insertion or removal of PT fluid, findings that indicate that PT fluid flow can have a substantial effect on the stability of the TGF system. Some insight into the basis of this phenomenon is provided by our previous investigations of the TGF-mediated LCO (12–16, 22). These modeling studies indicate that LCO will emerge for sufficiently large feedback loop gain magnitude (12), that the parameter regime that supports LCO may overlap the parameter regime of normal TGF operation, that a gain magnitude near that needed for LCO will produce maximal feedback compensation (16), and that LCO may augment NaCl delivery to the distal nephron (16). A finding of fundamental importance is that the emergence of LCO depends on key TGF system parameters and variables that, in turn, depend on the physiological state of the nephron or on the animal as a whole. For example, the parameters may be affected by an increase in systemic blood pressure, a resetting of the TGF response curve, a primary change in tubular transport, or an experimental intervention such as microperfusion of fluid into the PT. We have previously presented numerical simulations that indicate that sustained PT flow perturbations can elicit LCO or extinguish LCO (16). However, such simulations cannot determine how the feedback loop gain or the gain threshold at which LCO emerge is influenced by PT flow perturbations.

The principal objectives of this study were to analyze, in the context of our mathematical model, the effect of sustained PT flow perturbations on feedback loop gain, on the gain threshold at which LCO emerge, and on feedback compensation. By means of this analysis we provide a theoretical framework for understanding how fluctuations in physiological variables impact the stability of the TGF system and its regulatory efficacy.

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the “operating point” of the TGF response function. Frequently, that operating point is found to be near the steepest portion of the TGF response function (1); in the standard form of that function (a logistic curve that is equivalent to a scaled hyperbolic tangent function), the point of steepest slope corresponds to the point where the response function changes from concave down to concave up, i.e., to the point of inflection.

Also associated with steady-state flow, and thus with its corresponding operating point, is feedback gain, a measure of feedback signal amplification. Roughly speaking, gain can be measured by breaking the TGF (signal) loop (e.g., breaking the signal loop at the beginning of the thick limb), increasing the feedback loop signal by a small amount (e.g., increasing TAL flow by ΔFAL), and measuring the resulting change in the return signal (e.g., in a short-looped nephron, measuring the change in terminal descending limb flow, ΔFDL). Gain is then approximated by the resulting change divided by the signal increase (e.g., by ΔFDL/ΔFAL).

Two concepts of gain, steady-state gain (GSS) and instantaneous gain (−γ), have been applied to the TGF loop (13); each can be viewed as an interpretation of ΔFDL/ΔFAL (see APPENDIX A). Steady-state gain is the gain that has been measured in laboratory experiments; the concept of instantaneous gain arose from a theoretical analysis of a mathematical model of the TGF system (12, 13). We have previously shown that, under restricted circumstances, GSS is closely approximated by −γ (13).

For a given set of model parameters (see model below), there is a particular steady-state feedback loop configuration with an associated operating point and with associated values of −GSS and γ. In the context of our model’s normalized quantities, steady-state gain magnitude −GSS corresponds to the product of the magnitude of the slope of the feedback response function (see Eq. 2) at the operating point and the derivative of chloride concentration in luminal fluid alongside the MD with respect to TAL flow, also at the operating point; γ corresponds to the product of the slope of the feedback response function at the operating point, the derivative of chloride concentration at the MD with respect to TAL axial position, and the steady-state TAL fluid transit time.

A model steady state may be either stable or unstable. If a steady state is stable (stable steady state, SSS), model solutions will more and more closely approximate that steady state after a transient perturbation (as, e.g., a transient addition to SNGFR). If a
steady state is unstable (unstable steady state, USS), model solutions will more and more closely approximate LCO after a transient perturbation, no matter how small the perturbation.

For a sufficiently long delay in TGF signal transmission at the juxtaglomerular apparatus (JGA), the stability of a steady state is determined by the gain\(^1\)\(\gamma\) and by the critical gain \(\gamma_c\) (12). If \(\gamma < \gamma_c\), then the stable state is a (time-independent) steady state; if \(\gamma > \gamma_c\), then the stable state is an LCO. The critical gain \(\gamma_c\) can be determined by numerical experiments in which one varies \(\gamma\) to find the boundary between the stable and unstable steady states or by use of a characteristic equation, which can be derived from our model and which can be used to find a general expression for \(\gamma_c\) in terms of model parameters.

A steady state (whether stable or not), and thus its associated operating point, may be altered by non-TGF-regulated factors that increase effective SNGFR (and thus TAL flow), e.g., an increase in systemic blood pressure or an experiment in which fluid is inserted into the PT. A steady state may also be altered by a change in the slope of the feedback response curve, if the operating point does not coincide with the point of inflection. A convenient parameter for characterizing the slope of the response curve is the feedback sensitivity \(k\), which for the purposes of this study is defined to be the magnitude of the slope of the feedback response curve at its inflection point. We define the critical sensitivity \(k_c\) to be the sensitivity corresponding to a case in which \(\gamma = \gamma_c\). Thus, if \(k > k_c\), the model solution will tend toward an LCO; if \(k < k_c\), the model solution will tend toward the SSS.

A sustained flow perturbation or other sustained change in model parameters has the potential to change both \(\gamma\) and \(\gamma_c\) and thus change the relationship between them (i.e., change the sign of \(\gamma - \gamma_c\)). Thus a stable state may be affected by a perturbation, so that an SSS may become a USS, resulting in a stable LCO, or a state characterized by LCO may become an SSS. Figure 3 (see below) summarizes the dependence of stable model behavior on gain, operating point, flow perturbations, and TGF sensitivity.

**Mathematical Model**

Our model is given by the following system of coupled equations

\[
\frac{\partial}{\partial t} C(x, t) = - F(C_{MD}(t)) \frac{\partial}{\partial x} C(x, t) - \frac{V_{max} C(x, t)}{K_M + C(x, t)} - P(C(x, t) - C(x)) \\
F(C_{MD}(t)) = 1 + K_t \tanh \left( K_d (C - C_{MD}(t)) + \rho \right) \\
C_{MD}(t) = \int_{-\infty}^{t} \psi(t - s - \delta/2)C(1, s - \tau_p) \, ds
\]

Each equation is in nondimensional form (see Appendix B). The space variable \(x\) is oriented so that it extends from the entrance of the TAL \((x = 0)\), through the outer medulla, and into the cortex to the MD \((x = 1)\). Figure 1 gives a schematic representation of the model.

**Equation 1** is a partial differential equation for the chloride concentration \(C\) in the intratubular fluid of the TAL of a short-looped nephron. We assume that fluid entering the TAL has constant chloride concentration; thus, we assume that \(C(0, t)\) always equals 1. At time \(t = 0\), initial concentrations \(C(x, 0)\) (for \(x \in (0, 1)\)) and \(C(1, t)\) (for \(t \in (-\infty, 0)\)) must be specified. For \(t > 0\), the rate of change of that concentration depends on processes represented by the three right-hand terms in Eq. 1. The first term is axial advective chloride transport at intratubular flow speed \(F\). The second is transtubular efflux of chloride driven by active metabolic pumps situated in the tubular walls; that efflux is assumed to be approximated by Michaelis-Menten kinetics, with maximum transport rate \(V_{max}\) and Michaelis constant \(K_M\). The third term is transtubular chloride backleak, which depends on a specified fixed

\[\text{gain.}\]

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\(^1\)In a negative feedback system, gain is negative by convention. However, it is usually more convenient to think in terms of gain magnitude \(\gamma\), and for simplicity we will frequently refer to \(\gamma\) as the "gain."
extratubular chloride concentration profile \( C_e(x) \) and on chloride permeability \( P \).

Equation 2 describes fluid speed in the TAL as a function of effective luminal chloride concentration \( C_{\text{MD}} \) at the MD. This feedback relationship is an empirical equation well established by steady-state experiments (23). The constant \( C_1 \) is the inflection point of the TGF response curve; in our model, it is also the chloride concentration at the MD when \( F = 1 \) and the chloride concentration profile in the TAL has assumed a steady state. The positive constants \( K_1 \) and \( K_2 \) describe, respectively, the range of the feedback response and its sensitivity to deviations from the steady state.

The constant \( \rho \) represents a flow perturbation applied into Bowman’s space. Owing to the nondimensional model formulation and to our assumption that a fixed fraction of the glomerular filtrate is delivered to the TAL, \( \rho \) can represent a fractional flow perturbation applied into Bowman’s space or into the tubular lumen at the entrance of the TAL (in the nondimensional formulation, the TAL flow rate \( F \) and the SNGFR \( Q \) have the same value, because the dimensional \( F \) and \( Q \) have both been scaled by their respective base-case values).

