In vivo recordings of the LCO of the TGF system show system gain magnitude in the feedback loop (12, 18, 29). Theoretical studies indicate that LCO emerge because of the combination of time delays and sufficiently high concentration in the early distal tubule (11, 22, 24).}

and fluid flow, pressure, and tubular fluid chloride sure, fluid flow and pressure in the proximal tubule, variables, including glomerular capillary blood pres-

Limit-cycle oscillations and tubuloglomerular feedback regulation of distal sodium delivery

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Layton, H. E., E. Bruce Pitman, and L. C. Moore. Limit-cycle oscillations and tubuloglomerular feedback regulation of distal sodium delivery. Am. J. Physiol. Renal Physiol. 278: F287–F301, 2000.—A mathematical model was used to evaluate the potential effects of limit-cycle oscillations (LCO) on tubuloglomerular feedback (TGF) regulation of fluid and sodium delivery to the distal tubule. In accordance with linear systems theory, simulations of steady-state responses to infinitesimal perturbations in single-nephron glomerular filtration rate (SNGFR) show that TGF regulatory ability (assessed as TGF compensation) increases with TGF gain magnitude \( g \) when \( g \) is less than the critical value \( g_c \), the value at which LCO emerge in tubular fluid flow and NaCl concentration at the macula densa. When \( g > g_c \) and LCO are present, TGF compensation is reduced for both infinitesimal and finite perturbations in SNGFR, relative to the compensation that could be achieved in the absence of LCO. Maximal TGF compensation occurs when \( g \approx g_c \). Even in the absence of perturbations, LCO increase time-averaged sodium delivery to the distal tubule, while fluid delivery is little changed. These effects of LCO are consequences of nonlinear elements in the TGF system. Because increased distal sodium delivery may increase the rate of sodium excretion, these simulations suggest that LCO enhance sodium excretion.

Kidney, renal hemodynamics, mathematical model, nonlinear dynamics

Am. J. Physiol. Renal Physiol. 278: F287–F301, 2000. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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of the TGF system to compensate for perturbations in SNFGR. The results suggest that LCO limit the regulatory ability of the TGF system and that LCO may enhance time-averaged distal sodium delivery and renal sodium excretion.

**Glossary**

**Parameters**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_o$</td>
<td>chloride concentration at TAL entrance, mM</td>
</tr>
<tr>
<td>$C_{op}$</td>
<td>steady-state chloride concentration at MD, mM</td>
</tr>
<tr>
<td>$k$</td>
<td>sensitivity of TGF response, 1/mM</td>
</tr>
<tr>
<td>$K_M$</td>
<td>Michaelis constant, mM</td>
</tr>
<tr>
<td>$L$</td>
<td>length of TAL, cm</td>
</tr>
<tr>
<td>$P$</td>
<td>TAL chloride permeability, cm/s</td>
</tr>
<tr>
<td>$Q_{op}$</td>
<td>steady-state SNFGR, nl/min</td>
</tr>
<tr>
<td>$\Delta Q$</td>
<td>TGF-mediated range of SNFGR, nl/min</td>
</tr>
<tr>
<td>$r$</td>
<td>luminal radius of TAL, $\mu$m</td>
</tr>
<tr>
<td>$V_{max}$</td>
<td>maximum transport rate of chloride from TAL, nmol·cm⁻²·s⁻¹</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>fraction of SNFGR reaching TAL</td>
</tr>
<tr>
<td>$\delta$</td>
<td>distributed delay interval at JGA, s</td>
</tr>
<tr>
<td>$\tau_p$</td>
<td>discrete (or pure) delay interval at JGA, s</td>
</tr>
</tbody>
</table>

**Independent variables**

- $t$: time, s
- $x$: axial position along TAL, cm

**Specified functions**

- $C_o(x)$: extratubular chloride concentration, mM
- $\psi(t)$: kernel function for distributed delay (dimensionless)

**Dependent variables**

- $C(x, t)$: TAL chloride concentration, mM
- $C_{MD}(t)$: effective MD chloride concentration, mM
- $F(C_{MD}(t))$: TAL fluid flow, nl/min
- $S(x)$: steady-state TAL chloride concentration, mM

**METHODS**

Mathematical model and its solutions. We used mathematical simulations to evaluate how LCO may affect TGF-mediated regulation of fluid and NaCl delivery to the distal tubule. The simulations were based on a model formulation that we have used previously to study TGF system dynamics (18–21, 29). The model is illustrated in Fig. 3; model quantities are identified in the Glossary. For simplicity, only the chloride concentration is explicitly represented in the model (chloride is thought to be the principal ion sensed at the MD in the TGF response (31)). We assume that sodium is absorbed in parallel with chloride. Because the model TAL is assumed to have water-impermeable rigid walls, TAL tubular fluid flow rate is a function of time only and is equivalent to the flow rate past the MD. The mathematical formulation of the model is recapitulated in APPENDIX A; the numerical methods used to approximate solutions to the model and to compute quantities derived from those solutions are described in APPENDIX B.

Model parameter values, given in Table 1, are the same as those used in our previous studies; these parameter values serve as our “base-case” parameter set. Model results obtained with the base-case parameters are generally consistent with experimental measurements of nephron and TGF system function. In particular, the key system characteristics...
The change in the nature of the stable solution, as \( \gamma \) increases through \( \gamma_c \), is called a bifurcation. For the base-case parameters given in Table 1, the critical gain magnitude is \( \gamma_c \approx 3.24 \) (a method for determining the value of \( \gamma_c \) is given in Ref. 19). In model simulations, gain magnitude \( \gamma \) was varied by changing the steepness, but not the range, of the TGF response curve illustrated in Fig. 2A (see APPENDIX A).

Experiments indicate that there is a time delay in TGF signal transmission across the juxtaglomerular apparatus (j GA) (3); after a change in tubular fluid chloride concentration at the MD, no change is detected in SNGFR until after a discrete delay interval (\( \tau_p \) in our model) of about 2 s, and a full effect requires an additional distributed delay interval (\( \delta \)) of about 3 s. The incorporation of the j GA delay into the model is a necessary condition for a bifurcation to solutions with LCO; in the absence of a delay, the only stable model solution, for all \( \gamma > 0 \), is the steady-state base case (18). In some instances, we evaluated the effect of a perturbation on an LCO solution, relative to the effect of that same perturbation on an analogous steady-state solution. To obtain a steady-state with the same feedback gain magnitude that gives an LCO solution, we eliminated the j GA delay from the model to provide a hypothetical steady-state case.

Perturbations. Perturbations to SNGFR were simulated by adding or subtracting stipulated amounts to the base-case SNGFR \( (i.e., \text{to } Q_{op}) \), where it appears in the model equations; the precise role of \( Q_{op} \) in the model is set forth in APPENDIX A, Eq. A6. Perturbations were introduced, either transiently (to initiate LCO) or continuously, with a step function. Because the model formulation assumes that a fixed fraction of SNGFR reaches the TAL, a perturbation of \( Q_{op} \), as a percentage of \( Q_{op} \), is analogous to a perturbation, in vivo, at any site before the TAL, of the same percentage of the steady-state base-case fluid flow rate at that site.

