Published in final edited form as:

Math Biosci. 2011 April ; 230(2): 115–127. doi:10.1016/j.mbs.2011.02.004.

Feedback-Mediated Dynamics in a Model of Coupled Nephrons with Compliant Thick Ascending Limbs

Anita T. Layton, Matthew Bowen, Amy Wen, and Harold E. Layton

Department of Mathematics, Duke University, Durham, NC 27708-0320, USA

Abstract

The tubuloglomerular feedback (TGF) system in the kidney, a key regulator of glomerular filtration rate, has been shown in physiologic experiments in rats to mediate oscillations in thick ascending limb (TAL) tubular fluid pressure, flow, and NaCl concentration. In spontaneously hypertensive rats, TGF-mediated flow oscillations may be highly irregular. We conducted a bifurcation analysis of a mathematical model of nephrons that are coupled through their TGF systems; the TALs of these nephrons are assumed to have compliant tubular walls. A characteristic equation was derived for a model of two coupled nephrons. Analysis of that characteristic equation has revealed a number of parameter regions having the potential for differing stable dynamic states. Numerical solutions of the full equations for two model nephrons exhibit a variety of behaviors in these regions. Also, model results suggest that the stability of the TGF system is reduced by the compliance of TAL walls and by internephron coupling; as a result, the likelihood of the emergence of sustained oscillations in tubular fluid pressure and flow is increased. Based on information provided by the characteristic equation, we identified parameters with which the model predicts irregular tubular flow oscillations that exhibit a degree of complexity that may help explain the emergence of irregular oscillations in spontaneously hypertensive rats.

Keywords

kidney; hemodynamic control; negative feedback loop; delay differential equation; nonlinear dynamics; coupled oscillators

1. Introduction

The mammalian kidney plays a fundamental role in maintaining homeostasis, which involves maintaining blood pressure, electrolyte concentrations, blood plasma osmolality, and acid-base balance within the narrow limits that are compatible with survival. The nephron is the functional unit of the kidney. Each nephron consists of a bundle of capillaries, called a glomerulus, and a tubule having walls made up of a single layer of epithelial cells. A rat kidney contains ~35,000 nephrons [1]; a human kidney contains 0.30 to 1.4 million nephrons [2]. Blood is supplied to each glomerulus by an afferent arteriole. A filtrate of blood plasma is forced by blood pressure from the glomerulus into the nephron tubule, which begins the transformation of this filtrate of blood plasma into urine.

© 2011 Elsevier Inc. All rights reserved.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The single nephron glomerular filtration rate (SNGFR) is regulated by multiple mechanisms [3]. One such mechanism is the tubuloglomerular feedback (TGF) system, a negative feedback loop in which the chloride ion concentration is sensed by the macula densa, a specialized cluster of cells that are in the tubular wall and also near the afferent arteriole. According to the difference between the sensed Cl^- concentration and a target concentration [4], the muscle tension in the afferent arteriole is modified. The macula densa is near the end of the thick ascending limb (TAL), an important tubular segment for electrolyte homeostasis and for TGF function. The walls of the TAL, which are water-impermeable, vigorously pump NaCl from the TAL tubular fluid into the surrounding interstitium by means of active transepithelial transport, thereby diluting the tubular fluid.

If the chloride concentration in the TAL tubular fluid alongside the macula densa is above the target value, then TGF, through a sequence of signaling events, induces a constriction of the afferent arteriole, which results in a reduction in glomerular blood pressure and thus in SNGFR [3]. Conversely, if the chloride concentration in the tubular fluid alongside the macula densa is below the target value, then TGF acts to increase SNGFR. A full response of the afferent arteriole to a change in chloride concentration alongside the macula densa requires a few seconds [5]; a full response of tubular chloride concentration at the macula densa to a change in tubular flow appears to be approximately equal to the TAL fluid transit time.