Equation 3 represents time delays in the feedback pathway between the luminal fluid chloride concentration at the MD, \( C(1,t) \), and an effective MD concentration, \( C_{\text{MD}}(t) \), which is used to calculate the flow response that is mediated by AA smooth muscle. In a quasi-steady state, Eq. 2 provides a good description of the TGF response. However, dynamic experiments (2) show that a change in MD concentration does not significantly affect AA muscle tension until after a discrete (or pure) delay time \( \tau_p > 0 \), and then the effect is distributed in time so that a full response requires additional time, with greatest weight in the time interval \([t - \tau_p - \delta, t - \tau_p]\), where \( \delta > 0 \) is a second delay parameter. To simulate the pure delay followed by a distributed delay, the convolution integral given in Eq. 3 is used to describe the effective signal received by the AA at time \( t \) (22). The kernel function \( \psi_p \) for this integral is given by

\[
\psi_p(u) = \begin{cases} 
(1 + \cos(2\pi u/\delta))/\delta, & -\delta/2 \leq u \leq \delta/2 \\
0, & |u| > \delta/2 
\end{cases}
\]

(4)

With this function, a step change in \( C \) results in a sigmoidal increase in \( C_{\text{MD}} \) over a nondimensional time interval of \( \delta \).

Model parameters. A summary of parameters and variables, with their dimensional units as commonly reported, is given in the Glossary. The base-case parameters, which collectively represent the reference point for our parameter studies, are given in Table 1; the criteria for their selection and supporting references were given in Ref. 12. The extratubular chloride concentration is given in nondimensional form by \( C_e(x) = C_0(A_1e^{-A_1x} + A_2) \), where \( A_1 = (1 - C_0(1)/C_0)/(1 - e^{-A_3}) \), \( A_2 = 1 - A_1 \), \( A_3 = 2 \), and \( C_0(1) \) corresponds to a cortical interstitial chloride concentration of 150 mM. A graph of \( C_e \) for \( F = 1 \) was given in Fig. 1 of Ref. 12. The steady-state operating MD chloride concentration that corresponds to \( \rho = 0 \), which is \( C_1 \), can be calculated numerically using the TAL dimensions and transport parameters, with steady flow \( F = 1 \) in Eq. 1 for the time interval equal to the washout time of the TAL at that flow, 1 dimensionless time unit.

Model solutions. For the case of no perturbation, i.e., \( \rho = 0 \), a steady-state solution to Eqs. 1–4 may be obtained and sustained by fixing \( C(1,t) = C_1 \) for \( t > 0 \), fixing \( F \) at the base-case value of 1 for the TAL transit time interval (here, \( t \in [0,1] \)), solving Eq. 1 for that time interval (to obtain \( C(1,1) = C_1 \)), and then closing the TGF loop so that \( F \) is computed via Eqs. 2–4 for \( t > 1 \). Then \( F(C_{\text{MD}}(t)) \) will equal 1 for all time.

The steady state for \( \rho \neq 0 \) is more difficult to obtain because it corresponds to an unknown steady-state value of \( F \), a value that in general we will call \( F_{\text{op}} \) (thus \( \rho = 0 \) implies \( F_{\text{op}} = 1 \), whereas \( \rho \neq 0 \) implies \( F_{\text{op}} \neq 1 \)). Because we have previously established that, if \( \tau_p = 0 \) and \( \delta = 0 \) a model steady state is stable (12), \( F_{\text{op}} \) can be found directly by eliminating the delay at the MD (thus, \( C_{\text{MD}}(t) = C(1,t) \)) and solving Eqs. 1 and 2 until the solutions are sufficiently close to steady state. The long-time values of \( C_{\text{MD}}(t) \) and \( F(C_{\text{MD}}) \) will closely approximate \( C_{\text{op}} \) and \( F_{\text{op}} \).

Alternatively, one can consider the inverse problem in which one specifies \( F_{\text{op}} \) and finds the corresponding \( \rho \). Thus one can fix \( F = F_{\text{op}} \) in Eq. 1 for an interval equal to the TAL transit time at flow speed \( F_{\text{op}} \) (thus for \( t \in [0,1/F_{\text{op}}] \)), find \( C_{\text{op}} = C(1,1/F_{\text{op}}) \), assume that \( C(1,t) = C_{\text{op}} \) for \( t \leq 1/F_{\text{op}} \), and choose \( \rho \) so that for \( t > 1/F_{\text{op}} \), \( F(C_{\text{MD}}(t)) = F_{\text{op}} \) in Eq. 2. If the feedback loop is closed at \( t = 1/F_{\text{op}} \), then \( F(C_{\text{MD}}(t)) = F_{\text{op}} \) for all time.

When \( F_{\text{op}} \) is known, \( C_{\text{op}} \) may be obtained by solving the time-independent form of Eq. 1

\[
\frac{\partial}{\partial x} S(x,F) = -\frac{1}{F} \left( \frac{V_{\text{max}} S(x,F)}{K_M + S(x,F)} + P (S(x,F) - C_e(x)) \right)
\]

(5)

\( \text{This value, which is somewhat higher than typical cortical interstitial } Cl^- \text{ concentration, and which approximates interstitial } Na^+ \text{ concentration, is used because we model transport of only a single solute, taken to be } NaCl, \text{ and we assume that transport fluxes of } Cl^- \text{ and } Na^+ \text{ are identical.} \)
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where \( S \), the steady-state profile of the TAL chloride concentration \( C \), is considered to be a function of position \( x \) and steady flow \( F \), \( F \) is set equal to \( F_{\text{op}} \), the boundary condition corresponding to \( C(0, t) = 1 \) is \( S(0, F) = 1 \), and \( C_{\text{op}} = S(1, F_{\text{op}}) \). Steady-state profiles \( S(x, F) \), for various values of \( F \), were given in Fig. 1 of Ref. 12.

A dynamic solution to Eqs. 1–4 can be found by taking the corresponding steady-state solution as initial data and perturbing \( \rho \) transiently. The long-time solution, depending on model parameters, will tend either to a steady state or to an LCO.

**Steady-state gain.** The steady-state gain, which has been measured in experiments (1, 3), is expressed (13) in terms of dimensionless model variables as

\[
G_{\text{SS}} = \left. F'(C_{\text{op}}) \frac{\partial}{\partial F} S(1, F) \right|_{F = F_{\text{op}}}
\]

where the prime symbol indicates differentiation with respect to the argument of the TGF response function \( F \).

**Characteristic equation and instantaneous gain.** Information about the stability of a steady-state solution of the model TGF system (i.e., how it will respond to a transient perturbation) can be obtained from the model’s characteristic equation,

\[
1 = -\gamma e^{-\lambda_{\text{op}}(1-\varepsilon/2)} \left( \int_0^1 e^{-\lambda_{\text{op}}(1-\varepsilon)} \exp \left( \frac{-P}{F_{\text{op}}} \int_0^1 \frac{C(y)}{\frac{\partial}{\partial y} S(y, F_{\text{op}})} dy \right) dx \right) \times \left( \int_{-\varepsilon/2}^{\varepsilon/2} \psi(u)e^{-\lambda_{\text{op}}u} du \right)
\]

where the instantaneous gain magnitude \( \gamma \), in terms of normalized variables, is given by

\[
\gamma = \left. \frac{F'(C_{\text{op}})}{F_{\text{op}}} \frac{\partial}{\partial x} S(x, F_{\text{op}}) \right|_{x = 1}
\]

This characteristic equation generalizes previous versions (12, 15) by including the effect of \( F_{\text{op}} \), previously, we assumed \( F_{\text{op}} = 1 \) so that \( F_{\text{op}} \) did not appear. The method of derivation of the characteristic equation was explained previously (12, 13, 22).

There are two ways in which the characteristic equation may be used. First, given a particular set of model parameters, one can find the corresponding steady-state solution \( S(x, F_{\text{op}}) \), from that solution obtain \( \gamma \) via Eq. 8, substitute that \( \gamma \) in the characteristic equation, and then solve the characteristic equation (by techniques of numerical analysis) for the corresponding real and complex parts of the eigenvalues \( \lambda_n \) (\( n = 1, 2, 3, \ldots \)) that satisfy the characteristic equation when \( \lambda = \lambda_n \). If all the real parts of the \( \lambda_n \)’s are negative, then the model solution is an SSS. If the real part of any one of the eigenvalues is positive, then the steady state is a USS.

Conversely, given a set of model parameters, and the corresponding steady-state solution \( S(x, F_{\text{op}}) \), one can use the characteristic equation to solve for a particular value of \( \gamma \), the critical gain \( \gamma_c \), which corresponds to the transition between the SSS and the USS: \( \gamma_c \) is the smallest \( \gamma \) such that the real part of an eigenvalue \( \lambda_n \) equals 0. Thus, if one is given an external concentration \( C_e \), a steady-state profile \( S \) with associated \( F_{\text{op}} \) (a steady state based on the feedback response parameters), delay parameters \( \tau_p \) and \( \delta \), and backleak permeability \( P \), then one can determine the critical gain \( \gamma_c \), and compare it with the feedback gain \( \gamma \) (computed by Eq. 8) to determine whether a steady-state solution is stable; this is how the characteristic equation was used in this study. Because a steady-state solution and its corresponding operating values \( C_{\text{op}} \) and \( F_{\text{op}} \) are altered by a sustained flow perturbation introduced through \( \rho \), both \( \gamma \) and \( \gamma_c \) depend on \( \rho \) (see Fig. 3).