Starting from the steady-state base case, the model was perturbed as described above. After the solution reached a new steady state (or converged to an LCO), the steady (or time-averaged LCO) values of the TAL fluid flow rate, the chloride concentration at the MD, and the chloride delivery rate to the MD (and hence, into the distal tubule) were determined. Since the model includes only chloride concentration, we assumed that NaCl delivery is identical to the delivery of chloride.

Feedback compensation. The efficacy of TGF regulation was quantified by calculating feedback compensation, an index often used in experimental investigations (see, e.g., Refs. 14, 34). Feedback compensation is defined by

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

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

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

In the definition for magnification, \( Y \) is a system variable that is regulated by means of the feedback loop; \( \Delta Y \) is the change in the system variable \( Y \) in response to a change (i.e., a
perturbation) $\Delta X$ in another system variable $X$. The denominator of Eq. 2, $(\Delta Y/\Delta X)_{OL}$, 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 $(\Delta Y/\Delta X)_{CL}$ is the corresponding ratio when the loop is closed (closed-feedback-loop case, or CL). As $\Delta X$ tends to zero, $M$ converges to a ratio of derivatives. However, we retain the $\Delta$-notation, because this formulation is consistent with experimental studies, which necessarily entail measurable, and therefore large, finite perturbations.

A negative feedback loop usually operates so that the magnification $M$ will fall between 0 and 1, which is to say that the closed feedback loop will tend to reduce excursions in a controlled variable $Y$ arising from perturbations. In a system where there is complete feedback control, $M = 0$; for weak feedback control, $M$ is near 1. From the definition of magnification (Eq. 2), it follows that compensation (Eq. 1) is an index which assesses the degree of control afforded to the variable $Y$ by the feedback system, relative to the case where no feedback is present, when the system is presented with a specific perturbation of amplitude $\Delta X$. An index value of 100% corresponds to complete feedback compensation, whereas a value of 0% indicates no feedback compensation. (Our definition of magnification and compensation are based on Refs. 27 and 30.)

Thus, compensation is a measure of how nearly a feedback loop can return a system-regulated variable to its initial value when the system is perturbed, relative to the case where there is no feedback. In our context, we consider the regulated variables to be the tubular fluid flow rate, the tubular fluid chloride concentration, and their product, chloride delivery, all evaluated at the site of the MD. We consider the input signal to be SNGFR, and we consider the perturbations to be unspecified, non-TGF-induced changes in SNGFR. Alternatively, the perturbations may represent experimental interventions, such as the introduction of fluid into the proximal tubule by means of a micropipette in a freely flowing nephron.

Relationship between compensation and feedback loop gain. For a linear system, the relationship between compensation and feedback loop gain is important for this study and is easy to derive. Let $X$ represent the value of an input signal and suppose that, in response, the system produces an output signal $Y = \beta X$, where $\beta$ is a scalar. Suppose that at a base-case value $X_0$, the system output signal has the base-case value $Y_0 = \beta X_0$. Now suppose that $X_0$ is perturbed by an amount $\Delta X$. Then the output signal, in the absence of feedback (which is the open-feedback-loop case) would be $Y = \beta (X_0 + \Delta X)$, which differs from the base-case value $Y_0$ by the amount $\Delta Y = Y - Y_0$ in this specific linear case, $\Delta Y = \beta \Delta X$.

However, if a linear feedback loop is operative, then the input signal $X_0 + \Delta X$ can be corrected in part by the addition of the linear negative feedback term $-G_{ss}\Delta Y/\beta$, with negative gain $-G_{ss}$ (the subscript will be explained below). In this case, which is the closed-feedback-loop case, the output signal would be $Y = \beta (X_0 + \Delta X - G_{ss}\Delta Y/\beta)$. When this equation is solved for $\Delta Y$, one obtains $\Delta Y = \beta \Delta X/(1 + G_{ss})$. Thus the deviation from the base-case value $Y_0$ is reduced through feedback by a factor of $1/(1 + G_{ss})$.

We can now express compensation in terms of gain magnitude $G_{ss}$. By using the definitions of compensation and magnification given by Eqs. 1 and 2, we find

$$\text{Compensation} = \frac{\beta \Delta X/(1 + G_{ss})}{\Delta X} \times 100\%$$

(3)

which simplifies to

$$\text{Compensation} = \frac{G_{ss}}{1 + G_{ss}} \times 100\%$$

(4)

Thus in a linear negative feedback system, compensation and negative feedback gain magnitude $G_{ss}$ are simply related through Eq. 4, and the relationship is independent of the size of the perturbation $\Delta X$. Moreover, as gain magnitude $G_{ss}$ increases without bound, compensation approaches 100%.

In a nonlinear system, the relationship of gain to feedback compensation, as computed from the definition for compensation (Eqs. 1 and 2), will depend on the specific properties of the nonlinear elements. Thus, the relationship given by Eq. 4 for linear systems may only apply as $\Delta X$ tends to zero, where magnification becomes a quotient of derivatives. When we say, in the RESULTS section below, that compensation agrees with the predictions of linear systems theory, the agreement will be for a case where $\Delta X$ is taken sufficiently close to zero that the relationship obtained very nearly agrees with the relationship for linear systems given by Eq. 4. For an experimentally realizable perturbation, i.e., a finite perturbation, one may reasonably expect that compensation will depend on the magnitude of the perturbation.

Base-case and instantaneous gain. We have previously shown that in our model of the TGF system, a distinction must be made between steady-state gain magnitude and instantaneous gain magnitude; this technical point is treated in detail in Ref. 19 (see also APPENDIX A). The gain magnitude that determines the bifurcation of the system into LCO is the instantaneous gain magnitude, which we designate with the symbol $\gamma$. The gain magnitude $G_{ss}$ used in the calculation above corresponds to steady-state gain magnitude (thus the subscript). For the parameters in this study, the instantaneous gain $\gamma$ exceeds the steady-state gain $G_{ss}$ by $\sim 10.3\%$ (19). Thus, to be precise, when we say below that a calculated compensation agrees with the predictions of linear systems theory, we will mean that the result obtained by a calculation of the compensation by means of the definition (Eqs. 1 and 2) very nearly approximates the relation in Eq. 4, because $\Delta X$ has been taken sufficiently close to zero and $G_{ss}$ has been interpreted to be related to $\gamma$ by $G_{ss} = \gamma/1.103$.

Comparison and normalization of perturbed MD variables. In the presence of LCO but in the absence of sustained perturbations, model calculations show that the time-averaged tubular variable values at the MD (i.e., fluid flow, chloride concentration, chloride delivery), computed with the base-case parameters in Table 1, differ from the corresponding steady-state base-case values. For example, the time-averaged NaCl delivery rate differs from the steady-state NaCl delivery rate (see RESULTS, Table 2). Thus, to obtain a consistent and unambiguous interpretation of the definition of magnification (and corresponding compensation), we adopted the principle that a perturbed value should be compared to the nonperturbed value corresponding to the stable state of the system at the given gain magnitude $\gamma$. When $\gamma$ is less than $\gamma_{c}$, the stable state is nonoscillatory, the base case is the steady-state base case, and the tubular values at the MD are the steady-state values arising from the base-case parameters (see Table 2). When $\gamma$ exceeds $\gamma_{c}$, the stable state is oscillatory, the base case is a base-case LCO, and the base-case values of the tubular variables at the MD are the time-averaged values arising from the base-case parameters (these time-averaged values are a function of $\gamma$).

