Effect of tubular inhomogeneities on feedback-mediated dynamics of a model of a thick ascending limb

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One of the key mechanisms that mediate renal autoregulation is the tubuloglomerular feedback (TGF) system, which is a negative feedback loop in the kidney that balances glomerular filtration with tubular reabsorptive capacity. Tubular fluid flow, NaCl concentration and other related variables are known to exhibit TGF-mediated oscillations. In this study, we used a mathematical model of the thick ascending limb (TAL) of a short loop of Henle of the rat kidney to study the effects of (i) spatially inhomogeneous TAL NaCl active transport rate, (ii) spatially inhomogeneous tubular radius and (iii) compliance of the tubular walls on TGF-mediated dynamics. A bifurcation analysis of the TGF model equations was performed by deriving a characteristic equation and finding its roots. Results of the bifurcation analysis were validated via numerical simulations of the full model equations. Model results suggest that a higher TAL NaCl active transport rate or a smaller TAL radius near the loop bend gives rise to stable oscillatory solutions at sufficiently high TGF gain values, even with zero TGF delay. In addition, when the TAL walls are assumed to be compliant, the TGF system exhibits a heightened tendency to oscillate, a result that is consistent with predictions of a previous modelling study.

Keywords: kidney; renal hemodynamics control; negative feedback loop; delay differential equation; non-linear dynamics.

1. Introduction

The mammalian kidney plays a fundamental role in regulating the volume, osmolarity, mineral composition and acidity of the body by excreting water and inorganic electrolytes to achieve total body balance of these substances and to stabilize their concentrations in the blood (Eaton & Pooler, 2004). The functional unit of the kidney is the nephron, which consists of a bundle of filtering components, called a glomerulus, and a tubule made up of a single layer of epithelial cells. Blood is supplied to the glomerulus via an afferent arteriole. A filtrate of blood plasma is forced by blood pressure from the glomerulus into the nephron tubule, which initiates the transformation of this filtrate into urine.

The single nephron glomerular filtration rate (SNGFR), which is the volume of filtrate formed per unit time, is controlled by multiple mechanisms. One such mechanism is a negative feedback loop, called the tubuloglomerular feedback (TGF) system, which is a key regulator of glomerular filtration rate and the delivery of water and electrolytes to the distal nephron (Eaton & Pooler, 2004; Schnermann & Briggs, 2000). The TGF response is initiated by a deviation from a target value of the chloride concentration in the tubular fluid flowing past the macula densa, which is a cluster of specialized cells, located in the renal tubule wall near the end of the thick ascending limb (TAL) of the loop of Henle. If the macula densa chloride concentration is too low, TGF acts to restore it to target by inducing a vasodilation of the afferent arteriole and increases glomerular filtration pressure. A higher blood pressure

in the glomerulus increases SNGFR and raises the rate of fluid flow throughout the tubule. In particular, this leads to higher fluid flow rate in the TAL. (Conversely, if the macula densa chloride concentration exceeds the target value, the TGF system reduces glomerular filtration rate and consequently flow rate into the TAL. The resulting longer tubular transport time increases NaCl reabsorption along the TAL and lowers macula densa chloride concentration.) The TAL actively pumps out NaCl from tubular fluid into the interstitium. Because the TAL walls are nearly water impermeable, the active reabsorption of NaCl is not accompanied by water loss, and the tubular fluid NaCl concentration progressively decreases along the TAL. Thus, the TAL is an important segment of the TGF system, and its transport properties allow it to act as a key operator of the TGF system.

Experiments in the rat kidneys have indicated that TGF-mediated fluid flow in the nephrons exhibits either an almost time-independent steady state or a sustained limit cycle oscillations (LCOs) with a frequency of $\sim 20\text{--}50$ mHz. Mathematical models for the TGF system in a single short-looped nephron of the kidney have suggested that those LCO are a consequence of a Hopf bifurcation, which depends on physiological factors including feedback loop gain, feedback delay and the luminal volume of the TAL (Budu-Grajdeanu *et al.*, 2007; Layton *et al.*, 1991, 1995, 2006).

In a previous study (Layton, 2010), we developed a TGF model that represents the TAL having pressure-driven flow and compliant walls, and we used that model to investigate the impact of tubular wall compliance on TGF-mediated dynamics. These consideration were based on experimental observations indicating that *in vivo*, the TAL likely expands and contracts as luminal fluid flow rate changes, although the wall movements may be hindered by surrounding tubules and connecting tissues. Analysis of that model revealed complex parameter regions that allow a variety of qualitatively different model solutions: a regime having one stable time-independent steady-state solution; regimes having one stable oscillatory solution only and regimes having multiple possible stable oscillatory solutions. Model results suggest that the compliance of the TAL walls increases the tendency of the model TGF system to oscillate (Layton, 2010).

The previous study assumed that the TAL's luminal radius and maximum NaCl transport rate are spatially homogeneous (Layton, 2010). However, experimental evidence indicates that TAL radius and maximum transport rate vary along the TAL (Garg *et al.*, 1982; Knepper *et al.*, 1997). In this study, the impact of such inhomogeneities on the dynamic behaviours of the TGF system is examined by means of a characteristic equation that corresponds to a linearized model and via numerical simulations using the full TGF model. Our model results suggest that spatial inhomogeneity of the TAL physical and transport properties can have a significant impact on TGF dynamic behaviours; in particular, LCO can be sustained at zero TGF delay and sufficiently high gain values.

2. Mathematical model

2.1 Model formulation

We describe a mathematical model of a TGF system that represents a TAL with compliant tubular walls. The model is formulated as a boundary value problem and predicts tubular fluid rate, fluid pressure and tubular radius as functions of time and space. At the entrance of the TAL, pressure is given by the TGF response. At the end of the model tubule, fluid pressure is assumed to be known *a priori*. Because tubular fluid pressure at the macula densa is not well-known, we model a longer tubule to avoid this uncertainty: the model represents the portion of a short-loop nephron that extends in space from $x = 0$ at loop bend to $x = L_0$ at the end of collecting duct, where fluid pressure in rat can be inferred to be 1–3 mmHg, based on measurements in the interstitia, vessels and the pelvic space (Angell *et al.*, 1998;

TABLE 1 *Glossary*

Independent parameters	
α	TAL compliance (cm mmHg ⁻¹)
C_o	[Cl ⁻] at TAL entrance (mM)
P_o	Base case fluid pressure at the loop bend (mmHg)
β_0	Initial boundary value for $\beta_{0,1}(x)$ (μ m)
β_1	End boundary value for $\beta_{0,1}(x)$ (μ m)
β_2	End boundary value for $\beta_{1,2}(x)$ (μ m)
κ	TAL chloride permeability (cm s ⁻¹)
K_1, K_2	Parameters for TGF response
K_M	Michaelis constant (mM)
L_0	Length of model nephron (cm)
L	Length of TAL (cm)
μ	Fluid viscosity (g cm ⁻¹ s ⁻¹)
P_1	Pressure at end of nephron (mmHg)
Specified functions	
$\beta(x)$	Unpressurized TAL radius (μ m)
$R_{ss}(x)$	Steady-state TAL radius (μ m)
$V_{\max}(x)$	Maximum transport rate of chloride (nmole cm ⁻² s ⁻¹)
$C_e(x)$	Extracellular [Cl ⁻] (mM)
$P_o(t)$	Pressure at loop bend (mmHg)
$P_e(x)$	Extracellular pressure (mmHg)
Dependent parameters and variables	
t_o	Base case steady-state TAL transit time (s)
T_o	Steady-state TAL transit time (s)
C_{op}	Steady-state chloride concentration alongside MD (mM)
$C(x, t)$	TAL [Cl ⁻] (mM)
$P(x, t)$	Tubular fluid pressure (mmHg)
$Q(x, t)$	Tubular fluid flow (nl min ⁻¹)
$R(x, t)$	Luminal radius (μ m)

Gottschalk, 1952; Gottschalk & Mylle, 1957). The tubular walls are assumed to be compliant, with a radius that depends on transmural pressure gradient. We represent chloride ion (Cl⁻), which is known to be the principal tubular fluid signaling agent for TGF activation. Cl⁻ concentration is represented only along the TAL, from $x = 0$ at loop bend to $x = L$ at the macula densa ($L \leq L_0$). A schematic diagram for the TGF model is given in Fig. 1.