**Changing the physiological state: sustained flow perturbation and TGF sensitivity.** In this study, we assume that the model physiological state is determined by two parameters. The first parameter, the sustained flow perturbation \( \rho \) (which appears in Eq. 2), quantifies all non-TGF-related factors affecting SNGFR. Such factors include sustained microperfusion into the PT, changes in mean systemic arterial pressure, and changes in extracellular volume (ECV). The second parameter, the TGF sensitivity \( \kappa \), is defined to be the magnitude of the slope of the TGF response curve at its inflection point, which has been shown to depend on the physiological state (1, 29). By maximizing the slope of the TGF response given by Eq. 2 as a function of \( C_{\text{MD}} \), one finds that the dependence of model TGF sensitivity on model parameters is given by

\[
\kappa = F'(C_1) = -K_1K_2
\]

In this study, \( \kappa \) was varied by changing \( K_2 \).

**Feedback compensation.** The efficacy of model TGF regulation can be quantified by calculating feedback compensation, an index used in experimental investigations (10, 20, 26). Feedback compensation is defined by

\[
\text{compensation} = (1 - M) \times 100\%
\]

where \( M \), the magnification, is defined by

\[
M = \frac{\Delta Y}{\Delta X_{\text{CL}}}
\]

In the definition for magnification, \( Y \) is a system variable that is regulated by means of the feedback loop (e.g., distal chloride delivery); \( \Delta Y \) is the change in the system variable \( Y \) in response to a change \( \Delta X \) (i.e., a perturbation) in another system variable \( X \) (e.g., PT flow). The denominator of Eq. 11, \( \Delta Y/\Delta X_{\text{CL}} \), is the ratio of \( \Delta Y \) to \( \Delta X \) in the case where the feedback loop is open (open-feedback-loop case, or OL). The numerator \( \Delta Y/\Delta X_{\text{CL}} \) is the corresponding ratio when the loop is closed (closed-feedback-loop case, or CL). If \( \Delta X \) tends to zero, \( M \) converges to a ratio of derivatives; however, the \( \Delta \)-notation is retained because it is consistent with experimental studies, which necessarily entail measurable perturbations.

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Because the stable state of the model TGF system may be an LCO or a stable steady state, and the type of stable state (LCO or steady state) may differ for $\Delta X = 0$ and $\Delta X \neq 0$, “$\Delta Y$” requires further interpretation to be well defined. We thus adopt two conventions with respect to computing $\Delta Y$: 1) specific values of $Y$ are time averages that correspond to the stable state of the system under the given perturbation; and 2) changes in $Y$ are normalized with respect to the stable, nonperturbed state of the system. We thus set $\Delta Y = (Y_A - Y_0)/Y_0$, where $Y_0$ is the time-averaged value of $Y$ obtained from the stable state when $\Delta X = 0$, and $Y_A$ is the time-averaged value of $Y$ obtained from the stable state when $\Delta X \neq 0$ (if the stable state of the system is an LCO for zero perturbation, $Y_A$ is compared to the zero perturbation stable-state average, even if the perturbation $\Delta X$ has resulted in an SSS). We normalize with respect to $Y_0$ because $Y_0$, the time-averaged value of $Y$ obtained from the stable state when $\Delta X = 0$, may differ for the OL and CL model TGF systems.

If a steady state is the stable state, distal chloride delivery is computed as the product of the steady-state MD chloride concentration and the steady-state TAL fluid flow rate at the MD. If an LCO is the stable state, distal chloride delivery is computed as the time average of the product of the chloride concentration at the MD and the TAL fluid flow rate at the MD.

Numerical methods. Details of the numerical methods used to obtain results are given in Appendix C. The base-case parameter values (excepting those for $\rho$ and $\kappa$) were used in all calculations.

RESULTS

Steady-State Gain Is Well Approximated by Instantaneous Gain

Because we aim to make predictions that are related to experiments, it is incumbent on us to provide evidence that the instantaneous gain $\gamma$ that arises in our theoretical analysis can be estimated from experiments that measure steady-state gain.

Previously, we have calculated, for the case of no sustained flow perturbation and for base-case parameters, that the instantaneous gain magnitude $\gamma$ exceeds steady-state gain magnitude $-G_{SS}$ by $\sim 10\%$ (13). Here we report further calculations to demonstrate good agreement between instantaneous gain and steady-state gain in steady states corresponding to sustained flow perturbations over a range of feedback sensitivities. Some results of those calculations are illustrated in Fig. 2, which shows instantaneous and steady-state gain magnitudes $\gamma$ and $-G_{SS}$ for TGF sensitivities $k_4$ and $k_5$ (sensitivities that yield $\gamma = 4$ and $\gamma = 8$ at zero perturbation, $\rho = 0$). The value of $\rho$ used in this figure and subsequently in this section is the dimensional value of a sustained flow perturbation in early PT.

The relative difference between the two measures of gain, at zero perturbation, is $10.3\%$, for both $k_4$ and $k_5$.

![Diagram](https://example.com/diagram.png)

Fig. 3. Relationships among quantities that characterize the feedback loop and the stable state. White boxes, TGF system concepts; gray boxes, model analogs. A: parameters used to represent model physiological state. B: four quantities ($Q_{op}$, $C_{op}$, $\gamma$, $\gamma_c$) associated with a particular time-independent steady-state operating point. Each model quantity depends on variables in A, as implied by arrows. C: determination of stable state of model TGF system. If $\gamma > \gamma_c$, the time-independent steady state is the unstable steady state (USS), and a transient flow perturbation will lead to limit-cycle oscillations (LCO). If $\gamma_c > \gamma$, oscillation initiated by a transient flow perturbation will diminish in time and the model solution will converge to the time-independent stable steady state (SSS).
found that the relative differences tend to stabilize and perturbations tend to $-12$ nl/min. Indeed, for sensitivities greater than $\kappa_2$ and perturbations greater than $-8$ nl/min, the maximum relative difference was 12.4%. However, in the “corner” region marked off by sensitivities $\kappa$ such that $\kappa_1 \leq \kappa \leq \kappa_2$ and by perturbations $\rho$ such that $-10$ nl/min $\leq \rho \leq -8$ nl/min, the maximum relative difference was 16.3%. The difference between the two gain values in this corner is not likely to be physiologically significant, however, because in that region both values are less than $-1.2$ and their difference is never larger than $-0.14$.

By means of an analysis like that in Ref. 13, we found that $\gamma$ exceeds $-G_{SS}$ for all perturbations at a given sensitivity.

**Shape of the $\gamma$-Curve**

The dependence of $\gamma$ on flow perturbations and feedback sensitivity can be understood by considering the two steps involved in calculating $\gamma$ (Fig. 3). Given a flow perturbation $\rho$ and a feedback sensitivity $\kappa$, one determines the operating steady-state TAL flow $F_{op}$, and then one evaluates the three factors comprising $\gamma$ (given by Eq. 8): $F'(C_{op})$, $1/F_{op}$, and $\gamma(S(x), F_{op})|_{x=1}$.

The model's steady-state operating point is represented by $Q_{op}$ and $C_{op}$, where $Q_{op}$ is the effective SNGFR (which is defined to be the sum $Q + \rho$), and $C_{op}$ is the MD chloride concentration that corresponds to the steady-state TAL flow $F_{op}$. The calculation of the steady-state operating point is represented graphically in Fig. 4; the operating point corresponds to the intersection of the MD chloride concentration curve and the TGF response curve. Figure 4, A and B, illustrates the variation of the intersection point with sustained perturbation $\rho$ for two different feedback sensitivities $\kappa$. A sustained flow perturbation affects the location of the intersection point by translating the TGF response curve vertically (see Eq. 2). The range of operating points is smaller for the case of higher feedback sensitivity, because the higher sensitivity represents a stronger TGF response and thus a response with greater feedback compensation. Figure 4C explicitly shows the dependence of $Q_{op}$ and $F_{op}$ on $\rho$ for the feedback sensitivities used in Fig. 4, A and B. For the higher sensitivity $\kappa_2$, $F_{op}$ deviates less from the base-case TAL operating flow rate of 6 nl/min, compared with the lower sensitivity $\kappa_4$.

Varying $\rho$ and $\kappa$ has a substantial effect on $F'(C_{op})$ (the slope of the TGF response curve at the operating MD chloride concentration $C_{op}$) because of their impact on the steady-state operating point. As the perturbation $\rho$ increases over its full range, the operating point moves along the TGF response curve from a region of small slope to a region of large slope and then to another region of small slope (see Fig. 4, A and B). This is the primary reason that $\gamma$ depends on $\rho$ in the way shown in Fig. 5A1: $\gamma$ is largest for $\rho$ near zero, and $\gamma$ decreases as the magnitude of $\rho$ increases. Figure 5A1 also shows that, as long as the magnitude of the perturbation is not too large, $\gamma$ increases in magnitude as...
the TGF sensitivity increases. This results from the way that $\kappa$ affects $F'(C_{op})$. As $\kappa$ increases, the maximal slope of the response curve increases, which increases the slope of the response curve at the operating point. Thus $F'(C_{op})$, and hence $\gamma$, increases in magnitude as the TGF sensitivity increases, for values of $\rho$ that are not too large. The factors $1/F_{op}$ and $\frac{1}{x}S(x, F_{op})|_{x=1}$ do not change as much as $F'(C_{op})$ does when $\rho$ or $\kappa$ changes; thus the shape of the $\gamma$-curve primarily reflects the variation of the factor $F'(C_{op})$. However, the effects of the factors $1/F_{op}$ and $\frac{1}{x}S(x, F_{op})|_{x=1}$ can be seen in Fig. 5A1, which shows that the graph of $\gamma$ is not exactly symmetrical with respect to $\rho = 0$.