Following this principle, for cases where $\gamma > \gamma_{c}$ (and thus LCO are present), we interpret $\Delta Y$, in the closed-feedback-loop term of the definition of magnification (Eq. 2), to be
RESULTS

In the absence of sustained perturbations, LCO increase distal NaCl delivery. To determine the effect of LCO on distal fluid and NaCl delivery, we compared the rates at which fluid and chloride exited the model TAL segment in the steady-state case with corresponding time-averaged values during LCO. Results for gain magnitudes \( \gamma \) from 0 to 10 are illustrated in Fig. 4, where the time-averaged variables have been normalized by their corresponding steady-state base-case values. Nonnormalized results for selected values of \( \gamma \) are given in Table 2. At all gain magnitudes exceeding \( \gamma_0 \), fluid delivery was depressed slightly by LCO, with a maximum decrease of \(-0.5\%\) at \( \gamma = 10 \).

\[
\Delta Y = \frac{(Y - Y_0)}{Y_0},
\]

where \( Y \) is the time-averaged value resulting from a sustained perturbation, and where \( Y_0 \) is the time-averaged value when LCO are present but there is no sustained perturbation. Thus, \( Y_0 \) is considered to be the base-case value that is affected by the perturbation. Because LCO are never present when the feedback loop is open (and therefore non-functional), we interpret \( \Delta Y \) in \( \Delta Y/\Delta X \), where \( \Delta X \) is the steady-state value resulting from the perturbation and \( Y_0 \) is the steady-state base-case value.

The interpretation of \( \Delta Y \) when \( \gamma < \gamma_0 \) and therefore LCO are not present, is unambiguous, because the values of \( \Delta Y \) can be taken relative to identical steady-state values. Thus, when \( \gamma < \gamma_0 \) for both the closed-feedback-loop and open-feedback-loop terms, \( \Delta Y \) is interpreted as simply \( Y - Y_0 \), where \( Y_0 \) is the steady-state base-case value.

\[
\gamma = 4.\text{ This response was driven by the monotone increase in time-averaged chloride concentration, which results in a TGF-mediated suppression of SNGFR. In contrast, time-averaged chloride delivery exhibited a biphasic relationship with increasing gain magnitude. For small increases of } \gamma \text{ above } \gamma_0 \text{ chloride delivery decreased with time-averaged flow, but then it increased, reaching a value of 103.7\% of the steady-state base-case delivery rate for } \gamma = 10. \text{ Note that the time-averaged chloride delivery rate is the time-average of the product of the instantaneous flow rate and the instantaneous concentration, and that, except for the steady-state case, the product of time-averaged flow and the time-averaged concentration does not equal the time-averaged chloride delivery, as a result of phase differences in the waveforms for flow and chloride concentration (see, e.g., Fig. 5, below).}

The reason for the increase in time-averaged chloride delivery and the relative stability in fluid delivery can be seen in Fig. 5A, which shows the fluid flow, chloride concentration, and chloride delivery LCO waveforms at the MD, for \( \gamma = 5 \). The values in Fig. 5 were normalized by the steady-state base-case values of the fluid flow rate, chloride concentration, and chloride delivery rate. Although oscillations in fluid flow are relatively symmetric around the steady-state delivery rate, the oscillations of chloride concentration have sharp crests, relative to their troughs, and the concentration waveform is shifted upwards, relative to that of fluid delivery. In addition, the oscillations in chloride concentration exhibit a phase shift to the right. This phase shift, which has been observed in experiments (11), arises because a portion of the TAL fluid column must be expelled before the full effect of a change in TAL fluid flow rate is observed in MD concentration (21). The chloride delivery rate, the instantaneous product of fluid flow rate and chloride concentration, exhibits a phase shift as a result of the phase shift in chloride concentration. Moreover, chloride delivery has sharp crests, the sharpest among those of the exhibited waveforms, and these sharp crests, which rise well above the crests in normalized fluid flow rate, result in the increase in average chloride delivery rate, as compared to the steady-state chloride delivery rate.

Figure 5B provides further insight into how the nonlinear features of the TGF system contribute to the

---

Table 2. Base-case MD variables for selected gain magnitudes

<table>
<thead>
<tr>
<th>Gain Magnitude, ( \gamma )</th>
<th>Fluid Flow, ( \text{nl/min} )</th>
<th>( [\text{Cl}^-] ), ( \text{mM} )</th>
<th>Cl Delivery, ( \text{pmol/min} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steady state ( \gamma = \gamma_0 )</td>
<td>6.000</td>
<td>31.96</td>
<td>191.8</td>
</tr>
<tr>
<td>LCO 3.5</td>
<td>5.983</td>
<td>32.11</td>
<td>191.1</td>
</tr>
<tr>
<td>5</td>
<td>5.974</td>
<td>33.01</td>
<td>193.5</td>
</tr>
<tr>
<td>7</td>
<td>5.987</td>
<td>33.71</td>
<td>196.7</td>
</tr>
<tr>
<td>10</td>
<td>5.999</td>
<td>34.13</td>
<td>198.9</td>
</tr>
</tbody>
</table>

MD, macula densa; LCO, limit-cycle oscillations.
dissociation between the waveforms of fluid flow and chloride delivery. Figure 5B is a phase plot, where the instantaneous values for chloride delivery and fluid flow are plotted against each other. The result is a depiction of the trajectory of the oscillating system, which evolves in time in the direction indicated by the arrows. Trajectories for four values of $\gamma$ are shown, and the dots at the center of the plot give the time averages for both fluid flow and chloride delivery.

The excursions in flow are limited by the range of the TGF response, which in our model permits SNGFR to vary by no more than $\pm 30\%$ of the base-case value, $Q_{op}$. As gain magnitude $\gamma$ increases, the troughs in flow are limited at values of $\gamma = 5$ and greater, while the flow maxima continue to increase, becoming limited only at $\gamma = 10$. This asymmetry in the flow waveform, which arises because of the transport and transit-time nonlinearities in the TAL (see Fig. 2B and C), permits expansion of the trajectories in chloride delivery to higher maxima, while the minima of the trajectories are restricted.

In the presence of sustained perturbations, LCO result in larger deviations in distal NaCl delivery than in distal fluid delivery. For gain magnitude $\gamma = 5$, we computed the responses of MD variables to perturbations of up to $\pm 30\%$ of the steady-state flow rate. The percent deviations from corresponding steady-state base-case values are given in Table 3, which reports the unregulated responses for the open-feedback-loop case (OL), the hypothetical steady-state feedback-controlled responses (SS) obtained by removing the time delay at the JGA (see METHODS), and the LCO responses, based on time-averaged values.