The model equations are

$$\frac{\partial}{\partial x} P(x, t) = - \frac{8\mu}{\pi R(x, t)^4} Q(x, t), \quad (2.1)$$

$$\frac{\partial}{\partial x} Q(x, t) = - \left(2\pi R(x, t) \frac{\partial R}{\partial P} \right) \frac{\partial}{\partial t} P(x, t), \quad (2.2)$$

FIG. 1. Model represents three essential elements of the TGF pathway: (i) compliant TAL, which is modelled by (2.1–2.4) with spatially inhomogeneous R_{ss} and V_{max} , (ii) delay at the macula densa (right square) and (iii) TGF response function (left square). Symbols are identified in the Table 1. Perturbations enter as adjustments to hydrodynamics pressure $P(0, t)$ that drives flow into TAL entrance ($x = 0$) at time t . Oscillations in pressure result in oscillations in TAL flow $Q(x, t)$, radius $R(x, t)$ and tubular fluid chloride concentration $C(x, t)$.

$$\begin{aligned} \pi R^2(x, t) \frac{\partial}{\partial t} C(x, t) = & -2\pi R(x, t) C(x, t) \frac{\partial}{\partial t} R(x, t) - Q(x, t) \frac{\partial}{\partial x} C(x, t) \\ & - 2\pi R_{ss}(x) \left(\frac{V_{max}(x) C(x, t)}{K_M + C(x, t)} + \kappa (C(x, t) - C_e(x)) \right), \end{aligned} \quad (2.3)$$

$$R(x, t) = \alpha(P(x, t) - P_e(x)) + \beta(x), \quad (2.4)$$

$$P_o(t) = P_o + K_1 \tanh(K_2(C_{op} - C(L, t - \tau))). \quad (2.5)$$

Equations (2.1) and (2.2) describe intratubular fluid flow and pressure, where x is axial position along the nephron ($0 \leq x \leq L_0$). $P(x, t)$ denotes the tubular fluid pressure, $Q(x, t)$ is the tubular flow rate and $R(x, t)$ is the tubular radius. The inflow pressure $P_o(t) = P(0, t)$ is given by the TGF response, and the outflow pressure $P_1 = P(L_0, t)$ is considered fixed.

Equation (2.3) represents solute concentration in the TAL's tubular fluid, where $0 \leq x \leq L$. $C(x, t)$ denotes TAL tubular fluid chloride concentration; $C_e(x)$ is the extratubular (interstitial) chloride concentration, which is assumed to be fixed. The first term inside the large pair of parentheses in the third component corresponds to active solute transport characterized by Michaelis–Menten-like kinetics. The maximum active transport rate, $V_{max}(x)$, is assumed to be a function of the space. The other term represents transepithelial diffusion, which depends on transmembrane chloride permeability, κ . The steady-state TAL radius, $R_{ss}(x)$, is also a function of x , based on Fig. 13 in Knepper *et al.* (1997), which indicates an increase in radius along the inner strip of the outer medulla. The boundary condition $C(0, t) = C_o$ is considered to be fixed so that the fluid entering the TAL has a constant chloride concentration.

Equation (2.4) represents a compliant tube, such that its tubular luminal radius varies as a function of transmembrane pressure difference. $P_e(x)$ denotes the extratubular (interstitial) pressure, α specifies the degree of tubular compliance and $\beta(x)$ is the unpressurized TAL radius (see below).

Equation (2.5) defines the feedback-mediated loop bend pressure. K_1 denotes half of the range of pressure variation around its reference value P_o ; K_2 quantifies TGF sensitivity; the target concentration

C_{op} is the time-independent steady-state TAL tubular fluid chloride concentration alongside the macula densa when $P(0, t) = P_o$ (i.e. when $C_{op} = C(L, t - \tau)$) and $C(L, t - \tau)$ is the chloride concentration alongside the macula densa at the time $t - \tau$, where τ represents the TGF delay.

2.2 Model cases

To better understand the individual and combined effects of TAL NaCl active transport rate, tubular radius and tubular compliance on the TGF dynamics, we considered five cases: one spatially homogeneous model (H) and four spatially inhomogeneous models (IR, IT, IRT and CIRT):

- H:** spatially homogeneous TAL radius and maximum active chloride transport rate (i.e. constant R_{ss} and V_{max}); rigid TAL ($\alpha = 0$).
- IR:** spatially homogeneous maximum active chloride transport rate; spatially inhomogeneous TAL luminal radius (i.e. piecewise function R_{ss}); rigid TAL ($\alpha = 0$).
- IT:** spatially homogeneous TAL radius; spatially inhomogeneous maximum active chloride transport rate (i.e. piecewise function V_{max}); rigid TAL ($\alpha = 0$).
- IRT:** spatially inhomogeneous TAL radius; spatially inhomogeneous maximum active chloride transport rate; rigid TAL ($\alpha = 0$).
- CIRT:** spatially inhomogeneous TAL luminal radius; spatially inhomogeneous maximum active chloride transport rate; compliant TAL.

Profiles for TAL radii and maximum chloride transport rates for different model cases are given in Fig. 2.

2.3 Model parameters

Model parameters that are common to all five cases are given in Table 2. Parameters that differ among the cases are displayed in Table 3.

FIG. 2. TAL radius (A) and maximum active NaCl transport rate (B) for five considered cases: H, homogeneous; IR, inhomogeneous radius; IT, inhomogeneous transport; IRT, inhomogeneous radius and transport; CIRT, inhomogeneous radius and transport with the compliant walls. Defining parameters for each case are given in Table 3.

TABLE 2 *Model parameter values common to all five cases*

Parameter	Dimensional value
Independent parameters	
C_o	275 mM
Q_o	6 nl min ⁻¹
R_o	10 μ m
κ	1.5×10^{-5} cm s ⁻¹
L_0	2 cm
L	0.5 cm
μ	7.2×10^{-3} g cm ⁻¹ s ⁻¹
P_e	5 mmHg
P_o	10 mmHg
P_1	2 mmHg
t_o	15.708 s

TABLE 3 *Individual parameter values for five model cases. Arrows indicate that radius varies as a function of increasing x ; see Fig. 2*

Parameter (units)	H	IR	IT	IRT	CIRT
α (cm mmHg ⁻¹)	0	0	0	0	0.266×10^{-5}
R_{ss} (μ m)	10	$5 \rightarrow 10$	10	$5 \rightarrow 10$	$5 \rightarrow 10$
β_0 (μ m)	10	5	10	5	4.867
β_1 (μ m)	10	10	10	10	9.92
β_2 (μ m)	5.57	5.89	5.57	5.89	5.92
$V_{\max,1}$ (nmol cm ⁻² s ⁻¹)	14.50	14.50	25.86	25.86	25.86
$V_{\max,2}$ (nmol cm ⁻² s ⁻¹)	14.50	14.50	10.82	10.82	10.82
K_M (mM)	70	60.4	48.3	34.0	34.1
C_{op} (mM)	31.98	31.97	32.04	32.02	32.01
T_o (s)	15.70	13.79	15.70	13.79	13.79

For cases IT, IRT and CIRT, the TAL maximum active transport rate $V_{\max}(x)$ is assumed to decrease linearly over the first 30% of the TAL and then remain constant afterwards; i.e.

$$V_{\max}(x) = \begin{cases} V_{\max,1} - (V_{\max,1} - V_{\max,2})\frac{x}{0.3L}, & 0 \leq x \leq 0.3L, \\ V_{\max,2}, & 0.3L \leq x \leq L, \end{cases} \quad (2.6)$$

where $V_{\max,1}$ and $V_{\max,2}$ are parameters given in Table 3.