Shape of the $\gamma_c$-curve. Figure 5A2 illustrates the dependence of critical gain $\gamma_c$ on $\rho$ for sensitivities $\kappa_4$ and $\kappa_8$, a dependence computed by means of the characteristic equation, Eq. 7 ($\rho$ and $\kappa$ play a role in determining $\gamma_c$ through their influence on the steady-state TAL flow $F_{op}$; see Fig. 3). The decrease in $\gamma_c$ as a function of increasing perturbation largely arises from the accompanying increase in $F_{op}$, which increases the ratio of the JGA delay times ($\tau_p$ and $\delta$) to TAL fluid transit time, $1/F_{op}$ (based on an analysis similar to that given in Ref. 12). Because of the smaller variation of $\gamma_c$, relative to $\gamma$, as a function of $\rho$ (note the different scales on the vertical axes of Fig. 5, A1 and A2), variation of $\gamma$ primarily determines the sign of $\gamma - \gamma_c$ and thus the stable state of the model system (LCO or SSS).

Dependence of the Sign of $\gamma - \gamma_c$ on $\rho$

In Fig. 5, B1 and B2, the gain curves from Fig. 5, A1 and A2, are matched according to the sensitivities $\kappa_4$ or $\kappa_8$. For sustained flow perturbations between $a$ and $b$, or between $c$ and $d$, the stable state is an LCO ($\gamma > \gamma_c > 0$), and for $\rho$ outside those regions the model solution tends toward an SSS ($\gamma < \gamma_c < 0$). As TGF sensitivity $\kappa$ increases, the gain $\gamma$ changes more rapidly than critical gain $\gamma_c$, and therefore the range of values for which an LCO is the stable state increases as $\kappa$ increases. These results provide a theoretical explanation for a well-known observation: if the TGF response curve is sufficiently steep at its inflection point, and if the administered PT microperfusion is sufficiently small, then TGF mediates regular, sustained oscillations in tubular flow.

Critical Sensitivity Curve

The curve in Fig. 6A gives critical sensitivity as a function of perturbation $\rho$ (recall that, given a sensitivity $\kappa$, if there is a sustained perturbation $\rho$ such that the instantaneous gain $\gamma$ is equal to the critical gain $\gamma_c$, then that sensitivity $\kappa$ is defined to be the critical sensitivity $\kappa_4$ for that $\rho$; in Fig. 5B1, $\kappa_4$ equals the critical sensitivity for $\rho = a$ and $\rho = b$, and in Fig. 5B2, $\kappa_8$ is the critical sensitivity for $\rho = c$ and $\rho = d$). Because both $\gamma$ and $\gamma_c$ depend on $\rho$, the curve in Fig. 6A can be calculated directly only by means of exhaustive numerical simulations based on Eqs. 1–4. Therefore, we used the following implicit procedure to calculate points on the curve in Fig. 6A. We began by selecting a
Fig. 6. A: curve: dependence of critical sensitivity $\kappa_c$ on sustained flow perturbation $\rho$, where $\kappa_c$ is defined to be the feedback sensitivity for which $\gamma = \gamma_c$. For points (model physiological states) inside and above curve, the stable solution is an LCO; below and outside curve, solution is an SSS. Perturbations of size $a$ and $b$, and the hatched gray bar, correspond to the same features on Fig. 5B1; perturbations of size $c$ and $d$, and the solid gray bar, correspond to the same features on Fig. 5B2. By summarizing the locations of all points (model physiological states) where $\gamma = \gamma_c$, the black curve generalizes results in Figs. 5B1 and 5B2. This panel summarizes the dependence of $\gamma$ on $x$ and $y$.

B: numerical experiments for points S and T in A. In each experiment, TGF model was initialized at the steady state determined by $\rho$ and $k$. A transient pulse of 0.3 nl/min was added to the sustained perturbation, and the system was allowed to converge to its stable state. Because S is inside the region for which $\gamma > \gamma_c$, effective SNGFR develops LCO; T is outside this region, so the system returns to the SSS. C1: TGF response curves for perturbations of 5.0 and 9.5 nl/min; resulting steady-state operating points, at intersection with curve giving effective SNGFR as a function of MD concentration, correspond to points U and V in A. Solid circle indicates SSS; open circle indicates USS. C2: effect of instantaneous change in $\rho$ from point U to point V in A. Approximate amplitude range of LCO corresponds to wide gray curve in C1. D1: experimental record showing effect of a $-10$ nl/min perturbation of late PT flow on PT pressure: spontaneous oscillations are suppressed by perturbation, and the effect is reversible. D2: model results analogous to experiment in D1. Oscillatory state is taken to have zero perturbation and sensitivity $\kappa_y$, corresponding to point X in A; sustained perturbation of $-10$ nl/min, corresponding to point W in A, suppresses oscillation. E1: experimental record showing effect of a 7.5 nl/min perturbation in late PT flow on PT pressure: spontaneous oscillations increase in magnitude; effect is reversible. E2: model results analogous to experiment in panel E1. Initial LCO for model physiological state near critical sensitivity curve, point Y in A; 7.5 nl/min increase in $\rho$ causes magnitude of oscillation to increase. [D1 and E1 are reprinted from Holstein-Rathlou and Leyssac (Figs. 12 and 13 top in Ref. 5).]
value of $F_{op}$, next, we calculated the corresponding $\gamma_c$ via the characteristic equation (Eq. 7); finally, Eqs. 8 and 2 were used to determine the sensitivity $\kappa$ and the sustained perturbation $\rho$, respectively, needed to achieve the selected value $F_{op}$.

The points $a$, $b$, $c$, and $d$ on the $\rho$ perturbation axis in Fig. 6A are the same as those shown in Fig. 5, B1 and B2. The critical sensitivity corresponding to points $a$ and $b$ in Fig. 6A, $\kappa_B$, corresponds to the sensitivity $\kappa_s$ in Fig. 5, and the critical sensitivity $\kappa_s$ corresponding to points $c$ and $d$ in Fig. 6A is the sensitivity $\kappa_s$ used in Fig. 5. Thus, Fig. 6A can be viewed as a summary of the results of many experiments like those shown in Fig. 5, B1 and B2, although the curve given in Fig. 6A was calculated by means of the efficient and accurate technique described in the previous paragraph. Above the curve, the stable solution is an LCO, whereas below and outside the curve the stable solution is a steady state.

Critical Sensitivity Curve: Tests and Examples

Figure 6B gives the results of two numerical experiments conducted to test the critical sensitivity curve in Fig. 6A. Values of the sustained perturbation $\rho$ and the TGF sensitivity $\kappa$ were selected to place the model TGF system just inside (at point S) or just outside (at point T) the critical sensitivity curve. For each case, the model system was initialized at the time-independent steady-state operating point determined by those values (see Fig. 3). Then, a transient flow perturbation was applied at an elapsed time of 6 s. For the state just inside the curve, an LCO developed; however, for the state just outside the curve, the oscillations initiated by the transient flow perturbation rapidly decreased in amplitude and the solution approached an SSS. These tests confirm the explicit analytical results summarized in Figs. 5 and 6A.

Figure 6C1 depicts the effects of two different sustained flow perturbations, $\rho = 5.0$ and $\rho = 9.5$ nl/min, on the TGF response curve and on the corresponding operating points, indicated by the open and closed circles. The critical sensitivity curve in Fig. 6A and the points U and V corresponding to these perturbations predict that the stable state for $\rho = 9.5$ is a steady state (marked by the closed circle in Fig. 6C1) and that the stable state for $\rho = 5.0$ is an LCO (the associated unstable steady-state operating point is marked by the open circle in Fig. 6C1). The steady-state operating point for the smaller perturbation is closer to the steepest portion of the TGF response curve, compared with the operating point for the larger perturbation. Figure 6C2 illustrates the effect of suddenly changing (at time 4.0 min) the applied perturbation from $\rho = 9.5$ to $\rho = 5.0$ and then suddenly restoring the original perturbation (at time 8.0 min). Oscillations emerge and are damped out as predicted by the locations of points U and V in Fig. 6A. Note that the flow range of the oscillation, indicated by the thick gray curve in Fig. 6C1, occupies a significant portion of the sloped part of the TGF response function. This model simulation gives results similar to those obtained in experiments by Leyssac and Baumbach (18): in halothane-anesthe-

tized rats, PT pressure oscillations can be initiated or terminated by adding fluid to (or removing fluid from) the late PT via a micropipette.