The absence of feedback in the OL case led to large deviations from the steady-state base-case values. While the OL percent change in flow was equal, under model assumptions, to the percentage of the applied perturbation, the unregulated percentage changes of both chloride concentration and delivery were much larger. For flow perturbations of $-30\%$ and $30\%$, the changes in chloride concentration were $-53\%$ and $82\%$, respectively, while corresponding changes in chloride delivery were $-67\%$ and $137\%$. The high sensitivity of these two variables reflects the strong dependence of MD chloride concentration on fluid flow (see Fig. 2B) and the fact that distal chloride delivery is a product of both increased flow and increased concentration. In particular, for positive perturbations, increases in MD chloride concentration were much less muted by chloride back-leak along the TAL than were negative perturbations (see Figure 2 in Ref. 18).

Perturbations applied to the SS and LCO cases, which are both feedback-regulated, resulted in substantially smaller deviations from steady-state base case values than did perturbations applied to the OL case. However, the SS case exhibited smaller deviations from the steady-state base case than did the LCO case. This compensatory superiority was particularly marked for positive perturbations, where, for example, a perturbation of $+10\%$ led to SS increases (%) of 1.83, 4.57, and 6.48, in fluid flow, chloride concentration, and chloride delivery, respectively, while LCO increases (%) were 2.37, 9.77, and 10.6. Thus, while in either case the perturbation increased fluid delivery by about $2\%$, the perturbation in the case of LCO increased distal chloride delivery by $10.6\%$, which is 1.64 times larger than the SS increase of $6.28\%$. This suggests that LCO, by reducing feedback compensatory capability relative to the SS, increases NaCl delivery to the distal tubule, which may lead to enhanced NaCl excretion.

Calculations for $\gamma = 10$, again for a $+10\%$ perturbation, yielded SS increases (%) of 1.02, 2.52, and 3.57, in flow, chloride concentration, and chloride delivery, respectively, while LCO increases (%) were 2.85, 13.4, and 13.8. Thus the improved feedback compensation
that was afforded to the hypothetical SS case by increased gain magnitude resulted in an even larger disparity between chloride delivery in the SS and LCO cases.

In the presence of sustained infinitesimal perturbations in fluid flow, the regulatory ability of TGF is reduced by LCO. We next sought to quantify, by evaluating feedback compensation (the index defined in METHODS), how LCO may influence the regulatory function of the TGF system. We first examined the effect of LCO on TGF compensation for an infinitesimal perturbation in SNGFR, as a function of feedback gain magnitude $g$. Two cases were examined. In the first, LCO were prevented by eliminating the time delay at the JGA; in the second, the base-case time delay was used, which led to the emergence of LCO when gain magnitude exceeded $g_c = 3.24$.

Feedback compensation for fluid delivery to the MD is shown in Fig. 6. For gain magnitudes less than $g_c$, the degree of feedback compensation was identical in both cases and results agreed closely (differed by <0.01%) with the predictions of linear systems theory (see METHODS). Compensations for chloride concentration and delivery were identical with compensations for fluid delivery. However, the curves in Fig. 6 diverge near the bifurcation point. In the hypothetical steady-state case (SS), obtained when the JGA time delay was eliminated, feedback compensation for fluid flow continued to increase with gain in accordance with linear systems theory, and compensations for chloride concentration and delivery were identical with those for fluid delivery. In contrast, the onset of LCO reduced feedback compensation, starting at gain magnitudes just above $g_c$, in comparison to the control afforded by TGF when LCO were prevented. At a gain magnitude of 10, approximately equal to the highest published measurement of TGF gain (see Ref. 9), the reduction in feedback compensation for fluid flow was 19.1%. Compensation values for MD chloride concentration and delivery (results not shown in Fig. 6) were similar to those for fluid flow: the reductions in these compensations were 17.9% and 18.2%, respectively, relative to the hypothetical SS case for $g = 10$.

These results for infinitesimal perturbations suggest that LCO limit the ability of TGF to stabilize distal delivery of fluid and NaCl. Further, maximal regulatory efficacy is predicted to be achieved near $g_c$, the feedback gain magnitude where LCO emerge.

In the presence of sustained finite perturbations in fluid flow, the regulatory ability of TGF is reduced by LCO. As discussed in METHODS, the effects of finite perturbations may differ substantially from those elicited by infinitesimal perturbations, because of TGF system nonlinearities. Therefore, we also used the index of feedback compensation to quantify the impact of sustained, finite perturbations having physiologically relevant magnitudes. Such perturbations simulate a typical micropuncture protocol in which tubular fluid is added to, or removed from, the proximal tubule to permit estimation of feedback compensation (see, e.g., Refs. 27 and 33).

Figure 7 illustrates the responses in fluid flow, chloride concentration, and chloride delivery to perturbations of up to ±30% of steady-state flow rate. Three cases are shown for gain magnitude $g = 5$ in Fig. 7: open-feedback-loop responses where TGF was nonfunctional (squares), responses for steady flows where LCO were prevented by eliminating the time delay at the JGA (open circles), and time-averaged responses with LCO present (solid circles).

The responses to the perturbations, illustrated in Fig. 7, A–C, were normalized with respect to the corresponding base-case values at zero perturbation for either steady state or LCO, as appropriate (values given in Table 2 for $g < g_c$, or for $g = 5$, as appropriate). This different normalization convention, relative to Table 3, affects only the LCO case; the different convention was adopted because emphasis, in this context, was placed on regulatory capability around the base case in a given situation, either steady-state or LCO (see METHODS, Comparison and normalization of perturbed MD variables).

The open-feedback loop responses (Fig. 7, A–C, squares) exhibit the inherent sensitivity of the unregulated system, described above in conjunction with Table 3. The regulatory action of TGF can be seen in the smaller magnitudes of the closed-feedback-loop responses (both steady-state and LCO), relative to the corresponding open-loop responses. Furthermore, the magnitudes of the closed-loop steady-state responses were less than those obtained in the presence of LCO, except for the perturbations of ±25% and ±30%, because the curves become coincident as LCO are sup-
Fig. 7. Effects of finite perturbations of SNGFR on MD fluid flow rates, chloride concentrations, and chloride delivery rates, and associated feedback compensations, for TGF gain magnitude $\gamma = 5$. Effects of perturbations are computed for three cases: no TGF control of SNGFR (open loop), TGF control, but with LCO suppressed by eliminating MD delay (closed-loop, steady), and TGF control with LCO (closed-loop, oscillations). For perturbations of $\pm 25$ and $\pm 30\%$, the two closed-loop cases agree because LCO were suppressed by offsets from the center of the TGF response curve. A: flow rate at MD, given as a percentage of respective base-case, in response to flow perturbations in SNGFR, showing that TGF limits the effects of perturbations on fluid flow and that, in the model, LCO do not impair this effective TGF regulation. B: chloride concentration at MD, as a percentage of respective base-case. C: delivery rate of chloride ion by TAL flow to MD, as a percentage of respective base-case. B and C show that chloride concentration and delivery are much more sensitive to flow perturbations than is fluid flow, both in presence and absence of TGF regulation. Moreover, when TGF loop is closed, there is increased sensitivity to perturbations when LCO are present, compared to the absence of oscillations (i.e., steady flow). D–F: feedback compensation for fluid flow, chloride concentration, and chloride delivery, respectively. Horizontal bar is compensation predicted by linear systems theory, for $\gamma = 5$. For all three regulated variables, compensation when LCO are present is significantly less than compensation for steady flow. For chloride concentration and chloride delivery, compensation is more effective for positive perturbations than for negative perturbations, for both LCO and steady flow.