Experiments in normotensive rats have shown that nephron flow and pressure may exhibit regular oscillations having a period of ~30 s, and that these oscillations are mediated by TGF [6,7]. Mathematical models have indicated that the oscillations arise from a Hopf bifurcation: if feedback-loop gain is sufficiently large, and if the delay in TGF signal transmission is sufficiently long, then the stable state of the system is a limit cycle oscillation [8,9]. Experiments in spontaneous hypertensive rats have shown that TGF-regulated flow may exhibit irregular oscillations having characteristics of deterministic chaos [10,11]. We have previously hypothesized that those irregular oscillations arise, in part, from interactions among TGF systems of nearby nephrons, i.e., internephron coupling. It has been well documented that TGF systems in neighboring nephrons are coupled, and that coupling appears to arise from electrotonic conduction along the pre-glomerular vasculature [12,13,14]. Thus, if two nephrons have afferent arterioles that are nearby on the cortical radial artery, the contraction of one nephron's afferent arteriole tends to result in the contraction of the other nephron's afferent arteriole. An illustration of two coupled nephrons is shown in Fig. 1A.

Our previous detailed model investigations of coupled nephrons [15,16,17] consist of simple components that have been individually well-characterized in our publications [9,17,18]. In particular, the TAL is represented by a rigid tube with plug flow that carries only the chloride ion. *In vivo*, however, the TAL likely expands and contracts as luminal fluid flow rate changes, although the wall movements may be hindered by surrounding TALs and connecting tissues. To assess the impacts of tubular wall compliance on TGF dynamics, we developed a TGF model that represents a TAL having pressure-driven flow and compliant walls in a previous study. Analysis of that model revealed a complex parameter region that allows 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. Indeed, model results suggest that the compliance of the TAL walls increases the tendency of the model TGF system to oscillate.

A goal of this study is to better understand the flow dynamics in a system of coupled nephrons having compliant TAL walls. In particular, we investigate the extent to which TAL

wall compliance and internephron coupling contribute to the emergence of flow oscillations. For this analysis, we developed a model for coupled nephrons by coupling instances of our single-nephron model with compliant TAL walls [19]; the coupling is through the TGF systems of the single-nephron models. We then analyzed the coupled-nephron model by means of linearization and numerical simulations. For simplicity and tractability, we have limited the present investigation to two nephrons. For gains and delays encompassed within the normal and pathophysiologic regimes, the studies described herein predict that a variety of dynamic behaviors can arise in coupled nephron systems. We also investigate the role of internephron coupling in the generation of irregular TGF-mediated tubular flow oscillations. For this study, we identified a set of feedback parameters based on information provided by the characteristic equations. Using those parameters, we present simulation results that show that differing parameters gains and time delays between coupled nephrons, which merely reflect differences in nephron dimensions and TGF gains, can introduce doublet and triplet spectral peaks into the power spectrum, and generate irregular flow oscillations and complex power spectra similar to those observed in spontaneously hypertensive rats.

2. Mathematical Model

2.1. Model Equations and Parameters

We describe a mathematical model of a TGF system that represents a TAL with compliant walls. The model is formulated as a boundary value problem that predicts tubular fluid pressure, chloride concentration, and tubular radius as functions of time and space. At the entrance to the TAL, tubular fluid pressure is given by the TGF response. At the end of the model tubule, pressure is assumed to be known *a priori*. However, tubular fluid pressure at the macula densa is not well-characterized. To avoid that uncertainty, we model a longer tubule: 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 the 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 [20,21,22]. The tubular walls are assumed to be compliant, with a radius that depends on transmural pressure gradient. We represent chloride ion (Cl^-), the concentration of which alongside the macula densa is believed to be the principal tubular fluid signaling agent for TGF activation. Cl^- concentration is represented only along the TAL, which extends in space from $x = 0$ at loop bend to $x = L$ at the macula densa ($L < L_0$). The impact of coupling on a model nephron is represented through the dependence of its inflow pressure on coupled TGF responses. A schematic diagram for the model TGF system is given in Fig. 1.