The unpressurized tubular radius parameter $\beta(x)$ is given (in μ m) by the piecewise function:

$$\beta(x) = \begin{cases} \beta_{0,1}(x), & 0 \leq x \leq 0.3L, \\ \beta_1, & 0.3L \leq x \leq 1.5L, \\ \beta_{1,2}(x), & 1.5L \leq x \leq L_0, \end{cases} \quad (2.7)$$

where $\beta_{i,j}(x)$ denotes a cubic polynomial defined in $x_0 \leq x \leq x_1$ such that $\beta_{i,j}(x_0) = \beta_i$ and $\beta_{i,j}(x_1) = \beta_j$, and $\beta'(x_0) = \beta'(x_1) = 0$ (Layton & Layton, 2003). The parameters β_i and β_j are chosen such that

in the time-independent steady state (when Q is a constant in time and space), the model yields a target TAL radius and a target outflow pressure $P(L_0)$.

Extratubular concentration is specified by

$$C_e(x) = C_o(A_1 \exp(-A_3(x/L)) + A_2), \quad (2.8)$$

for $0 \leq x \leq L$. We set $A_1 = (1 - C_e(L)/C_o)/(1 - \exp(-A_3))$, $A_2 = 1 - A_1$ and $A_3 = 2$. $C_e(L)$ corresponds to a cortical interstitial concentration of 150 mM.

2.4 Numerical method

To simulate the tubular fluid motions, we take the spatial derivative of (2.1) and use the resulting equation to eliminate the fluid flow gradient term $\partial Q/\partial x$ from (2.2). That yields an advection–diffusion equation for the pressure P

$$\frac{\partial}{\partial t} P - \frac{R^2}{4\mu \frac{\partial R}{\partial P}} \frac{\partial}{\partial x} R \frac{\partial}{\partial x} P = \frac{R^3}{16\mu \frac{\partial R}{\partial P}} \frac{\partial^2}{\partial x^2} P, \quad (2.9)$$

subject to the boundary conditions $P(0, t) = P_o(t)$ and $P(L_0, t) = P_1$.

As in Layton (2010), (2.9) was advanced in time using numerical method that is second order in space and time. Numerical solutions to the solute conservation equation (2.3) were previously described in Layton & Pitman (1994) and Layton *et al.* (1997): a spatially second-order essentially non-oscillatory (ENO) method was used in conjunction with Heun's method to obtain a method that was second order in both space and time. A time step of $\Delta t = 1/320$ s was applied on a spatial grid of 1280 subintervals, which yield a space step of $\Delta x = L_0/1280 = 2/1280$ cm.

3. The characteristic equation

After a transient perturbation in renal blood pressure, which may be caused by various circumstances such as an animal's breathing, heart beat and movement, nephron fluid pressure (or flow) may tend to an approximation of a time-independent steady state, or it may evolve into an LCO. To predict the asymptotic behaviour of the *in vivo* tubular fluid dynamics that occurs in response to a perturbation, one may perform a direct computations of the numerical solution to the model equations (2.3), (2.4) and (2.9), for a given set of model parameter values. However, because such computations involve long-time integration of a system of coupled partial differential equations, they can be time consuming and may not be feasible if one wishes to attain a thorough understanding of the systemic dependence of model behaviour on the parameter values that fall within the physiologic range. Thus, as an alternative, we derived and analysed a characteristic equation from a linearization of the model equations.

We first non-dimensionalize (2.9) and (2.3). We use the following reference base case units: unit length along the TAL, $L = 0.5$ cm; unit fluid pressure at the loop bend, $P_o = 10$ mmHg; unit luminal radius of TAL, $R_o = 10$ μ m; unit chloride concentration, $C_o = C(0, t) = 275$ mM; unit flow rate in the TAL, $Q_o = 6$ nl min⁻¹; unit time, $t_o = \pi R_o^2 L / Q_o = 5\pi$ s (the TAL fluid transit time at flow rate Q_o). We define $\tilde{x} = x/L$, $\tilde{t} = t/t_o$, $\tilde{\tau} = \tau/t_o$, $\tilde{C} = C/C_o$, $\tilde{C}_e = C_e/C_o$, $\tilde{Q} = Q/Q_o$, $\tilde{V}_{\max} = V_{\max}/(C_o Q_o/(c_{A_o} L))$, $\tilde{K}_M = K_M/C_o$, $\tilde{\kappa} = \kappa/(Q_o/(c_{A_o} L))$, $\tilde{P} = P/P_o$, $\tilde{R} = R/R_o$, $\tilde{R}_{ss} = R_{ss}/R_o$, $\tilde{\mu} = \mu/(\pi P_o R_o^4 / Q_o L)$, $\tilde{\alpha} = \alpha P_o / R_o$, where $c_{A_o} = 2\pi R_o$. Then, expressing (2.9) and (2.3) in terms of

non-dimensional variables, simplifying and dropping the tildes, we obtain

$$\frac{\partial}{\partial t} P - \frac{R^2}{4\mu\alpha} \frac{\partial}{\partial x} R \frac{\partial}{\partial x} P = \frac{R^3}{16\mu\alpha} \frac{\partial^2}{\partial x^2} P, \quad (3.1)$$

$$R^2 \frac{\partial}{\partial t} C = -2RC \frac{\partial}{\partial t} R - Q \frac{\partial}{\partial x} C - R_{ss} \left(\frac{V_{\max} C}{K_M + C} + \kappa(C - C_e) \right). \quad (3.2)$$

We now linearize (3.1) by assuming infinitesimal perturbations in C , P and R :

$$C(x, t) = C_{ss}(x) + \epsilon C_\epsilon(x, t), \quad (3.3)$$

$$P(x, t) = P_{ss}(x) + \epsilon P_\epsilon(x, t), \quad (3.4)$$

$$R(x, t) = R_{ss}(x) + \epsilon R_\epsilon(x, t), \quad (3.5)$$

where $\epsilon \ll 1$, and $C_{ss}(x)$, $P_{ss}(x)$ and $R_{ss}(x)$ denote the steady-state Cl^- concentration, pressure and radius, respectively. From non-dimensionalized forms of (2.1) and (2.4), one can show that

$$R_{ss}^4 \frac{\partial}{\partial x} P_{ss} = -8\mu, \quad (3.6)$$

$$R_{ss} = \alpha(P_{ss} - P_e) + d\beta, \quad (3.7)$$

$$R_\epsilon = \alpha P_\epsilon, \quad (3.8)$$

where $d = \frac{\beta_0}{R_0}$. Note that steady-state tubular flow rate is normalized to 1. Then, by taking the spatial derivative of (3.6), we get

$$R_{ss}^3 \frac{\partial^2}{\partial x^2} P_{ss} = -4R_{ss}^2 \frac{\partial}{\partial x} R_{ss} \frac{\partial}{\partial x} P_{ss} = \frac{32\mu}{R_{ss}^2} \frac{\partial}{\partial x} R_{ss}. \quad (3.9)$$

Also, substituting (3.7) into (3.6), we obtain an equation for P_{ss}

$$(\alpha(P_{ss} - P_e) + d\beta)^4 \frac{\partial}{\partial x} P_{ss} = -8\mu. \quad (3.10)$$