Table 3. Deviations of MD variables from steady-state base-case, for gain magnitude $\gamma = 5$

<table>
<thead>
<tr>
<th>Perturbation, %</th>
<th>Fluid Flow, %</th>
<th>[Cl$^-$], %</th>
<th>Cl$^-$ Delivery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OL SS LCO</td>
<td>OL SS LCO</td>
<td>OL SS LCO</td>
</tr>
<tr>
<td>$-30$</td>
<td>$-30$ -7.05</td>
<td>$-52.5$ -16.5</td>
<td>$-66.7$ -22.4</td>
</tr>
<tr>
<td>$-25$</td>
<td>$-25$ -5.39</td>
<td>$-47.3$ -12.8</td>
<td>$-60.4$ -17.5</td>
</tr>
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<td>$30$ 6.64</td>
<td>82.1 17.1</td>
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OL, open-feedback-loop; SS, steady state. *LCO are suppressed by the perturbation.
Input into the nephron. The system may be especially efficacious at stabilizing oscillations, the nonlinear features of the TGF system can both limit and enhance regulation and that the feedback compensation was reduced in comparison to the steady-state case, except at perturbations of +25% and ±30%, where LCO are suppressed. As in the steady-state case, the compensation curve for average fluid flow is relatively symmetrical, whereas those for average chloride concentration and delivery are skewed, thereby indicating that the system is better able to compensate for positive perturbations than for negative perturbations. The asymmetrical curves reflect the higher open-feedback loop sensitivities of MD chloride concentration and distal chloride delivery to increases in TAL flow rate, relative to decreases in flow rate (already noted above for the steady-state case). As a result of those sensitivities, the LCO compensation values for chloride concentration and chloride delivery, corresponding to positive perturbations, exceeded those for fluid flow. However, as a consequence of the high inherent sensitivity of chloride delivery to flow perturbations, the increments in average distal chloride delivery during LCO were larger, as a percentage of base-case delivery, than the increments in average fluid flow. These results illustrate yet again that the regulated variable most affected by LCO is distal chloride delivery.

The marked nonlinearity of the TGF system is evident in the distortion of the waveforms obtained in the perturbed cases compared with the case of no perturbation. Not only were LCO in both flow and chloride concentration translated to generally higher or lower levels, but also the waveforms underwent fundamental shape changes as the excursions approached the limits of the TGF feedback response curve and as the flow decreased to values where the NaCl concentration at MD approached its minimum level. Chloride delivery, which is the product of fluid flow and concentration, was shaped by the phase difference between flow and chloride concentration, and the phase lag was influenced by the sign and magnitude of the perturbation.
For example, note that the chloride delivery waveform for zero perturbation has a shoulder region on the rising portion of the curve. For the negative perturbation, this inflection is more pronounced and is shifted to the right on the waveform.

**DISCUSSION**

Adequacy of the model. This investigation has shown that a simple model of TGF, a model that describes a seemingly straightforward proportional negative feedback control system, can exhibit complex behavior when its nonlinear elements are engaged by perturbations of physiologically relevant magnitude. However, the applicability of the model results and of the model predictions discussed below depends upon whether our model of the TGF system provides an adequate representation of the key features of the TGF system in vivo. The model is based on conservation of mass and on experimental data from volume-replete rats (18, 29); the model’s assumptions have been previously examined in substantial detail in Refs. (18-21, 29). The model’s properties and previous predictions have agreed well with published experimental measurements, including feedback system gain magnitude (19), the approximate feedback gain value needed to support LCO (18), and the temporal characteristics of the LCO waveform (21). The nonlinearities in the model are responsible for these temporal characteristics, which distort the LCO waveform in distinctive ways and which underlie the results of this study. Thus the similarity of in vivo recordings of LCO, which generally exhibit these temporal characteristics (21), lends substantial support to the adequacy of the model.

Model predictions. This investigation of the impact of LCO on the regulatory role of the TGF system has yielded a number of predictions that are of potential physiological importance. First, the model predicts that, if LCO are suppressed, then the TGF system will be particularly effective in stabilizing distal NaCl delivery when SNGFR is increased above its steady-state base-case value by a perturbation (Fig. 7F). Indeed, because of system nonlinearities, the model feedback compensation in this case exceeds that of a linear system with equivalent feedback gain magnitude. However, the model’s ability to maintain distal NaCl delivery in the case of a reduction in SNGFR is less than that of a linear control system. In contrast to this asymmetrical compensation for NaCl, feedback compensation for distal fluid delivery is symmetrical (Fig. 7D) and is similar to that reported in experimental studies (9, 33, 34, 35).

Second, the model predicts that the onset of LCO, in the absence of a sustained perturbation, results in increases in time-averaged distal NaCl delivery, while time-averaged distal fluid delivery is little affected (Fig. 4). Although the magnitude of the increment in distal NaCl delivery is modest, this behavior illustrates yet again that the nonlinear elements in the TGF system can result in a dissociation of the regulation of fluid and electrolyte delivery to the distal nephron.

Third, the model predicts that LCO markedly reduce the ability of the TGF system to compensate for perturbations in SNGFR, both infinitesimal and finite (Figs. 6 and 7). In vivo, the kidney is continually perturbed by substantial fluctuations in blood pressure, and one important role of the TGF system is its participation in the autoregulation of renal blood flow and GFR (26). Any decrease in the efficacy of renal autoregulation, as a consequence of the development of LCO, would result in increased fluctuations in the baseline level of SNGFR and distal delivery of water and solutes. Indeed, the model predicts that a sustained perturbation in SNGFR would in some cases result in nearly double the increments in time-averaged fluid and/or NaCl delivery into the distal nephron, when compared with an otherwise similar case where LCO are absent (Table 3 and related results in text for γ = 10).

Fourth, the model predicts that maximal regulatory efficacy will be attained at the phase transition boundary between steady and oscillatory flows, that is, when the feedback gain magnitude γ nearly equals the critical gain magnitude γc (Fig. 6). This implies that the relatively low feedback gain of the TGF system, as typically measured experimentally (see below) is sufficient to result in near optimal TGF regulation of distal delivery of fluid and NaCl. Increasing the feedback gain above γc does not further increase TGF compensation, because LCO emerge.

These four predictions, taken together, suggest that LCO have the capacity to increase time-averaged NaCl delivery to the distal nephron while time-averaged distal fluid delivery is not increased or is increased fractionally less than NaCl. The potential for LCO to differentially increase time-averaged distal NaCl delivery and thus enhance sodium excretion is considered in a separate subsection (see below).