Figure 6D1, adapted from a study by Holstein-Rathlou and Leyssac (5), illustrates PT pressure from an experiment in a halothane-anesthetized Sprague-Dawley rat. The stable state approximated an LCO, but the removal of PT fluid (a perturbation of $-10$ nl/min) resulted in an SSS (albeit with a superimposed high-frequency oscillation from respiration). The removal of the perturbation resulted in a return to an LCO, and the response to the perturbation was repeatable. Figure 6D2 contains analogous results from our model. We began in a state, corresponding to X in Fig. 6A, in which the steady-state operating point is at the inflection point of the TGF response curve; moreover, because $\kappa > \kappa_c$, the stable state is an LCO. The introduction of a $-10$ nl/min flow perturbation$^3$ resulted in the relocation of the steady-state op-

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$^3$Because our flow perturbation is applied into Bowman’s space, our $-10$ nl/min perturbation would correspond to a somewhat smaller negative flow perturbation applied in the late PT, owing to proximal absorption. Thus, in our simulations, the TGF system is perturbed somewhat less than in Ref. 5; this difference, however, does not affect our results qualitatively.
erating point to the point corresponding to W in Fig. 6A, and that steady state was stable. The removal of the perturbation resulted in a return to an LCO. The flow pattern in Fig. 6D2 is very similar to the pressure pattern in Fig. 6D1, except that the model oscillation has a higher frequency. However, by a temporal rescaling of our base-case parameters, that aspect of the experimental record could be matched also.

Figure 6E1, also adapted from Ref. 5, illustrates PT pressure from an experiment in which a 7.5 nl/min flow perturbation increased the amplitude of oscillations in a preexisting LCO. Figure 6E2 shows results from our model that predict this pattern. If one initializes the model at a steady-state operating point that corresponds to a sustained negative perturbation and that is inside the LCO regime, but is near the bifurcation boundary (e.g., an operating point corresponding to point Y in Fig. 6A), and then one introduces a positive perturbation that results in a steady-state operating point nearer the point of inflection (e.g., corresponding to point Z in Fig. 6A);
then the increased gain results in a stronger feedback response and an increased oscillation amplitude.

**Relationship to Previous Work**

In our previous model investigations (12, 13, 15, 22), \( F_{op} \) was always set to the (nondimensional) base-case value of 1. Thus \( F_{op} \) did not appear in the earlier versions of the characteristic equation, and the steady-state concentration profile \( S \) (which depends on \( F_{op} \)) was a fixed function. In Ref. 12 (with \( F_{op} \) equal to 1), we used the characteristic equation to determine the dependence of \( \gamma_c \) on the JGA delay \( \tau \).

In the present study, the delays \( \tau_p \) and \( \delta \) were fixed, and we studied the dependence of \( \gamma_c \) on \( F_{op} \) (more precisely, we studied how \( \gamma_c \) depends on \( \rho \) and \( \kappa \), which determine \( F_{op} \)). For each value of \( F_{op} \), there is a curve, analogous to the \( n = 1 \) bifurcation curve in Ref. 12, that gives the dependence of \( \gamma_c \) on \( \tau = \tau_p + \delta/2 \), as illustrated in Fig. 7. For \( \rho = 0 \) and any sensitivity, \( F_{op} \) equals 1, and therefore the middle curves in Fig. 7, \( A \) and \( B \), are the same (these two middle curves are analogous to the curve labeled \( n = 1 \) in Fig. 4 of Ref. 12).

**Along the Critical Sensitivity Curve, Chloride Compensation and Distal Chloride Delivery Change Abruptly as One Passes from Stable Steady State to Stable LCO**

For the ranges of \( \rho \) and \( \kappa \) used in Fig. 6A, feedback compensation and delivery of chloride to the MD were computed as described in METHODS and APPENDIX C. Column \( B \) in Fig. 8 illustrates results for sensitivity \( \kappa_S \). Figure 8B1 is a reproduction of Fig. 5B2, Fig. 8B2 is feedback compensation for chloride delivery to the MD corresponding to sensitivity \( \kappa_S \), and Fig. 8B3 is chloride delivery to the MD for sensitivity \( \kappa_S \). In Fig. 8, B2 and B3, the solid curve corresponds to the stable-state case (LCO or SSS); the dashed curve corresponds to the steady-state case where LCO have been suppressed in the USS regime by setting \( \tau_p \) and \( \delta \) equal to 0.

Column \( A \) in Fig. 8, which illustrates model results for the stable-state case, is analogous to results in Column \( B \) but also represents the role of variable \( \kappa \), as shown by the axes accompanying Fig. 8A4. Column \( C \) illustrates analogous results for the case in which LCO are suppressed.

Figure 8A1 is analogous to Fig. 5, B1 and B2. The (mostly) upper surface is the gain \( \gamma \) as a function of sustained perturbation \( \rho \) and sensitivity \( \kappa \); the lower surface, largely hidden by the upper surface, is the critical gain \( \gamma_c \). The bold line that appears on each surface corresponds to sensitivity \( \kappa_S \). The intersection of these surfaces corresponds to the critical sensitivity curve exhibited in Fig 6A; specifically, the projection of that intersection onto the \( \rho-\kappa \) plane, shown in Fig. 8A4, is the critical sensitivity curve. Figure 8C1 is the same figure as Fig. 8A1, but here the intersection of these surfaces is interpreted to mark the boundary between SSS and USS. In a physiological context, the unstable steady state is unrealizable, because frequent transient perturbations, always present in a living system, will result in LCO. However, the unrealizable USS is

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**Fig. 9.** A: model predictions for TGF compensation, assessed in terms of water delivery to the MD, for both stable and steady states; model predictions for TGF compensation, assessed in terms of chloride delivery to the MD, for stable state. Gray chloride compensation curve is same as solid black curve in Fig. 8B2. Water compensation is more symmetrical as a function of sustained flow perturbation \( \rho \), than chloride compensation. B: model predictions for water delivery to the MD for both stable and steady states; model prediction for chloride delivery to the MD for stable state. Gray chloride delivery curve is same as solid black curve in Fig. 8B3. LCO affect distal water delivery much less than distal chloride delivery. C: maximum difference between stable-state percent distal delivery and steady-state percent distal delivery, for \(-12 \text{ nl/min} \leq \rho \leq 12 \text{ nl/min} \), as a function of TGF sensitivity \( \kappa \). Model predicts that LCO cause greater change in distal chloride delivery than in distal water delivery and that this discrepancy becomes more pronounced as TGF sensitivity increases.
useful for evaluating the effects of stable oscillations, relative to steady-state operation.

Figure 8A2 illustrates model predictions for TGF compensation, assessed in terms of chloride delivery to the MD, assuming stable-state operation: compensation decreases abruptly when a change in \( \rho \) or \( \kappa \) moves the model physiological state from just outside to just inside the critical sensitivity curve. In contrast, steady-state operation yields a smooth relationship between compensation and the independent variables \( \rho \) and \( \kappa \), as shown in Fig. 8, B2 and C2. Thus, in the USS parameter regime, the effect of LCO, relative to steady-state operation, is to reduce the system’s capacity to keep chloride delivery to the MD within a narrow range around the zero-perturbation chloride delivery.

Figure 8A3 illustrates model predictions for chloride delivery to the site of the MD. Comparison with Fig. 8, B3 and C3, indicates that, in the presence of LCO, perturbations result in an asymmetric delivery change. Positive perturbations result in increased chloride delivery relative to the USS, whereas negative perturbations result in decreased delivery relative to the USS.

Figure 9 shows that water delivery to the MD, as a function of perturbation, varies much less than chloride delivery to the MD, and that the variation of water compensation with respect to perturbation is more symmetrical than that of chloride compensation. For \( \kappa = \kappa_8 \), Fig. 9, A and B, shows water compensation and delivery as a function of perturbation, both for stable-state and for steady state, along with the stable-state result for chloride. Figure 9C shows the maximum difference, over sustained perturbations of \(-12\) to \(12\) nl/min, between percent stable-state and percent steady-state delivery as a function of sensitivity, for both chloride and water: the model predicts that LCO affect MD chloride delivery much more than they affect water delivery.