Next, we substitute (3.4) and (3.5) into (3.1) and keep only the $O(\epsilon)$ terms

$$\begin{aligned} R_{ss} \frac{\partial}{\partial t} P_\epsilon - \frac{1}{4\mu\alpha} \left(R_{ss}^3 \frac{\partial}{\partial x} R_\epsilon \frac{\partial}{\partial x} P_{ss} + R_{ss}^3 \frac{\partial}{\partial x} R_{ss} \frac{\partial}{\partial x} P_\epsilon + 3R_{ss}^2 R_\epsilon \frac{\partial}{\partial x} R_{ss} \frac{\partial}{\partial x} P_{ss} \right) \\ = \frac{1}{16\mu\alpha} \left(R_{ss}^4 \frac{\partial^2}{\partial x^2} P_\epsilon + 4R_{ss}^3 R_\epsilon \frac{\partial^2}{\partial x^2} P_{ss} \right). \end{aligned} \quad (3.11)$$

Simplifying (3.11) from (3.6) and (3.9), we obtain the following advection–diffusion equation for P_ϵ ,

$$\frac{\partial}{\partial t} P_\epsilon + \frac{\partial}{\partial x} P_\epsilon \left(\frac{2}{R_{ss}^2} - \frac{1}{4\mu\alpha} R_{ss}^2 \frac{\partial}{\partial x} R_{ss} \right) - 2P_\epsilon \left(\frac{1}{R_{ss}^3} \frac{\partial}{\partial x} R_{ss} \right) = \frac{R_{ss}^3}{16\mu\alpha} \frac{\partial^2}{\partial x^2} P_\epsilon, \quad (3.12)$$

subject to the boundary conditions:

$$P_\epsilon(0, t) = P'(C_{\text{op}})C_\epsilon(1, t - \tau), \quad (3.13)$$

$$P_\epsilon(L_0, t) = 0. \quad (3.14)$$

As in the previous studies (Layton *et al.*, 1991, 1997, 2006; Pitman *et al.*, 1993; Layton, 2010), we assume that $C_\epsilon(x, t)$ can be written as $C_\epsilon = f(x) e^{\lambda t}$, for some function $f(x)$ and a constant λ . Thus,

$$P_\epsilon(0, t) = P'(C_{op})f(1)e^{\lambda(t-\tau)}. \quad (3.15)$$

Assuming that the solution for (3.12–3.14) has the form

$$P_\epsilon(x, t) = P'(C_{op})f(1)g(x)e^{\lambda(t-\tau)}, \quad (3.16)$$

and substituting into (3.12), we obtain the second-order differential equation for $g(x)$

$$\frac{R_{ss}^3}{16\mu\alpha}g''(x) - \left(\frac{2}{R_{ss}^2} - \frac{1}{4\mu\alpha}R_{ss}^2\frac{\partial}{\partial x}R_{ss}\right)g'(x) + \left(\frac{2}{R_{ss}^3}\frac{\partial}{\partial x}R_{ss} - \lambda\right)g(x) = 0, \quad (3.17)$$

with boundary conditions $g(0) = 1$ and $g(L_0) = 0$.

Next, we linearize the solute conservation equation (3.2),

$$\begin{aligned} (R_{ss} + \epsilon R_\epsilon)^2 \frac{\partial}{\partial t}(C_{ss} + \epsilon C_\epsilon) = & -2(R_{ss} + \epsilon R_\epsilon)(C_{ss} + \epsilon C_\epsilon) \frac{\partial}{\partial t}(R_{ss} + \epsilon R_\epsilon) \\ & + \frac{(R_{ss} + \epsilon R_\epsilon)^4}{8\mu} \frac{\partial}{\partial x}(P_{ss} + \epsilon P_\epsilon) \frac{\partial}{\partial x}(C_{ss} + \epsilon C_\epsilon) \\ & - R_{ss} \left(\frac{V_{\max}(C_{ss} + \epsilon C_\epsilon)}{K_M + C_{ss} + \epsilon C_\epsilon} + \kappa(x)(C_{ss} + \epsilon C_\epsilon - C_e) \right). \end{aligned} \quad (3.18)$$

Note that at steady state $\epsilon = 0$, the steady-state solutions satisfies

$$\frac{R_{ss}^4}{8\mu} \frac{\partial}{\partial x} P_{ss} \frac{\partial}{\partial x} C_{ss} = (K(C_{ss}) + \kappa(C_{ss} - C_e))R_{ss}, \quad (3.19)$$

where the active transport term is denoted by $K(C) = \frac{V_{\max}C}{K_M + C}$.

Keeping only the $O(\epsilon)$ terms in (3.18), we arrive at the evolution equation for C_ϵ

$$\begin{aligned} R_{ss}^2 \frac{\partial}{\partial t} C_\epsilon = & -2\alpha R_{ss} C_{ss} \frac{\partial}{\partial t} P_\epsilon + \frac{R_{ss}^3}{8\mu} \left(4R_\epsilon \frac{\partial}{\partial x} P_{ss} \frac{\partial}{\partial x} C_{ss} + R_{ss} \frac{\partial}{\partial x} P_{ss} \frac{\partial}{\partial x} C_\epsilon + R_{ss} \frac{\partial}{\partial x} P_\epsilon \frac{\partial}{\partial x} C_{ss} \right) \\ & - (K'(C_{ss}) + \kappa) R_{ss} C_\epsilon. \end{aligned} \quad (3.20)$$

Substituting $C_\epsilon = f(x) e^{\lambda t}$, $P_\epsilon(x, t) = P'(C_{op})f(1)g(x) e^{\lambda(t-\tau)}$ and $R_\epsilon(x, t) = \alpha P_\epsilon(x, t)$ into the above equation and cancelling out $e^{\lambda t}$, we obtain

$$\begin{aligned} \lambda R_{ss}^2 f(x) = & \frac{R_{ss}^3}{8\mu} (R_{ss} P'_{ss} f' + R_{ss} C'_{ss} P'(C_{op})f(1)g'(x) e^{-\lambda\tau} + 4\alpha C'_{ss} P'_{ss} P'(C_{op})f(1)g(x) e^{-\lambda\tau} \\ & - 2\alpha \lambda R_{ss} C_{ss} P'(C_{op})f(1)g(x) e^{-\lambda\tau} - (K'(C_{ss}) + \kappa) R_{ss} f(x). \end{aligned} \quad (3.21)$$

Applying (3.6) and rearranging,

$$\begin{aligned} & f'(x) + ((K'(C_{ss}) + \kappa) R_{ss} + \lambda R_{ss}^2) f(x) \\ & = e^{-\lambda\tau} P'(C_{op})f(1) \left(\frac{R_{ss}^4}{8\mu} C'_{ss} g'(x) - 2\alpha g(x) \left(\lambda R_{ss} C_{ss} + 2 \frac{C'_{ss}}{R_{ss}} \right) \right). \end{aligned} \quad (3.22)$$

Recall we have fixed Cl^- concentration at the loop bend (i.e. $x = 0$). Thus,

$$C_\epsilon(0, t) = f(0) e^{\lambda t} = 0 \Rightarrow f(0) = 0. \quad (3.23)$$

Given the initial condition (3.23), the solution for (3.22) can be found to be

$$f(s) = \exp\left(-\int_0^s P(x) dx\right) \left(\int_0^s Q(x) \exp\left(\int_0^x P(y) dy\right) dx\right), \quad (3.24)$$

where

$$P(x) = (K'(C_{ss}) + \kappa)R_{ss} + \lambda R_{ss}^2, \quad (3.25)$$

$$Q(x) = e^{-\lambda \tau} P'(C_{op}) f(1) \left(\frac{R_{ss}^4}{8\mu} C'_{ss} g'(x) - 2ag(x) \left(\lambda R_{ss} C_{ss} + 2 \frac{C'_{ss}}{R_{ss}} \right) \right). \quad (3.26)$$

Setting $s = 1$ and cancelling the factor $f(1)$, we arrive at the characteristic equation

$$1 = e^{-\lambda \tau} P'(C_{op}) \int_0^2 \left(\frac{R_{ss}^4}{8\mu} C'_{ss} g' - 2ag \left(\lambda R_{ss} C_{ss} + 2 \frac{C'_{ss}}{R_{ss}} \right) \right) \times \exp\left(-\int_x^2 (K'(C_{ss}) + \kappa)R_{ss} + \lambda R_{ss}^2 dy\right) dx. \quad (3.27)$$