Finally, a general, overarching prediction is that TGF system nonlinearities play a significant role in the regulatory function of TGF. Even if the results and specific predictions arising from the model simulations are not confirmed in full detail in subsequent, more comprehensive, simulations, or by in vivo experiments, new insights should nonetheless emerge in such investigations, for evidence of the potential of nonlinearities to distort TGF waveforms is manifest in experimental records (21).

Model predictions and measured gains. Thirteen steady-state measurements of TGF gain or compensation are collected in Table 4. The measured values are

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Reference</th>
<th>Compensation, %</th>
<th>Gain</th>
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<tr>
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<td>2</td>
<td></td>
<td>3.2</td>
</tr>
<tr>
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<td>9</td>
<td></td>
<td>3.1</td>
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<td>Moore and Mason</td>
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<td>64, 64, 71</td>
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<tr>
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<td>58, 63, 70</td>
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<td>34</td>
<td>63</td>
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</tr>
<tr>
<td>Thomson et al.</td>
<td>35</td>
<td>45</td>
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TGF, tubuloglomerular feedback.
These measurements cluster just below where the model predicts that LCO emerge. Five of the data points, states associated with enhanced renal sodium excretion, are distributed below the critical gain magnitude, \( \gamma_c \) (two compensations of 70% superimpose). These measurements were obtained in hydropenia or extracellular volume depletion, states associated with enhanced renal sodium retention. The nearness of the measurements to \( \gamma_c \) suggests that the TGF system in hydropenia or volume depletion may operate, on average, at or near maximal regulatory efficiency, at least in terms of the stabilization of distal fluid and sodium delivery.

Of course, TGF gain magnitude in some individual nephrons will be greater than the means reported in Table 4 and illustrated in Fig. 9. Furthermore, barbiturate anesthetics, which were used in the majority of the studies cited in Table 4, may attenuate the sensitivity and magnitude of the TGF response (23). Thus LCO are likely to be present in a substantial subset of nephrons, as shown by Holstein-Rathlou and coworkers (8, 9, 11, 13, 15, 37, 39). The model predicts that regulation of the delivery of fluid and NaCl to the distal nephron will be less effectively regulated in these oscillating nephrons, and as the proportion of oscillating nephrons increases, renal sodium excretion may also increase (see below).

This potential natriuretic effect of LCO may be relevant to hypertension; several studies in genetically hypertensive rat strains have revealed both high TGF gains and spontaneous oscillations in a substantial fraction, or in a majority, of nephrons (4, 10, 14, 15, 37, 38). Indeed, in these hypertensive animals, the oscillations, which usually appear to be chaotic but can also take the form of LCO, may act to enhance sodium excretion and thereby limit the degree of hypertension. However, it should be noted that the effect of chaotic oscillations in tubular fluid flow on distal sodium delivery is unknown and is not addressed in this study.

The natural evolution of the operating point of a system to a region near a bifurcation is an organizational characteristic of some types of complex systems; it is called “self-organizing criticality” (1). In this context, the term “criticality” refers to a system poised near a boundary where the stability of the system changes, and “self-organizing” indicates that the system spontaneously moves to a critical state. The function of a system exhibiting self-organizing criticality may change abruptly when the phase transition is crossed, as in this study when LCO emerge. Because small changes in key parameters can lead to marked functional changes in systems exhibiting self-organized criticality, they are thought to be particularly effective and adaptable regulatory systems.

The pattern shown in Fig. 9, where the TGF system in hydropenia or volume depletion appears to operate near the boundary of a phase transition, suggests that this system may exhibit self-organizing criticality. Moreover, our model suggests an advantageous consequence of operating near the bifurcation boundary: TGF compensation is maximal there. Although the questions of whether nephrons exhibit self-organizing behavior and what mechanisms might mediate such behavior are intriguing, the answers are unknown.

Potential effects on sodium excretion. The model predicts that LCO tend to increase time-averaged NaCl delivery to the distal nephron, attributable to both waveform distortion and reduced TGF regulatory ability. This prediction leads to an important question: Would an LCO-mediated increase in delivery of NaCl to the distal nephron also increase renal sodium excretion? This is a complex issue that involves several considerations.

The first concerns the linkage between distal sodium delivery and renal sodium excretion. The distal nephron exhibits some degree of short-term load adaptation, driven by increased luminal sodium concentration (16, 36), which would tend to attenuate a perturbation in distal sodium delivery. On the other hand, a rise in average tubular fluid NaCl concentration at the MD, subsequent to the emergence of LCO, will suppress renin secretion (31) and thereby tend to enhance sodium excretion. In addition, it is well established that perturbations in tubular flow driven by fluctuations in blood pressure are associated with acute changes in renal sodium excretion, a phenomenon called pressure natriuresis (6, 7). Moreover, renal sodium excretion is a process that exhibits integral characteristics, in that...
the effects of small increases in sodium excretion accumulate over time until the losses are sufficient to reduce arterial blood pressure and/or alter renal sodium handling to reestablish long-term sodium balance (6, 7). Hence, it is reasonable to expect that the decrease in TGF regulatory ability associated with LCO will result in parallel changes in distal sodium delivery and renal sodium excretion that are physiologically significant.

Experiments have shown that LCO in tubular fluid chloride concentration persist well into the early segment of the distal tubule in the rat (11, 24). Thus, a second consideration concerning the effect of LCO on renal sodium excretion is the response of the transporting cells in the distal nephron to oscillations in tubular fluid NaCl concentration and tubular fluid flow. Although we could not find any experimental data concerning the influence of such oscillations on sodium transport in the distal nephron, some evidence suggests that there may be significant effects. In many types of sodium-transporting tight epithelial cells, apical sodium entry does not passively follow changes in mucosal sodium concentration. Rather, apical membrane sodium permeability varies inversely with mucosal sodium concentration (5, 25, 32). Several mechanisms may be involved in this phenomenon, which appears to stabilize cytosolic sodium activity and which has been referred to as "feedback inhibition" (25, 32). The decrease in sodium permeability initiated by increased luminal sodium concentration has a time constant on the order of a few seconds (5). Because this time interval is much shorter than the period of the LCO (20 to 50 s; Ref. 10), apical sodium permeability may vary in time and decrease, in its time-averaged value, when time-averaged luminal sodium concentration increases. Thus, apical sodium uptake may not directly track the oscillations in luminal sodium concentration; consequently, the ability of the tubular epithelial cells to adapt to an LCO in sodium load may be limited, in comparison to the ability to adapt to an increased steady load.