Fluid motion along the model nephron indexed by i is modeled as the flow of an incompressible fluid along a narrow compliant tube. Intratubular fluid flow and pressure are described by the coupled partial differential equations

$$\frac{\partial}{\partial x} P_i(x, t) = - \frac{8\mu}{\pi R_i(x, t)^4} Q_i(x, t), \quad (1)$$

$$\frac{\partial}{\partial x} Q_i(x, t) = - \left(2\pi R_i(x, t) \frac{\partial R_i}{\partial P_i} \right) \frac{\partial}{\partial t} P_i(x, t), \quad (2)$$

where $P_i(x, t)$ is the tubular fluid pressure, $Q_i(x, t)$ is the tubular volume flow, and $R_i(x, t)$ is the tubular radius. As given by Eq. (1), which represents Poiseuille flow along a narrow tubule in the zero Reynold's number limit, fluid flow along the model nephron is driven by axial pressure gradient. Equation (2) represents the incompressibility constraint or continuity

of mass. The inflow pressure $P_{\text{in}}(t) = P_i(0, t)$ is given by the TGF response (see below), and the outflow pressure $P_{\text{out}} = P_i(L_0, t)$ is considered fixed.

Solute conservation in the i -th TAL's tubular fluid is represented by a hyperbolic partial differential equation

$$\pi R_i(x, t)^2 \frac{\partial}{\partial t} C_i(x, t) = -2\pi R_i(x, t) C_i(x, t) \frac{\partial}{\partial t} R_i(x, t) - Q_i(x, t) \frac{\partial}{\partial x} C_i(x, t) - 2\pi R_{o,i} \left(\frac{V_{\text{max},i} C_i(x, t)}{K_{M,i} + C_i(x, t)} + \kappa_i (C_i(x, t) - C_e(x)) \right), \quad (3)$$

where $0 \leq x \leq L$, $C_i(x, t)$ is TAL tubular fluid chloride concentration, $C_e(x)$ is the extratubular (interstitial) chloride concentration, and $R_{o,i}$ is the steady-state TAL radius. The two terms inside the large pair of parentheses on the right-hand side correspond to active solute transport characterized by Michaelis-Menten-like kinetics (characterized by maximum transport rate $V_{\text{max},i}$ and Michaelis constant $K_{M,i}$) and transepithelial diffusion (characterized by backleak permeability κ_i). The boundary condition $C_{o,i} = C_i(0, t)$ is considered fixed.

To represent a compliant tube, the tubular luminal radius is allowed to vary as a function of transmural pressure difference:

$$R_i(x, t) = \alpha_i (P_i(x, t) - P_e(x)) + \beta_i(x), \quad (4)$$

where $P_e(x)$ is the extratubular (interstitial) pressure, α_i specifies the degree of tubular compliance, and $\beta_i(x)$ is the tubular radius when the pressure gradient is zero (see below).

In model nephron i , the inflow pressure at the loop bend, $P_i(0, t)$, is a function of the TGF-mediated pressure response of the nephron itself in addition to the coupled TGF responses of nearby nephrons. Accordingly, we model the TGF-mediated pressure at the loop bend by the equation

$$P_i(0, t) = P_{i,0} + K_{1,i} \tanh(K_{2,i}(C_{\text{op}} - C_i(L, t - \tau_i))) + \sum_{j \neq i} \phi_{ij} K_{1,j} \tanh(K_{2,j}(C_{\text{op}} - C_j(L, t - \tau_j))), \quad (5)$$

where ϕ_{ij} characterizes the strength of the coupling between nephrons i and j ; $K_{1,i}$ denotes half of the range of pressure variation around the reference pressure, $P_{i,0}$, for nephron i ; $K_{2,i}$ quantifies the TGF sensitivity; C_{op} is the time-independent steady-state TAL tubular fluid chloride concentration alongside the macula densa when $P_i(0, t) = P_{i,0}$; and $C_i(L, t - \tau_i)$ is the chloride concentration alongside the macula densa (of nephron i) at the time $t - \tau_i$, where τ_i is the TGF delay.