The results in Figs. 8 and 9 generalize results in Ref. 16 by showing that the pattern reported there holds for a substantial range of physiologically plausible sensitivities and by relating the stability thresholds observed in Ref. 16 to the conceptual framework developed in Refs. 12 and 13.4

4A small difference in the calculation algorithm for stable-state compensation and percent delivery, relative to the calculations in Ref. 16, should be acknowledged. For stable-state compensation and percent delivery in this study, in calculating the difference between the deliveries associated with perturbed and nonperturbed states, the zero-perturbation reference was always taken to be the delivery associated with the zero-perturbation stable state. In contrast, in Ref. 16, the state of the perturbed solution (steady state or LCO) was noted and then compared with the corresponding state (steady state or LCO) at zero perturbation. For steady-state compensation and percent delivery, in both this study and in Ref. 16, the time-independent steady states (stable or not) were compared with steady-state zero-perturbation reference values. As a result of this small methodological difference, the stable-state compensation and delivery values do not agree exactly with the steady-state values for perturbations that push the system outside the critical sensitivity curve, but the disagreement, which is visible at the left and right of Fig. 8, B2 and B3, (for perturbations of magnitude greater than \(-8\) nl/min) is slight. Although it is not clear which standard is more appropriate

DISCUSSION

The principal aim of this study was to use a mathematical model to better understand the effects of sustained flow perturbations on the TGF system. This study predicts that such perturbations affect the feedback gain (a determinant of feedback signal strength), the critical gain (the gain value that must be exceeded to elicit sustained, regular, TGF-mediated LCO in nephron flow), and, through the relationship of gain to critical gain, the stable state of the system (LCO or stable steady state) and feedback compensation (an index that assesses the degree of control provided by a feedback system). In particular, the model analysis permits the identification of the boundary between stable steady-state flow and stable oscillatory flow in terms of the feedback sensitivity and the PT flow perturbation; we call this boundary the “critical TGF sensitivity curve.” Along this curve, the model predicts that feedback compensation, assessed in terms of chloride delivery to the distal nephron, changes abruptly, decreasing as one moves from a stable steady state to LCO.

In previous studies (12–16), we have noted limitations of the model framework used in this study. Many simplifying assumptions are required to obtain a model formulation that is amenable to analytical treatment, e.g., derivation of a characteristic equation (Eq. 7). For example, fractional volume absorption in the PT and descending limb is assumed to be constant and equal to the parameter \( \alpha \); thus our model assumes perfect glomerulotubular balance (GTB). Moreover, by using a simple expression to represent saturable, concentration-dependent epithelial transport, we have avoided the detailed representation of tubular epithelial cells. Nonetheless, our previous studies have suggested that our model is effective in predicting and explaining the results of laboratory experiments. Thus we believe that the model results from this study provide insight into the relationships among key physiological parameters and the state of fluid flow in the nephron.

Because this work is specifically concerned with perturbations in proximal nephron flow, a concern that may be raised is that our assumption of perfect GTB may not be strictly applicable. However, we believe that this simplification is appropriate for our relatively simple model and for the specific goals of our investigation, for two reasons. First, the capability to solve and utilize a characteristic equation is of inestimable value in understanding the fundamental behavior of this system and in verifying numerical methods and simulation strategies. At present, it is unclear whether the analytical treatment afforded by our characteristic equation can be extended to encompass representations of flow-dependent PT absorption. Second, we do not know how PT transepithelial transport may be affected by TGF-mediated flow oscillations, which have short periods relative to the time intervals used in steady-state measurements. In contrast, for comparison, the results indicate that the differing standards yield similar results and essentially the same predictions.
both experimental and theoretical studies indicate that the TAL has a major influence on the dynamic behavior of the TGF loop in its action as a transducer of flow changes into concentration changes (6, 15).

However, notwithstanding our assumption of perfect GTB, this study offers substantive insight into the effects of changes in PT absorption on the stability and dynamics of TGF. The basis for this assertion is that a sustained perturbation in flow applied into Bowman’s capsule will have the same effect on Henle’s loop flow and, hence, TGF dynamics, as a change in PT absorption. Thus our graphical results can be used to predict the effects on TGF stability of perturbations that are ascribed in part or in whole to deviations from GTB. For example, a change Δ in absolute PT absorption can be represented in Fig. 8 by means of the perturbation value $\rho = -\Delta \alpha$, which results in a change $-\Delta$ in TAL flow. Nevertheless, an effort to develop a comprehensive, dynamic mathematical model of PT absorption is clearly warranted by recent studies (27, 30, 31), which indicate that the abnormal regulation, chronic adaptation, and dysfunction of PT absorption can play a major role in the pathogenesis of hemodynamic abnormalities in renal disease.

Holstein-Rathlou and Leyssac (5) have also used a mathematical model to simulate the effects of sustained perturbations on the stability of TGF-mediated LCO. However, our work extends their early work on this problem and is distinguishable from theirs in important ways. First, their model study was based on numerical simulations and used informal conceptual reasoning to explain the effects of perturbations. In contrast, in the context of our model and its characteristic equation, we have provided a rigorous analysis and explicit predictions in terms of parameter values for the effects of perturbations; our numerical simulations confirm the accuracy of the analysis. Second, Holstein-Rathlou and Leyssac included in their model a detailed submodel of glomerular filtration and somewhat less detailed submodels of AA dynamics and the PT; the dynamics of the TAL and TGF signaling delays were represented by an implicitly defined third-order lag function. In contrast, we included a detailed model of spatially distributed TAL dynamics, which our previous studies have shown to introduce substantial dynamic complexity (12–16). We did not model filtration or AA dynamics but, as in the model by Holstein-Rathlou and Leyssac (5), we incorporated the standard empirical TGF response function, although in terms of tubular chloride concentration at the MD rather than fluid flow into the loop of Henle. The relative simplicity of our model allows us to formulate a rigorous and detailed conceptual framework and make predictions with an economy of assumptions. Finally, none of the stability regions identified by Holstein-Rathlou and Leyssac by means of numerical simulation corresponds to the effects of sustained flow perturbations (i.e., none is analogous to Fig. 6A). However, the experimental pressure records of LCO given by Holstein-Rathlou and Leyssac in Figs. 10–14 of Ref. 5 provide invaluable examples of the phenomena predicted in our study, and the model simulations given in those figures are analogous to our simulations; indeed, Figs. 12 and 13 in Ref. 5 correspond to our Fig. 6, D and E, respectively.

A significant result of this study is the prediction that the feedback compensation afforded by the TGF system might be significantly reduced, compared with the compensation associated with steady-state5 flow, by LCO. Laboratory experiments appear to support

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\[ \text{Effective SNDR (nl/min)} \]

\[ V_{\text{MD}} \text{ (mM)} \]

\[ \text{Volume Expansion} \]

\[ \text{Resetting} \]

\[ \text{Volume Contraction} \]

\[ (O) \]

\[ (C) \]

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Fig. 10. An interpretation of change in extracellular volume (ECV) status. A: gray curve, reference TGF response curve; solid black response curves, change in ECV results in vertical translation of TGF response curve and change in steepness (sensitivity) of response curve (change in model parameters $\rho$ and $\kappa$). Dashed curves, TGF response curve resets to align steady-state operating point with inflection point of response curve. Width of black path represents magnitude of distal NaCl delivery for steady-state flow; width of gray path represents magnitude of distal NaCl delivery for stable oscillatory flow. Solid curve is critical sensitivity curve from Fig. 6A. Open circles represent states of volume expansion and contraction that are sufficient to drive the model physiological state outside the critical sensitivity curve, and thus LCO are suppressed. Dashed curves: in response to horizontal resetting of the TGF response curve, the critical sensitivity curve resets horizontally. After resetting, the open circles are again within the regime that supports LCO.

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5 The steady-state compensation curves shown in Figs. 8, B2 (dashed curve) and C2, and B4 (dashed curve) are roughly comparable to the “fractional compensation” curves published by Thomson and Blantz (25). Both our compensation curves and Thomson and Blantz’s fractional compensation curves imply that, assuming steady-state flow, when the operating point of the TGF system is near the inflection point of the TGF response curve, the TGF system exhibits its strongest feedback and is most able to compensate for flow perturbations.
this hypothesis: transfer function analyses relating renal arterial blood pressure to renal blood flow indicate that a decrease in compensation is associated with an ~30-mHz oscillation, an oscillation that can reasonably be inferred to arise from TGF-mediated oscillations in many nephrons (8, 9). Our previous simulation studies using a fixed TGF sensitivity also indicated that LCO could lead to reduced TGF regulation of chloride delivery to the distal nephron (16). The present study extends these earlier findings by showing that this phenomenon is robust, in that it occurs over a wide range of physiologically reasonable TGF sensitivities (from $-\kappa_3$ to $\kappa_{10}$, the sensitivities that yield feedback loop gain magnitudes of 3 and 10, respectively, when there is zero PT flow perturbation; see Fig. 8).

The traditional view is that TGF, in conjunction with GTB, acts to match the filtered load with the absorptive capacity of the nephron, thereby stabilizing both SNGFR and the delivery of sodium into the distal nephron. Furthermore, in response to disturbances in extracellular fluid volume, systemic neurohumoral mechanisms are activated that modify TGF sensitivity and operating point, as well as proximal and distal nephron sodium absorption, resulting in an appropriate adaptive change in distal sodium delivery and renal sodium excretion (19, 24, 29). However, the results of the present study suggest that TGF regulation of distal sodium delivery may be more complex, owing to the prediction that TGF regulatory efficacy can change rapidly (within tens of seconds) when the dynamic state of the nephron changes (e.g., from steady flow to LCO).

One example of this additional complexity is that the response to an increase in tubular fluid load will depend on both the initial dynamic state of the individual nephron and the amplitude of the perturbation. If the initial TGF sensitivity is sufficiently high and the TGF system is oscillating, then small perturbations will likely not change the dynamic state of the nephron, and the degree of feedback regulation of distal sodium delivery and SNGFR afforded by TGF will correspond to the reduced TGF system compensation with LCO. In contrast, a large perturbation sufficient to quench LCO will result in an increase in TGF feedback compensation, which should enhance the autoregulatory response, thereby protecting the renal microcirculation from large surges in blood flow and intravascular pressure.