A third consideration is that the parameters that determine whether LCO emerge in our model are not fixed; rather, they are strongly influenced by the physiological state of the nephron. These parameters may be reset by alterations in a number of physiological variables, including the factors that influence renal sodium transport. For example, alterations in dietary sodium intake, effective circulating volume, and arterial blood pressure influence renal hemodynamics and sodium transport via changes in levels of renal nerve activity, angiotensin II, and atrial natriuretic peptide. These factors alter nephron flow and can shift the operating point and sensitivity of the TGF system (31). Such functional changes can affect the key parameters that determine whether a nephron will exhibit steady flow or LCO. These key parameters are the system time delays, including delays related to tubular fluid flow rate (18, 29), and the gain magnitude, which is a function of the steepness of the TGF response curve and the slope of the chloride concentration profile in TAL flow along the MD in the steady state (18, 19). Hence, the physiological state of an animal can affect both the propensity for LCO to emerge and the rate of sodium transport in the distal nephron.

Summary. The findings of this study support the accepted view that TGF plays a key role in the regulation of the delivery of fluid and electrolytes to the distal nephron. However, these findings also predict a rich ensemble of behaviors that may mediate a differential regulation of fluid and electrolyte delivery to the distal nephron and differential compensatory responses to positive and negative perturbations in SNFGR. A notable prediction is that the emergence of LCO in vivo will enhance the delivery of NaCl into the distal nephron and thereby tend to enhance sodium excretion.

Both experimental tests and additional modeling studies are warranted to further elucidate the functional significance of the behaviors of this complex, nonlinear negative-feedback control system.

APPENDIX A

Mathematical Model

Model equations. The model is formulated as a system of coupled equations

\[ \frac{\partial}{\partial t} C(x, t) = -F(C_{MD}(t)) \frac{\partial}{\partial x} C(x, t) \]

\[ \quad - \frac{V_{\text{max}} C(x, t)}{K_{\text{m}} + C(x, t)} - P(C(x, t) - C_{\text{d}}(x)) \]  

\[ F(C_{MD}(t)) = 1 + K_1 \tanh(K_2(C_{\text{cop}} - C_{MD}(t))) \]  

\[ C_{MD}(t) = \int_{-\infty}^{t} \psi_b(t - s - \delta/2)(1, s - \tau_p) \, ds \]

Each equation is in nondimensional form (see Normalization of equations, below). 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 3 gives a schematic representation of the model.

Equation A1 is a partial differential equation for the chloride concentration \( C \) in the intratubular fluid of the TAL of a short-looped nephron. At time \( t = 0 \), initial concentrations \( C(x, 0) \) (for \( x \in [0, 1] \)) and \( C(1, t) \) (for \( t \in (0, 0) \)) must be specified. We assume the boundary condition \( C(0, t) = 1 \), meaning that fluid entering the TAL has constant chloride concentration. The rate of change of that concentration at \( x \in (0,1) \) depends on processes represented by the three right-hand terms in Eq. A1. The first term is axial convective chloride transport at intratubular flow speed \( F \). The second is transepithelial efflux of chloride driven by active metabolic pumps situated in the tubular walls; that efflux is approximated by Michaelis-Menten kinetics, with maximum transport rate \( V_{\text{max}} \) and Michaelis constant \( K_{\text{m}} \). The third term is transtubular chloride backleak, which depends on a specified fixed extratubular chloride concentration profile \( C_{\text{d}}(x) \) (see below) and on chloride permeability \( P \).

Equation A2 describes fluid speed in the TAL as a function of the effective luminal chloride concentration \( C_{MD} \) at the MD (see below). This feedback relation is an empirical equation well-established by steady-state experiments (31). The constant \( C_{\text{cop}} \) is the steady-state chloride concentration obtained at the MD when \( F = 1 \). 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.

Equation A3 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_MD(t), which is used to calculate the flow response that is modulated by smooth muscle of the afferent arteriole (AA). In quasi-steady state, Eq. A2 provides a good description of the TGF response. However, dynamic experiments (3) show that a change in MD concentration does not significantly affect AA muscle tension until after a discrete (or pure) delay time \( \tau_p \), 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 \( \delta \) is a second delay parameter. To simulate the pure delay followed by a distributed delay, the convolution integral given in Eq. A3 is used to describe the effective signal received by the AA at time \( t \) (29). The kernel function \( \psi \) for this integral is given by

\[
\psi_i(u) = \begin{cases} 
(1 + \cos(2\pi u/3))/3, & -\delta/2 \leq u \leq \delta/2 \\
0, & |u| > \delta/2
\end{cases}
\]  

(A4)

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

A steady-state solution to Eqs. A1–A4 may be obtained by setting \( F = 1 \) for 1 unit of normalized time (the transit time of the TAL at flow speed 1), starting at \( t = 0 \), to give the steady-state operating concentration \( C_{op} = C(1, 1) \) at the MD. If one specifies that \( C(1, t) = C_{op} \) for \( t \leq 1 \), then the initial flow to the TAL, \( F \), is fixed at 1 for all previous time. The feedback loop can then be closed at \( t = 1 \). If the system remains unperturbed, then the system solution will not vary in time. The steady-state TAL concentration profile \( C \) is denoted by \( S(x) \).

Normalization of equations. The dimensional forms of Eqs. A1 and A2 are given by

\[
\frac{\partial}{\partial t} C(x, t) = \frac{F(C_{MD}(t))}{\pi r^2} \frac{\partial}{\partial x} C(x, t) - (2r) \left( \frac{V_{max} C(x, t)}{K_M + C(x, t)} + P \left( C(x, t) - C(x_0) \right) \right)
\]  

(A5)

and

\[
F(C_{MD}(t)) = \alpha \left[ Q_{op} + \frac{\Delta Q}{2} \tanh \left( \frac{k}{2} \left( C_{op} - C_{MD}(t) \right) \right) \right]
\]  

(A6)

where \( r \) is the tubular radius, \( x \) is the (dimensionless) fraction of SNFGR reaching the TAL, \( Q_{op} \) is the steady-state operating SNFGR, \( \Delta Q \) is the TGF-mediated range of SNFGR, and \( k \) is the sensitivity of the TGF response (18). To express these equations in nondimensional form, let \( x = \frac{x}{L} \), \( t = \tau_p t_o \), \( F = r/f(A_0 f_o, \bar{C}(\xi, \tilde{t})) = C(x, t) C_{op} - C(x_0) C_{op} \), \( \xi = x/L \), \( \tilde{t} = t/L \), \( C_{op} = F(C_{MD}(t))F_{op} \), \( F_{op} = \frac{V_{max}}{K_M + V_{max}} \), \( K_{op} = K_{MD} \), \( P = P_{op} \), \( K_1 = K_2 = 2 \), \( C_{op} = C_{op} \), \( \bar{C}(\xi, \tilde{t}) = \frac{\tilde{t}_p}{\tilde{t}_o} \tilde{t} = \tilde{t}_o \), \( \delta = \tilde{t}_o \), and \( \psi_i(\tilde{t}) = \frac{\psi_i}{\psi_i(1/t_o)} \), where \( L \) is TAL length and the quantities subscripted with an “\( \bar{\} \)” are conveniently chosen reference values: \( A_0 = \frac{\pi}{2}, \tau_o = A_0 L/F_{op}, C_{op} = C(0), F_{op} = Q_{op}, V_{max} = F_{op}/(2F_{op}), P = F_{op}/(2F_{op}), \) and \( Q_{op} = Q_{op}. \) With these conventions, \( \tau_o \) is the filling time (and thus the transit time) of the TAL at flow rate \( F_{op} \) and \( V_{max} \) is the rate of solute convection into the inlet of the TAL at flow rate \( F_{op} \), divided by the area of the sides of the TAL.