To facilitate a comparison of (3.27) with the characteristic equations derived for homogeneous rigid and compliant TAL models (Layton *et al.*, 1991; Layton, 2010), we apply (3.6–3.19) to obtain

$$\frac{d}{dx} C_{ss} = -(K(C_{ss}) + \kappa(C_{ss} - C_e))R_{ss}. \quad (3.28)$$

Taking the spatial derivative of (3.28) yields

$$\begin{aligned} C''_{ss} &= -(K'(C_{ss})C'_{ss} + \frac{V'_{\max} C_{ss}}{K_M + C_{ss}} + \kappa(C'_{ss} - C'_e))R_{ss} - (K(C_{ss}) + \kappa(C_{ss} - C_e))R'_{ss} \\ &= -(K'(C_{ss})C'_{ss} + \frac{V'_{\max} C_{ss}}{K_M + C_{ss}} + \kappa(C'_{ss} - C'_e))R_{ss} + \frac{R'_{ss} C'_{ss}}{R_{ss}}. \end{aligned} \quad (3.29)$$

Dividing by C'_{ss} from both sides and rearranging,

$$(K'(C_{ss}) + \kappa)R_{ss} = -\frac{C''_{ss}}{C'_{ss}} + \frac{R'_{ss}}{R_{ss}} + \kappa \frac{C'_e}{C'_{ss}} R_{ss} - \frac{V'_{\max} C_{ss}}{(K_M + C_{ss})C'_{ss}} R_{ss}. \quad (3.30)$$

Substituting (3.30) into (3.27) and simplifying, we finally get

$$\begin{aligned} 1 &= \frac{\gamma e^{-\lambda \tau}}{R_{ss}(1)} \int_0^1 \left(\frac{R_{ss}^5}{8\mu} g' - 2ag \left(\lambda R_{ss}^2 \frac{C_{ss}}{C'_{ss}} + 2 \right) \right) \\ &\quad \times \exp\left(-\int_x^1 \left(\frac{\kappa C'_e}{C'_{ss}} + \lambda R_{ss} - \frac{V'_{\max} C_{ss}}{(K_M + C_{ss})C'_{ss}} \right) R_{ss} dy\right) dx, \end{aligned} \quad (3.31)$$

where $\gamma \equiv P'(C_{\text{op}})C'_{ss}(1)$ is the TGF gain and $g(x)$ satisfies (3.17). Equation (3.31) assumes compliant TAL walls and allows spatially varying radius and maximum active transport, i.e. it applies to the CIRT case. It can also be simplified and applied to other cases.

In the rigid tube limit where $\alpha \rightarrow 0$, (3.31) reduces to

$$1 = \frac{\gamma e^{-\lambda\tau}}{8\mu R(1)} \int_0^1 R^5 g' \exp\left(-\int_x^1 \left(\frac{\kappa C'_e}{C'_{ss}} + \lambda R - \frac{V'_{\max} C_{ss}}{(K_M + C_{ss})C'_{ss}}\right) R dy\right) dx. \quad (3.32)$$

Note that R_{ss} is replaced by R for a rigid TAL.

Multiplying (3.17) by $16\mu\alpha$ and setting $\alpha = 0$, it becomes

$$g''(x) + 4\frac{R'}{R}g'(x) = 0, \quad (3.33)$$

with boundary conditions $g(0) = 1$ and $g(L_0) = 0$. Solving for $g(x)$, we get

$$g(x) = -\frac{1}{C_1} \int_0^x \frac{1}{R^4} dy + 1, \quad (3.34)$$

$$g'(x) = -\frac{1}{C_1 R^4(x)}, \quad (3.35)$$

where $C_1 = \int_0^{L_0} R^{-4} dx$. Plugging (3.35) into (3.32),

$$1 = -\frac{\gamma}{8\mu C_1} \frac{e^{-\lambda\tau}}{R(1)} \int_0^1 R \cdot \exp\left(-\int_x^1 \left(\frac{\kappa C'_e}{C'_{ss}} + \lambda R - \frac{V'_{\max} C_{ss}}{(K_M + C_{ss})C'_{ss}}\right) R dy\right) dx. \quad (3.36)$$

With appropriate functions for R and $V_{\max}$, (3.36) can be applied to the IR, IT and IRT cases. From Appendix A, the characteristic equation for a TGF model of a rigid TAL with inhomogeneous radius and maximum active transport is given by

$$1 = -\gamma_{\text{rigid}} \frac{e^{-\lambda\tau}}{R(1)} \int_0^1 R \cdot \exp\left(-\int_x^1 \left(\frac{\kappa C'_e}{C'_{ss}} + \lambda R - \frac{V'_{\max} C_{ss}}{(K_M + C_{ss})C'_{ss}}\right) R dy\right) dx, \quad (3.37)$$

where $\gamma_{\text{rigid}} \equiv Q'(C_{\text{op}})C'_{ss}(1)$. Comparing (3.36) and (3.37), we see that in the limit $\alpha \rightarrow 0$, the gain factors for the rigid and compliant TAL models is related by

$$\gamma = 8\mu C_1 \gamma_{\text{rigid}}. \quad (3.38)$$

Furthermore, if we assume for the rigid TAL model that $V_{\max}$ and R are spatially homogeneous, then (3.36) by setting $R = 1$ and $V'_{\max} = 0$ becomes

$$1 = -\frac{\gamma}{8\mu L_0} e^{-\lambda\tau} \int_0^1 e^{-\lambda(1-x)} \exp\left(-\int_x^1 \frac{\kappa C'_e}{C'_{ss}} dy\right) dx, \quad (3.39)$$

which was also derived in Layton (2010). Equation (3.39) will be used for the H case.

4. Model results

We first compare the steady-state behaviours of the five cases. We computed steady-state TAL tubular fluid chloride concentration profiles $C_{ss}(x)$, using TAL transport parameters corresponding to each of the five cases, by integrating (3.28). Those $C_{ss}(x)$ profiles, and the external chloride concentration profile $C_e(x)$, are shown in Fig. 3. Among the five cases, the IT case, which has the highest transport rate, given by the product $V_{\max}R$, near the loop bend, yields a $C_{ss}(x)$ that has the steepest slope near $x = 0$. As a result, along the cases with rigid TAL walls, the IT case has the highest degree of instability in the near-zero delay parameter regime, as we will see below.

To better understand the effects of inhomogeneities of TAL on the dynamics of the TGF system, we used the model's characteristic equations (3.31, 3.36 and 3.39) to predict parameter boundaries that separate qualitatively differing dynamic behaviours. Then, we used numerical solutions of the full (non-linear) model (2.3, 2.4 and 2.9) to validate and to supplement the information provided by the characteristic equations.

A solution to the characteristic equation (3.31) is a number in an infinite series $\lambda_1, \lambda_2, \dots$, where $\lambda_n \in \mathbb{C}$. The real and imaginary parts of λ_n correspond to the strength and frequency, respectively, of a solution of the model equations. We determined parameter regions that have differing combinations of signs of $\text{Re}(\lambda_n)$ by computing values of $\gamma - \tau$ pairs that correspond to $\text{Re}(\lambda_n) = 0$, i.e. roots that may indicate a solution bifurcation or transition between stable solution behaviours. These $\gamma - \tau$ pairs, in $\gamma - \tau$ plane, were obtained for five model cases by using the corresponding characteristic equations. Bifurcation results for the H and IR cases are shown in Figs 4 and 5. Results for each of the four models with inhomogeneous parameters (IR, IT, IRT and CIRT) are given in Fig. 6. Note that Figs 5B and 6A are the same but with different γ -axis scales for the comparison with other model cases.