Another, more involved example of the potential physiological importance of the dynamic state of the nephron is TGF adaption to changes in extracellular fluid volume. Figure 10 presents a graphical illustration of the transitions from euvolemma to ECV expansion and contraction. Volume expansion results in renal vasodilation, reduced TGF sensitivity, increased filtration, and reduced proximal and distal nephron sodium absorption. In terms of our model, these effects correspond to an increase in $\rho$ and a decrease in $\kappa$, whereas opposite changes are associated with volume contraction. As shown in Fig. 10A, when $\rho$ increases, the TGF response curve translates upward, and when $\kappa$ decreases, the slope of the response curve at its inflection point decreases. During volume expansion, the model physiological state follows a path in the $\rho - \kappa$ plane similar to that given by the dashed straight line in Fig. 10B. The resulting effect on distal sodium delivery is illustrated in Fig. 10B by the relative thicknesses of the black and gray regions surrounding the dashed straight line. This is a qualitative representation of the difference between the distal NaCl deliveries associated with steady-state and stable oscillatory flow (see Fig. 9B). Given a point $(\rho, \kappa)$ along the dashed straight line, the width of the black path represents the magnitude of the distal NaCl delivery associated with steady-state flow, and the width of the gray path represents the magnitude of the distal NaCl delivery associated with stable oscillatory flow.

The model predicts that, after volume expansion (or contraction), NaCl delivery changes immediately and significantly, and if the model physiological state does not leave the interior of the critical sensitivity curve after the perturbation, the presence of LCO accelerates the TGF system’s response to the initiating challenge. That is, LCO result in distal NaCl delivery changes that are both appropriate for the ECV status and larger than the changes that would occur under steady-state flow conditions, thereby shortening the time required for a return to euvolemma. The open circles in Fig. 10B represent states of volume expansion and contraction that are sufficient to suppress LCO and thus drive the model physiological state outside the critical sensitivity curve (the solid curve in the middle of Fig. 10B). The subsequent response of systemic neurohumoral systems (e.g., renin-angiotensin system, renal sympathetic nerve activity, ANF) will enhance distal sodium delivery. In addition, as illustrated in Fig. 10A, the TGF response curve will be reset horizontally over the following 10–60 min, which will act to restore the TGF operating point near the point of maximum TGF sensitivity (26, 28, 29). Concurrent with the horizontal resetting of the TGF response curve, the critical sensitivity curve resets horizontally and changes slightly. Figure 10B illustrates the resetting of the critical sensitivity curve (the dashed curves that represent the reset critical sensitivity are not exact and are for illustrative purposes only). After resetting of the critical sensitivity curve, the open circles are again inside the region of stable oscillations. The reestablishment of LCO will then again facilitate the excretion of the excess sodium during volume expansion and enhance sodium conservation during volume contraction. The model thus suggests that an additional effect of TGF resetting may be to maintain the dynamic state of the nephron when extracellular fluid volume is altered.

As discussed above, volume expansion, as well as acute and chronic hypertension, is associated with reduced proximal absorption. However, in the present version of our model, fractional proximal and descending limb volume absorption (represented by the parameter $\omega$) was kept constant. Thus more detailed consideration of the physiological impact of LCO on the TGF
system will require $\alpha$ to be variable and flow dependent, a modification that may preclude an analytical treatment of model system stability. We predict that this modification to our model will increase the relative contribution of LCO to the regulation of distal sodium delivery, owing to the synergistic effects of reduced upstream volume absorption and reduced TGF compensation when LCO are present. We also note that the effect of LCO on sodium excretion, and thus on sodium balance, depends on the correlation between distal sodium delivery and sodium excretion. Whether LCO-related changes in distal sodium delivery correspond to changes in sodium excretion depends on factors such as the response of distal tubular epithelial cells to LCO in tubular flow rate (16).

The results presented here suggest that the TGF system may display a wider range of functional behavior than previously thought. The differences in distal sodium delivery associated with steady-state and oscillatory flow may give the TGF system the flexibility needed to participate in the regulation of sodium balance while at the same time homeostatically regulating GFR. Further investigation with more detailed modeling and clever experimentation will be required to evaluate the physiological importance of the nonlinear dynamics of the TGF system. The analytical strategy and the numerical tools developed in this study will greatly facilitate such efforts.

APPENDIX A

Steady-State and Instantaneous Gain

Steady-state gain, denoted by $G_{SS}$, corresponds to a case in which one introduces a sustained step perturbation in the feedback loop signal (here taken to be fluid flow rate) and waits sufficiently long for a new steady state to be established before one quantifies the return signal (13). Then, $G_{SS}$ is approximated by the resulting change divided by the signal increase; that approximation approaches the exact value of $G_{SS}$ as the signal increase tends to zero. Thus, in a short-looped nephron, if the feedback loop is broken at the entrance to the TAL, the feedback signal is the tubular flow rate, and $G_{SS}$ is given by the limit

$$\lim_{\Delta F_{SL} \to 0} \frac{\Delta F_{SL}}{\Delta F_{AL}}$$

where, for each sustained change of $\Delta F_{AL}$, one uses the associated steady-state (i.e., full-response) value of $\Delta F_{SL}$.

Instantaneous gain, denoted by $-\gamma$, corresponds to a case in which one instantaneously increases the feedback response through the JGA by means of a change in the feedback loop signal and obtains the full corresponding change in the return signal, subsequent to delays in the feedback pathway (13). Then, $-\gamma$ is the value of the change in the return signal divided by the signal increase as that increase tends to zero. Thus, if the feedback loop is broken at the entrance to the TAL, one may use the same limit expression as given above in Eq. A1, but 1) $\Delta F_{AL}$ is interpreted to be the fractional magnitude of an instantaneous insertion of fluid in the TAL entrance (i.e., a Dirac delta function perturbation, which results in an instantaneous shift of the fluid column) and $\Delta F_{SL}$ is interpreted to be the fractional response in descending limb flow, assuming that the TAL chloride concentration at the MD arising from the fluid insertion remains fixed; and 2) delays at the JGA are ignored.

APPENDIX B

Normalization of Equations

The dimensional forms of Eqs. 1 and 2 are given by

$$\frac{\partial}{\partial t} C(x, t) = -\frac{F(C_{M0}(t))}{\pi r^3} \frac{\partial}{\partial x} C(x, t)$$

$$+ \left( \frac{2r}{r} \right) \left( \frac{V_{\text{max}} C(x, t) - C(x, t)}{K_M + C(x, t)} + P C(x, t) - \tilde{C}_t(x) \right)$$

and

$$F(C_{M0}(t)) = \alpha \left( q_o + \frac{\Delta Q}{2} \right) \tanh \left( \frac{k}{2} \left( C_t - C_{M0}(t) \right) \right) + \rho$$

where $r$ is the tubular radius, $\alpha$ is the (dimensionless) fraction of SNGFR reaching the TAL, $q_o$ is the steady-state (operating) SNGFR when $\rho = 0$ (i.e., the base-case SNGFR), $\Delta Q$ is the TGF-mediated range of SNGFR, $\rho$ is the primitive sensitivity of the TGF response, and $\rho$ is a flow perturbation applied into Bowman’s space (12).

To express these equations in nondimensional form, let $\tilde{x} = x(L/L), \tilde{t} = t/t_o, \tilde{r} = r/(2r), \tilde{C}(\tilde{x}, \tilde{t}) = C(x, t)/C_o, \tilde{C}_t(\tilde{x}) = C_t(x)/C_o, \tilde{C}_{M0}(t) = C_{M0}(t)/C_o, F(\tilde{C}_{M0}(t)) = F(C_{M0}(t))/F_o, \tilde{P} = \alpha \tilde{P}F_o, \frac{V_{\text{max}}}{V_{\text{max}O}} = \frac{V_{\text{max}O}}{V_{\text{max}O}}, K_M = K_M/C_o, \tilde{P} = \tilde{P}F_o, K_1 = \Delta Q/2Q_o, K_2 = kC_o/2, \tilde{C}_o = C_o/C_o, \tilde{\delta} = \tilde{\delta}t_o, \tilde{\tau} = \tilde{\tau}t_o, \tilde{\delta} = \tilde{\delta}t_o,$ and $\tilde{\psi}(\tilde{\psi}) = \psi_o(\psi)/1(t_o)$, where $L$ is the TAL length and the quantities subscripted with an “O” are conveniently chosen reference values: $A_o = \pi r^2, t_o = A_o/L/F_o, C_o = C(0, t), F_o = F_t = \alpha Q_o, (V_{\text{max}O} = F_oC_o/(2\pi rL)), \tilde{P}_o = \tilde{P}F_o/(2\pi rL),$ and $Q_o = Q_t$. With these conventions, $t_o$ is the filling time (and thus the transit time) of the TAL at flow rate $F_o$, and $(V_{\text{max}O})$ is the rate of solute advection into the inlet of the TAL at flow rate $F_o$, divided by the area of the walls of the TAL.