When Eqs. A5 and A6 are rewritten in dimensionless terms and the tilde symbols are dropped, Eqs. A1 and A2 follow directly. The dimensional form of Eqs. A3 and A4 are the same as their nondimensional forms.

Model parameters. A summary of parameters and variables, with their dimensional units as commonly reported, is given in the Glossary. The values of model base-case parameters are given in Table 1; the criteria for their selection and supporting references were given in Ref. 18. The extratubular concentration is given in nondimensional form by \( C_e(x) = C_{op}(e^{-A_1 x} + A_2), \) where \( A_1 = (1 - C_{op}(1/C_{op})(1 - e^{-A_1 x})) \), \( A_2 = 1 - A_3, A_3 = 2, \) and \( C_{op} \) corresponds to a cortical interstitial concentration of 150 mM. Graphs of \( C_e \) and the steady-state luminal profile \( S(x) \) were given in Fig. 1 of Ref. 18. The steady-state operating concentration \( C_{op} \) was calculated numerically using the TAL dimensions and transport parameters, with steady flow \( F = 1 \) in Eq. A1.

Gain magnitude. A bifurcation in model solution can occur when the magnitude of the instantaneous gain \( \gamma \) of the feedback response exceeds a critical value, \( \gamma_c \) (18). The instantaneous gain is given by \(-\gamma = k_{11}k_{22}S'(1), \) where \( k_{11}k_{22} \) is a measure of the strength of the feedback response and \( S'(1) \) (a negative quantity) is the slope of the steady-state chloride concentration profile at the MD. In a negative feedback loop, the feedback gain is negative by convention; thus the phrase “gain magnitude” is used when referring to \( \gamma \). The instantaneous gain, investigated in Ref. 19, corresponds to the maximum reduction in SNFGR resulting from an instantaneous shift of the TAL flow column toward the MD, under the assumption that the response in SNFGR is also instantaneous.

The critical gain magnitude \( \gamma_c \) can be determined from the model’s characteristic equation, given in Ref. 21. In this study, we assume that all parameters affecting the gain are fixed except for the sensitivity of the TGF feedback response \( k \), which is used to vary \( \gamma \) through the equation \( K_2 = KC/2 \). Thus, by increasing \( k \), \( \gamma \) is increased.

**APPENDIX B**

Numerical Methods

Methods are identified by corresponding figure numbers.

Figure 1A. This curve was computed from the model equations given in APPENDIX A. Equation A1 was solved using a second-order essentially nonoscillatory (ENO) scheme, coupled with Heun’s method for time advance. This algorithm yields solutions that exhibit second-order convergence in both space and time (17). The integral of Eq. A3 was evaluated by the trapezoidal rule. The numerical time and space steps in normalized units were \( \Delta x = 1/640 \) and \( \Delta t = (320 \times t_o)^{-1} \), where \( t_o \) is the steady-state TAL transit time in seconds (see APPENDIX A, Normalization of equations). These time steps, which correspond to dimensional values of \( \Delta x = 7.8125 \times 10^{-4} \) cm and \( \Delta t = 3.125 \times 10^{-3} \) s, were used for all dynamic calculations required for Figs. 1, 2, 4–9, and for Tables 2 and 3. The high degree of numerical grid refinement was required, both to faithfully represent the nonlinearities that are embodied in the model equations (21) and to compute with sufficient accuracy the time-averaged fluid flow rates, chloride concentrations, and chloride delivery rates. Oscillations in Fig. 1A were initiated by a brief transient perturbation of \( F \). The waveform was recorded only after the oscillation had converged to a LCO.

Figure 2A. This standard curve was obtained by evaluating Eq. A6, with \( F = F_{op} \).

Figure 2B. To obtain data for this curve, Eq. A1 was solved for specified constant values of fluid flow, \( F \). For each value of \( F \), a steady-state concentration profile was obtained for \( C \). The curve was constructed by plotting the concentration values at
the MD as a function of the values of SNGFR, Q, which are given by $Q = \frac{F}{\alpha}$. 

Figure 2C. To obtain data for Fig. 2C, Eq. A1 was solved for a sustained sinusoidal flow given by $F = \alpha Q = \alpha Q_{o}(1 + 0.30 \sin(2\pi t/22))$, where $t$ is in seconds. The resulting concentration waveform at the MD was recorded after the initial concentration profile had passed out of the model TAL.

Figure 4. LCO were computed for integer values of $\gamma$ (dots on curves) and for additional values between 3 and 4. Oscillations were initiated by a brief transient perturbation (-10% of steady-state base-case flow). Waveforms were recorded for analysis only after oscillations were indistinguishable from LCO; to ensure this convergence, simulations were conducted for 968,640 time steps, corresponding to about 50.5 min of simulated oscillations, before waveforms were recorded. Slightly more than two periods of each waveform were recorded (periods differ slightly as a function of $\gamma$). The period of each waveform was determined, and two periods of each were used to compute the time averages of MD variables. Simpson’s rule was used to approximate the integrals that represent the time averages.

Figure 5. Selected waveforms computed for Fig. 4 were used for Fig. 5.

Figure 6. Compensation was evaluated at integer values of $\gamma$ and at selected other values to produce sufficiently smooth curves. The hypothetical steady-state case for $\gamma > \gamma_c$ was computed by replacing Eq. A3 with the nondelay relation $C_{MD}(t) = C(t, t)$, for the cases where the stable solution to the model equations is a steady state, i.e., for $\gamma \leq \gamma_c$ and for cases where both $\gamma > \gamma_c$ and the MD delay was eliminated (marked “SS” in the figure), compensation was evaluated from the defining equations (Eqs. 1 and 2) as follows: sustained perturbations of $-0.01$ and $+0.01$ of the value for $Q_{o}$ were added to $Q_{o}$, solutions were computed until new steady-states were achieved; $\Delta Y$ was identified with changes in the MD variables $F, C$, or $FC$; centered difference quotients $\Delta Y / \Delta X$, with $\Delta X = 0.02$, were computed for both open and closed feedback loop (open loop corresponds to $\gamma = 0$); compensation was then computed using Eqs. 1 and 2. For the oscillatory cases, sustained perturbations of $-0.01$ and $+0.01$ of the value for $Q_{o}$ were also added to $Q_{o}$; solutions were computed until LCO had been attained, and averages were computed (as for Fig. 4); $\Delta Y$ was identified with changes in the time-averages of the MD variables $F, C$, or the product $FC$; centered difference quotients $\Delta Y / \Delta X$ were computed for the closed-feedback-loop cases (open-loop cases had already been computed for the steady-state compensations), using the normalization conventions described in the METHODS; compensation was then computed using Eqs. 1 and 2.

Figure 7. The values represented were computed by the methods already described for Figs. 4 and 6.

Figure 8. Waveforms computed for Fig. 7 were used in Fig. 8.

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