H case. Consider first the H case. For sufficiently small γ or τ such that (γ, τ) that fall below all curves $\text{Re}(\lambda_n) = 0$ in Fig. 4, the time-independent steady-state solution, indicated by ' $\rho_n < 0$ ', is the only stable solution to which any initial solution, or any transient perturbation of a steady-state solution converges. For (τ, γ) values above the curve $\text{Re}(\lambda_n) = 0$ for some n , a perturbation of the steady-state

FIG. 3. Steady-state tubular fluid chloride concentration profiles in TAL, computed from (3.28), for the five cases, corresponding to the parameter sets in Table 3. $x = 0$, loop bend; $x = 0.5$ cm, macula densa. At steady state, the chloride concentration profiles for the IRT and CIRT cases are identical. The profile for C_e (dotted) is included for comparison.

FIG. 4. Root loci for H case.

FIG. 5. Root loci for H case (A) and IR case (B). MD chloride concentration oscillations corresponding to the points **W** and **X** are given in Fig. 7W and X.

solution results in an LCO. An example is the region where $\text{Re}(\lambda_2) > 0$ and $\text{Re}(\lambda_1) < 0$, and where an LCO of a frequency corresponding to either $\text{Im}(\lambda_1)$ or $\text{Im}(\lambda_2)$ can be elicited. Model solutions can exhibit multiple stable dynamic modes, i.e. multistability (Layton *et al.*, 2009), as the crossings of the root curves introduce new parameter regimes (Layton *et al.*, 2006).

IR case. Figure 5B exhibits bifurcation curves for the IR case, where TAL radius is given as a piecewise increasing function. A comparison between Fig. 5A (H case) and B reveals that the inhomogeneous TAL radius lowers the root curves. But more surprisingly, oscillatory states become attainable at $\tau = 0$ and sufficiently large γ ($\gamma > 22.35$ for the linearized IR model). In contrast, root curves do not cross the γ -axis in the H case, which implies that, with $\tau = 0$, a transient perturbation always result in a steady-state solution. These results imply that a non-zero delay is not a necessary condition for the emergence of LCO, and that the spatial distribution of TAL radius is an important bifurcation parameter.

FIG. 6. Root loci for IR case (A), IT case (B), IRT case (C) and CIRT case (D). MD chloride concentration oscillations corresponding to the points **Y** and **Z** in panel B are given in Fig. 7Y and Z; MD chloride concentration oscillations corresponding to the points **P1** and **Q1** in panel C, and to **P2**, **Q2** and **R2** in panel D are given in Fig. 8.

To better understand the effects of spatial inhomogeneity in TAL radius and its role in the emergence of LCO at zero TGF delay, we analyse (3.2) for the IR case. Replacing the steady-state radius R_{ss} by a piecewise increasing function $R(x)$ and dividing by R^2 , (3.2) becomes

$$\frac{\partial}{\partial t} C = -\frac{Q(C(1, t - \tau))}{R^2} \frac{\partial}{\partial x} C - \frac{1}{R} (K(C) + \kappa(C - C_e)). \quad (4.1)$$

Because the TAL walls are assumed rigid, $\frac{\partial R}{\partial t} = 0$. Suppose that at time t , the TAL $[Cl^-]$ profile approximates the steady-state profile, but the macula densa $[Cl^-]$, denoted by C_{MD} , falls slightly below C_{op} . TGF then acts to increase the inflow fluid flow rate at the entrance of the proximal tubule. If $\tau = 0$, then tubular flow Q increases instantaneously at the loop bend ($x = 0$) and throughout the TAL.

Because $\frac{\partial C}{\partial x} < 0$, a larger Q implies that the right-hand side of (4.1) is positive; thus, C is increasing in time. However, when R is smaller near the loop bend, chloride concentration will change faster near the loop bend than at the macula densa. Consequently, for large γ values, the TGF response may induce a rapid rise in chloride concentration near the loop bend, which, after the TAL transit time, results in an increase in C_{MD} that may be sufficiently large so that it exceeds C_{op} . An LCO thus results. An analogous argument can be made for the case where C_{MD} exceeds C_{op} slightly.

Note that if TAL radius is larger near the loop bend, chloride concentration changes faster near the macula densa than near the loop bend; thus, no LCO will be generated at zero delay.

IT case. We then computed root curves for the IT case, where TAL maximum active NaCl transport rate V_{max} is specified as a piecewise decreasing function. Compared to the IR case, root curves for IT case, shown in Fig. 6B, are even lower, with the curves crossing the γ -axis at as low a γ value as 5.47 (compare with Fig. 5B for the IR case). For the parameter values considered, these results suggest that spatially inhomogeneous V_{max} reduces the stability of the TGF system, to a greater extent than spatially inhomogeneous TAL radius.

IRT case. The IRT case represents both inhomogeneous TAL radius and V_{max} . A smaller TAL radius near the loop bend lowers the stability of the system at small delays. However, a smaller TAL circumference also reduces the effective TAL transport rate. As shown in Fig. 6C, these two factors result in crossings of the root curves with the γ -axis at γ values that are finite but larger than the IT case, with a smallest γ value of 6.25 (compare to 5.47 for the IT case).

The explanation of the emergence of LCO at zero τ value for the IT and IRT cases is similar to that for the IR case: a higher V_{max} near the loop bend implies a larger $\frac{\partial C}{\partial t}$ there, compared to at the macula densa. As a result, following a perturbation in Q , C_{MD} may overshoot, which leads to LCO.

CIRT case. In the CIRT case, TAL compliance is introduced with $\alpha = 0.226 \times 10^{-5}$ cm mmHg $^{-1}$, taken to be 1/5 of measured compliance in isolated tubule studies (Young & Marsh, 1981). This choice of the TAL compliance is based on the consideration that the effective compliance of the TAL tubular walls may be lowered *in vivo* by various factors including the possible tethering of the TAL to other tubules via the interstitial matrix, resistance of the renal capture and resistance exerted by neighbouring TALs that may be oscillating in sync (Layton, 2010). Root curves are shown in Fig. 6D for $n \leq 5$. Compared with the IRT case (Fig. 6C), root curves corresponding to the CIRT case suggest that TAL compliance increases the tendency of the system to oscillate, especially at high frequency. This is consistent with a previous study that used a simple TAL model with homogeneous TAL transport (Layton, 2010). Indeed, if we used the isolated tubule TAL compliance ($\alpha = 1.33 \times 10^{-5}$ cm mmHg $^{-1}$), oscillatory states became attainable at even lower (possibly unphysiologically low) gain values (~ 1.5 at $\tau = 0.2$); result not shown.

Crossing of the root curves with the γ -axis has not been revealed in previous TGF models that include a detailed representation of the TAL but not the spatial inhomogeneity of its transport properties and dimension (Layton *et al.*, 1991; Layton, 2010). To validate the emergence of LCO at zero delay revealed in the present model, we computed numerical solutions to the full model equations (2.3, 2.4, and 2.9) for selected values of gain γ and delay τ . We chose two points from each of the IR and IT cases, labelled **W** and **X** in Fig. 5B, and **Y** and **Z** in Fig. 6B. These points were all chosen along the γ -axis and correspond to the following pairs of delays and gains: $(\tau_W, \gamma_W) = (0, 15)$, $(\tau_X, \gamma_X) = (0, 25)$, $(\tau_Y, \gamma_Y) = (0, 4)$ and $(\tau_Z, \gamma_Z) = (0, 9)$. The time profiles for the macula densa $[Cl^-]$ following a transient perturbation are shown in Fig. 7. Points **W** and **Y**, which lie within the ' $\rho_n < 0$ ' regime and at $\tau = 0$, correspond to time-independent steady states. Points **X** and **Z**, which lie above ' $\rho_4 = 0$ ' and ' $\rho_3 = 0$ ', respectively, correspond to oscillatory solutions; see Fig. 7X and Z. These results indicate an

FIG. 7. Sample solutions for points (**W**: $\tau = 0$, $\gamma = 15$), (**X**: $\tau = 0$, $\gamma = 25$) from Fig. 5B (IR case, $\alpha = 0$), and (**Y**: $\tau = 0$, $\gamma = 4$) and (**Z**: $\tau = 0$, $\gamma = 9$) from Fig. 6B (IT case, $\alpha = 0$), obtained via numerical simulations using full model equations (2.3, 2.4 and 2.9).

agreement between the full model and the linearized model: that LCOs may be obtained at zero TGF delay for some spatially varying TAL transport rates and dimensions.