When Eqs. B1 and B2 are rewritten in dimensionless terms and the tilde symbols are dropped, Eqs. 1 and 2 follow directly. The dimensional forms of Eqs. 3 and 4 are the same as their nondimensional forms.

The instantaneous gain magnitude $\gamma$ has dimension form

$$\gamma = \frac{L}{F_{op}} \frac{F'(C_{op})}{S(x, F_{op})}_{x=L}$$

and the nondimensionalization procedure of the previous paragraph yields Eq. 8.

APPENDIX C

Numerical Methods

Methods are identified by corresponding figure numbers. Figure 2. Data for these two curves were calculated using Eqs. 6 and 8. The steady-state operating points, which are needed in Eqs. 6 and 8, were calculated from the values of $\rho$ and $\kappa$ using an iterative procedure. Given an initial estimate for $C_{op}$, an estimate for $F_{op}$ was calculated using Eq. 2, and this approximate $F_{op}$ was then substituted for $F$ in Eq. 5, which was solved using the standard explicit fourth-order Runge-Kutta method (RK4) to obtain $S(1, F)$. The approximate $C_{op}$ for the next iteration was taken to be a weighted average of the previous estimate and this value of $S(1, F)$, thus guaranteeing convergence of the iterates to the actual steady-state operating values $C_{op}$ and $F_{op}$. The factor $L/F_{op}$ in Eq. 6 was calculated using the five-point

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centered differentiation formula (CDF), and the factor \( \frac{1}{2} S(x, F_{op})_{x=1} \) in Eq. 8 was calculated using the five-point right-hand differentiation formula (RDF). In both Eqs. 6 and 8, \( F'(C_{op}) \) could be calculated analytically.

**Figure 4.** Figure 4, A and B. The TGF response curves were calculated using Eq. 2, with the nondimensional TAL flow rate values scaled by \( Q_m = 30 \) nl/min. The MD chloride concentration curves were calculated by multiplying the effective SNGFR by a to yield the TAL flow rate \( F \), substituting this value of \( F \) in Eq. 5 and solving for \( S(1, F) \), using RKM.

**Figure 5.** The steady-state operating TAL flow rates \( F_{op} \) were calculated using the iterative method described for Fig. 2. The corresponding values of \( Q_{op} \) were given by \( Q_{op} = F_{op}/\alpha \).

**Figure 5A.** Instantaneous gain values were calculated by the method described for Fig. 2.

**Figure 5A2.** Critical gain values were calculated using Eq. 7. Given values of \( \rho \) and \( \kappa \), the steady-state operating TAL flow rate \( F_{op} \) and steady-state TAL chloride concentration profile \( S(x, F_{op}) \) were calculated using the iterative method described for Fig. 2. Equation 7 was then solved for \( \gamma \), after setting \( \lambda = 0 \), using a method based on that summarized in Appendix C of Ref. 13.

**Figure 5, B1 and B2.** These curves are the same as those given in Fig. 5, A1 and A2, but they are matched according to TGF sensitivity \( \kappa \).

**Figure 6.** Figure 6A. The critical sensitivities in this figure were calculated using the following algorithm. Given an operating TAL flow rate \( F_{op} \), calculate the steady-state TAL chloride concentration profile \( S(x, F_{op}) \) (using Eq. 5 and RKM), set \( C_{op} = S(1, F_{op}) \), and calculate \( \frac{1}{2} S(x, F_{op})_{x=1} \) using RDF. Then, after calculating the critical gain \( \gamma_{\text{crit}} \) associated with \( F_{op} \) (by solving Eq. 7 by the method described for Fig. 5A2), calculate \( \gamma = \gamma_{\text{crit}}, S(x, F_{op})_{x=1} \) (Eq. 8, with \( \gamma = \gamma, \) and \( m = F'(C_{op}) \)); \( m \) is the required slope of the TGF response curve at the operating MD chloride concentration \( C_{op} \). Next, solve the equation \( m = F'(C_{op}) \), using Newton’s method, for the necessary sensitivity parameter \( \kappa \) and set \( \kappa_{\text{crit}} = K_{\text{crit}} \). Finally, calculate the sustained perturbation \( \rho \) associated with \( F_{op} \) and \( \kappa_{\text{crit}} \) using Eq. 2; i.e., let \( \rho = F_{op} - (1 + K_{\text{crit}} \tan(K_{\text{crit}} C_{\text{crit}})) \).

**Figure 6B.** To obtain data for Fig. 6B, Eq. 1 was solved for values of \( \rho \) and \( \kappa \) that correspond to points S and T in Fig. 6A. After initialization at the time-independent steady state associated with points S and T, an additional transient Gaussian flow perturbation was applied at \( t = 6 \) s. Equation 1 was solved by means of a second-order, essentially nonoscillatory (ENO) scheme, coupled with Heun’s method for the time advance. This algorithm yields solutions that exhibit second-order convergence in both space and time (21). The numerical time and space steps in normalized units were \( \Delta t = 1/640 \) and \( \Delta x = (320 \times t_0)^{-1} \), where \( t_0 \) is the base-case steady-state TAL transit time in seconds. These mesh widths, which correspond to dimensional values of \( \Delta x = 7.8125 \times 10^{-7} \) cm and \( \Delta t = 3.125 \times 10^{-3} \) s, were used for all dynamic calculations.

The high degree of numerical grid refinement was required both to faithfully represent the nonlinearities that are embodied in the model equations (15) and to compute with sufficient accuracy the time-averaged chloride and water delivery rates used in the calculation of compensation values.

**Figure 6C1.** The TGF response curves and the MD chloride concentration curve were calculated using the method described for Fig. 4A and B.

**Figure 6, C2, D2, and E2.** Data for these figures were calculated by solving Eq. 1 for values of \( \rho \) and \( \kappa \) that correspond to the points U, V, W, X, Y, and Z in Fig. 6A, using the method described for Fig. 6B. For each of these figures, the model solution was stable at time \( t = 0 \).

**Figure 7.** For each fixed value of \( \tau \), data for these curves were calculated from the given values of \( \rho \) and \( \kappa \) using the method described for Fig. 5A2. The value of \( \tau = \tau_p + \delta/2 \) was changed by making proportional changes in the values of \( \tau_p \) and \( \delta \); in particular, the ratio \( \tau_p/\delta = 2/3 \) was maintained, so that \( \tau_p = 4\sqrt{\pi} \) and \( \delta = 6\sqrt{\pi} \).

**Figure 8.** Figure 8, A1, B1, and C1. Data for these figures were calculated using the methods described for Figs. 2 and 5A2. **Figure 8, B2 and B3.** For a given sustained perturbation \( \rho \) and TGF sensitivity \( \kappa \), average steady-state chloride deliveries were used to calculate chloride compensation (according to Eq. 10) and chloride percent distal delivery \( (100\% \times (Y_A - Y_0)/Y_0) \). Closed-loop stable states were found by solving Eq. 1 for sufficiently large simulated time; i.e., for enough time either to allow transient behavior to die out or to let stable oscillatory behavior fully develop (Eq. 1 was solved using the method described for Fig. 6B). If an LCO was the stable state, the waveform was recorded only after the oscillation had reached full amplitude and was indistinguishable from the limit-cycle trajectory (the model equations were solved for a minimum of 10 simulated minutes, and then two-cycle averages were calculated at 2-min intervals until the relative change in those averages was, in most cases, <0.0001). Open-loop stable states were found by solving Eq. 5, using RKM, with \( F = 1 + \rho \). For stable states that were LCOs, the average chloride delivery was calculated, using the trapezoidal rule, over two complete cycles of the oscillation. For stable steady states, the average chloride delivery was equal to the stable constant value. The zero-perturbation value of chloride compensation was interpreted to be the average of the compensation values for \( \rho = \pm 0.25 \) nl/min.

**Figure 8, C2 and C3.** For a given sustained perturbation \( \rho \) and TGF sensitivity \( \kappa \), average steady-state chloride deliveries were used to calculate steady-state chloride compensation (according to Eq. 10) and steady chloride percent delivery \( (100\% \times (Y_A - Y_0)/Y_0) \). Closed-loop steady states were found using the method described for Fig. 2, and open-loop steady states were found by solving Eq. 5, using RKM, with \( F = 1 + \rho \). The zero-perturbation value of steady-state chloride compensation was found by interpreting Eq. 11 as the ratio of two derivatives and using CDF to calculate the magnification M.

**Figure 8, B2 and B3.** These curves were taken from Fig. 8A2, A3, C2, and C3 (\( \kappa = \kappa_{\text{crit}} \)).

**Figure 8, A4 and C4.** This curve is the critical sensitivity curve from Fig. 6A.

**Figure 9.** Figure 9, A and B. Compensation and percent delivery values were calculated as described for Fig. 8.

**Figure 9C.** Maximum difference was calculated between percent stable-state delivery and percent steady-state delivery, for both chloride and water.

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**DISCLOSURES**

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