We performed similar simulations for the IRT case, for two points along the γ -axis, with $(\tau_{P1}, \gamma_{P1}) = (0, 4)$ and $(\tau_{Q1}, \gamma_{Q1}) = (0, 8)$; and for the CIRT, for the following τ - γ pairs: $(\tau_{P2}, \gamma_{P2}) = (0.2, 1)$, $(\tau_{Q2}, \gamma_{Q2}) = (0.4, 2.5)$ and $(\tau_{R2}, \gamma_{R2}) = (0, 8)$. Locations of these points are indicated in Fig. 6C and D. The macula densa $[\text{Cl}^-]$ time profiles corresponding to these points are displayed in Fig. 8. Both points **P1** and **P2**, which lie below all root curves, i.e. within the ' $\rho_n < 0$ ' regime, yield a time-independent steady state. Points **Q1**, **Q2** and **R2** correspond to oscillatory solutions with frequencies that depend on the γ values.

5. Analysis of the characteristic equation

In this section, we provide a rigorous justification for the existence of roots along the γ -axis in TAL models with inhomogeneous radius and maximum transport rate. To that end, we analyse the characteristic equations corresponding to the H, IR and IT cases and compare the results. First, we examine the

FIG. 8. Sample solutions for points (**P1**: $\tau = 0$, $\gamma = 4$) and (**Q1**: $\tau = 0$, $\gamma = 8$), from Fig. 6C (IRT case, $\alpha = 0$), and for points (**P2**: $\tau = 0.2$, $\gamma = 1$), (**Q2**: $\tau = 0.4$, $\gamma = 2.5$) and (**R2**: $\tau = 0$, $\gamma = 8$) from Fig. 6D (CIRT case, $\alpha = 0.226 \times 10^{-5} \text{ cm mmHg}^{-1}$), obtained via numerical simulations using full model equations (2.3, 2.4 and 2.9).

characteristic equation (3.39) for the H case and show that no root exists for $\tau = 0$ and some $\gamma > 0$. By setting $\tau = 0$ and $\rho = 0$ (where $\lambda = \rho + i\omega$), we simplify (3.39) to

$$1 = -\frac{\gamma}{8\mu L_0} \int_0^1 e^{-i\omega(1-x)} \exp\left(-\int_x^1 \frac{\kappa C'_e}{C'_{ss}} dy\right) dx. \quad (5.1)$$

Substituting $e^{-i\omega(1-x)} = \cos(\omega(1-x)) - i \sin(\omega(1-x))$ into (5.1), we note that if a root exists for some $\gamma > 0$ and some ω , then the imaginary part of the resulting equation must satisfy

$$0 = \int_0^1 \sin(\omega(1-x)) \exp\left(-\int_x^1 \frac{\kappa C'_e}{C'_{ss}} dy\right) dx, \quad (5.2)$$

where the sine term in the integrand is oscillatory and the exponential term corresponds to the amplitude of integrand, which we denote by $F(x)$.

Consider the amplitude part of $F(x)$, i.e. $\exp(-f(x))$, where $f(x) = \int_x^1 \frac{\kappa C'_e}{C'_{ss}} dy$. Note that $f(x)$ is a decreasing function of x because C'_{ss} and C'_e are positive, and $f(1) = 0$; thus, $\exp(-f(x))$ is an increasing function of x . Next, note that $\sin(\omega(1-x))$ is oscillatory with equidistant x -intercepts, i.e. the n th root of $\sin(\omega(1-x)) = 0$ is given by

$$x_n = 1 - \frac{2\pi n}{\omega} \text{ such that } x_n - x_{n-1} = x_{n-1} - x_{n-2}.$$

It follows that $F(x)$ is an oscillatory function with increasing amplitude, such that the area bounded by a pair of consecutive x -intercepts increases and switches sign. Thus, the total positive and negative areas do not cancel, which implies that the right side of (5.2) is never zero and a root does not exist for a positive γ . Consequently, in the H case, the root curves do not cross the γ -axis.

Now consider the characteristic equation for the IR case:

$$1 = \frac{\gamma}{8\mu C_1} \frac{e^{-\lambda\tau}}{R(1)} \int_0^1 R \cdot \exp\left(-\int_x^1 \frac{\kappa C'_e}{C'_{ss}} R + \lambda R^2 dy\right) dx. \quad (5.3)$$

Setting $\tau = 0$ and $\rho = 0$, the above equation becomes

$$1 = \frac{\gamma}{8\mu C_1} \frac{1}{R(1)} \int_0^1 R \cdot \exp(-g_1(x)) \exp(-i\omega h_1(x)) dx, \quad (5.4)$$

where $g_1(x) = \int_x^1 \frac{\kappa C'_e}{C'_{ss}} R dy$ and $h_1(x) = \int_x^1 R^2 dy$.

For a root to exist for some $\gamma > 0$ and $\omega > 0$, the imaginary part of (5.4) must satisfy

$$0 = \int_0^1 R \cdot \exp(-g_1(x)) \sin(\omega h_1(x)) dx. \quad (5.5)$$

Similar to $f(x)$ in (5.2), $g_1(x)$ is decreasing so that $R \cdot \exp(-g_1(x))$ is an increasing function. However, when we consider the distance between x -intercepts of $\sin(\omega h_1(x))$, we must take into account the concavity of $h_1(x)$. Because $h_1''(x) = -2RR' < 0$, $h_1(x)$ is concave down and the distance between consecutive x -intercepts is decreasing. Thus, even though the amplitude of the integrand is increasing from 0 to 1, some ω may exist such that the positive and negative areas exactly cancel. Indeed, for

$\omega = 13$ and with non-dimensional parameters, the right-hand side of (5.5) is evaluated to be 0.0169; for $\omega = 14$, the right-hand side of (5.5) is -0.0109 . Thus, by the intermediate value theorem, the right-hand side vanishes for some $\omega \in (13, 14)$. This result explains the crossing of the γ -axis by the root curves in the IR case.

Consider the opposite radius variation, where R ‘decreases’ from the loop bend to the macula densa. Then, the distance between two consecutive roots of $\sin(\omega h_1(x)) = 0$ increases as x increases due to $h_1''(x) > 0$. This implies that the positive and negative areas do not cancel and the right-hand side of (5.5) should be positive for any ω . Thus, for this radius function, we expect no γ -crossings; that was confirmed by numerical solution of the characteristic equation (results not shown).

Finally, consider the characteristic equation for IT case:

$$1 = -\frac{\gamma}{8\mu C_1} e^{-\lambda\tau} \int_0^1 e^{-\lambda(1-x)} \exp\left(-\int_x^1 \frac{\kappa C'_e}{C'_{ss}} - \frac{V'_{\max} C_{ss}}{(K_M + C_{ss}) C'_{ss}} dy\right) dx. \quad (5.6)$$

Setting $\tau = 0$ and $\rho = 0$, (5.6) becomes

$$1 = \frac{\gamma}{8\mu C_1} \int_0^1 \exp(-g_2(x)) \exp(-i\omega h_2(x)) dx, \quad (5.7)$$

where $g_2(x) = \int_x^1 \frac{\kappa C'_e}{C'_{ss}} - \frac{V'_{\max} C_{ss}}{(K_M + C_{ss}) C'_{ss}} dy$ and $h_2(x) = 1 - x$.

If a root exists for some $\gamma > 0$ and $\omega > 0$, then the imaginary part of (5.7) must satisfy

$$0 = \int_0^1 \exp(-g_2(x)) \sin(\omega h_2(x)) dx. \quad (5.8)$$

Similar to the H case, $\sin(\omega h_2(x))$ is oscillatory with equidistant x -intercepts. However, the second term in the integrand of g_2 , $\frac{V'_{\max} C_{ss}}{(K_M + C_{ss}) C'_{ss}}$, is non-negative because $V'_{\max} \leq 0$; thus, $g_2(x)$ has both increasing and decreasing parts and so does $\exp(-g_2(x))$. As a result, despite the equidistant distribution of the x -intercepts of $\sin(\omega h_2(x))$, the amplitude part of the integrand in (5.8) is not monotonic, so that some ω may exist such that the positive and the negative areas exactly cancel. This result indicates the existence of γ -intercepts by the root curves in the IT case.

6. Discussion

We have used a mathematical model of the TGF system of the rat kidney to investigate the potential impacts of two types of TAL spatial inhomogeneity on the TGF-mediated dynamics. The introduction of inhomogeneities in tubular radius or in maximum chloride transport rate gives rise to oscillatory solutions at zero TGF delays and sufficiently high TGF gain values. The introduction of tubular wall compliance, even at a compliance that is only 1/5 of isolated tubule measurement, further increases the tendency of the system to oscillate.

A key finding of this study is that the spatial dependence of TAL radius and NaCl transport rate is an important bifurcation parameter. It is generally believed that a TGF system may generate an LCO solution *for sufficiently long delays and for sufficiently high gains*. In contrast, our model indicates that, provided that the TAL radius is smaller or the maximum TAL NaCl reabsorption rate is higher

near the loop bend, a non-zero (or, sufficiently long) delay is not necessary for the emergence of LCO. With spatially inhomogeneous TAL radius and NaCl transport that are consistent with experimental measurements, both the linearized model and the full non-linear model predict oscillatory solutions at zero TGF delay and sufficiently high TGF gains (see Figs 5B and 6). It is noteworthy that the IR case predicted a crossing of the γ -axis at $\gamma = 22.35$, which is much higher than the gain values of ~ 3.5 determined experimentally in normotensive rats, and is likely unphysiological. The γ -intercepts predicted in the IT, IRT and CIRT cases, which are around 4–6, are more physically plausible.

The present model assumes that TAL radius and transport properties may vary over the first 30% of the TAL, which corresponds to the inner-stripe segment. The length of the TAL that exhibits spatial varying properties, assumed fixed in the present study, appears to be yet another bifurcation parameters. The precise effects of that length on the dynamic behaviors of the TGF system are outside the scope of this study; nonetheless, our preliminary results suggest that the longer the length, the higher the tendency of the system to oscillate.

The TAL plays an essential role in the production of a concentrated urine by the kidney. The NaCl transported by the TAL raises interstitial osmolality in the outer medulla by a factor of ~ 2 , relative to blood plasma osmolality. Anatomic findings have revealed a smaller TAL radius in the inner stripe, which may be a consequence of complex medullary architecture there, as the loops of Henle of short-looped nephrons turn within a narrow band near the outer inner medullary boundary, and tubules and vessels shift positions before entering the inner stripe. Nonetheless, the effect of the reduced TAL radius in the inner stripe on TAL NaCl transport capacity is more than compensated by the higher maximum active NaCl transport rate there. Modelling studies have indicated that the higher TAL transport rate in the inner stripe has a larger concentrating effect, compared to a TAL with the same average maximum active NaCl transport rate (Layton & Layton, 2005).

Budu-Grajdeanu *et al.* (2007) used a mathematical model of the rat TAL to predict the impact of TAL spatial inhomogeneities in TAL on the transduction of oscillatory tubular fluid flow into oscillatory tubular fluid chloride concentration. They found that with the introduction of inhomogeneities, tubular fluid chloride concentration oscillations tend to be less sinusoidal and more distorted than in the homogeneous case and to thus have stronger harmonics. They hypothesized that inhomogeneities in TAL radius and NaCl transport will markedly increase the degree of spatial dependency of TAL reabsorption, and the inclusion of the inhomogeneities in a model of TGF will produce even more complex dynamics. That hypothesis is supported by the results of the present study.

The present model represents the TAL in detail, because tubular fluid chloride concentration, a key signal for TGF, exhibits the largest change, by far, along the TAL. Other components of the TGF loop are represented by means of simple phenomenological representations: the actions of the proximal tubule and descending limb of a short-looped nephron were modelled by a linear function that represents glomerular tubular balance in proximal tubule and water absorption from the descending limb (Layton, 2002). Because water and NaCl transport along the proximal tubule and descending limb impacts fluid flow rate and NaCl transport along the TAL, those transport processes may affect TGF mediation. Thus, the roles of those segments in renal autoregulation are worthy of further investigation.

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Appendix A. Derivation of 3.37

Below we present a derivation of the characteristic equation (3.37) for a rigid TAL with spatially varying radius and maximum transport rate. The solute conservation equation, in non-dimensional form, is given

by Layton *et al.* (1991)

$$R^2 \frac{\partial}{\partial t} C + Q(C(1, t - \tau)) \frac{\partial}{\partial x} C = -R(K(C) + \kappa(C - C_e)), \quad (\text{A.1})$$

where $K(C) = \frac{V_{\max} C}{K_M + C}$. Q represents the fluid flow through the tube, which depends on the macula densa chloride concentration at an earlier time $t - \tau$, i.e. $Q = Q(C(L, t - \tau))$.

We linearize (A.1) by applying (3.3) and gathering $O(1)$ and $O(\epsilon)$ terms, respectively,

$$\frac{d}{dx} C_{ss} = -\frac{R}{Q_{\text{op}}} (K(C_{ss}) + \kappa(C_{ss} - C_e)), \quad (\text{A.2})$$

$$R^2 \frac{\partial}{\partial t} C_\epsilon + Q_{\text{op}} \frac{\partial}{\partial x} C_\epsilon + Q'(C_{\text{op}}) C_\epsilon(1, t - \tau) \frac{\partial}{\partial x} C_{ss} = -(K'(C_{ss}) + \kappa) R C_\epsilon, \quad (\text{A.3})$$

where $Q(C_{ss}(1))$ and $C_{ss}(1)$ have been replaced with Q_{op} and C_{op} , respectively. Now by setting $Q_{\text{op}} = 1$ plugging $C_\epsilon = f(x)e^{\lambda t}$ into (A.3), we obtain

$$f'(x) + f(x)(\lambda R + \kappa + K'(C_{ss}))R = -Q'(C_{\text{op}})C'_{ss}f(1)e^{-\lambda\tau}. \quad (\text{A.4})$$

This first-order linear differential equation has the solution

$$f(s) = -\exp\left(-\int_0^s (K'(C_{ss}) + \kappa)R + \lambda R^2 dx\right) \left(\int_0^s Q'(C_{\text{op}})C'_{ss}f(1)e^{-\lambda\tau} \exp\left(\int_0^x (K'(C_{ss}) + \kappa)R + \lambda R^2 dy\right) dx\right), \quad (\text{A.5})$$

where we have used the boundary condition $f(0) = 0$. Setting $s = 1$ and cancelling the factor $f(1)$ yields

$$1 = -Q'(C_{\text{op}})e^{-\lambda\tau} \int_0^1 C'_{ss} \exp\left(-\int_x^1 (K'(C_{ss}) + \kappa)R + \lambda R^2 dy\right) dx. \quad (\text{A.6})$$

Finally, using (A.2), one can obtain (3.37) where $\gamma_{\text{rigid}} \equiv Q'(C_{\text{op}})C'_{ss}(1)$.