Parameters and numerical method—Base-case values for model parameters are given in Table 1. The luminal radius parameter $\beta_i(x)$ is given (in μm) by the piecewise function:

$$\beta_i(x) = \begin{cases} \beta_{0,i} & 0 \leq x \leq 1.5 \times L \\ \beta_{1,i}(x) & 1.5 \times L \leq x \leq L_0 \end{cases} \quad (6)$$

where $\beta_{1,i}(x)$ is a cubic polynomial such that $\beta_{1,i}(x = 1.5 \times L) = \beta_{0,i}$ and $\beta_{1,i}(x = L_0) = \beta_{2,i}$ [23]. The values $\beta_{0,i}$ and $\beta_{2,i}$ are chosen such that at steady state (i.e., with Q assumed constant) the model yields a target average TAL radius and a target outflow pressure $P_i(L_0)$.

Extratubular concentration is specified by $C_e(x) = C_o(A_1 \exp(-A_3(x/L)) + A_2)$, where $A_1 = (1 - C_e(L)/C_o)/(1 - \exp(-A_3))$, $A_2 = 1 - A_1$, and $A_3 = 2$, and where $C_e(L)$ corresponds to a cortical interstitial concentration of 150 mM. Graphs for $C_e(x)$ and the steady-state profile $C(x)$ were given in Figure 1 of Ref. [9].

To compute an approximation for the tubular fluid motion, we take the spatial derivative of Eq. (1) and use the resulting equation to eliminate the fluid flow gradient term $\partial Q_i/\partial x$ from Eq. (2). That yields an advection-diffusion equation for the pressure P_i

$$\frac{\partial}{\partial t} P_i(x, t) - \frac{R_i(x, t)^2}{4\mu \frac{\partial R_i}{\partial P_i}} \frac{\partial}{\partial x} R_i(x, t) \frac{\partial}{\partial x} P_i(x, t) = \frac{R_i(x, t)^3}{16\mu \frac{\partial R_i}{\partial P_i}} \frac{\partial^2}{\partial x^2} P_i(x, t), \quad (7)$$

subject to the boundary conditions $P(0, t) = P_{in}(t)$ and $P(L_0, t) = P_{out}$. As in [19], Eq. (7) was advanced in time using a numerical method that is second order in space and time.

Numerical solutions to the solute conservation equation (3) were computed by means of a spatially second-order ENO method applied in conjunction with Heun's method to obtain a method that was second-order in both space and time [24,25]. A time step of $\Delta t = 1/320$ s was applied on a spatial grid of 1280 subintervals, which yielded a space step of $\Delta x = L_0/1280 = 2/1280$ cm.

3. The Characteristic Equation

An animal's breathing, heart beat, and movement almost constantly perturb renal blood pressure. Following a perturbation, tubular fluid pressure (or flow) may tend to an approximation of a time-independent steady state, or it may evolve into a LCO. Given a set of model parameters, one may predict the asymptotic behavior of the *in vivo* tubular fluid dynamics that occurs in response to a perturbation by means of a direct computation of the numerical solution to the model equations Eqs. (3), (4), and (7). However, such computations can be time-consuming and may not be feasible if one wishes to attain a thorough understanding of the systematic dependence of model behavior on the parameter values that fall within the physiologic range. Thus, as an alternative, we derived and analyzed a characteristic equation from a linearization of the model equations.

We first nondimensionalize Eqs. (7) and (3) for the *i*th nephron by letting $\tilde{x} = x/L$, $\tilde{t} = t/T_o$, $\tilde{\tau} = \tau/T_o$, $\tilde{c}_{A_o} = c_{A_o}/c_{A_o} = 1$ (where $c_{A_o} = 2\pi R_o$), $\tilde{C}_i = C_i/C_o$, $\tilde{C}_e = C_e/C_o$, $\tilde{Q}_i = Q_i/Q_o$, $\tilde{V}_{max,i} = V_{max,i}/(C_o Q_o/(c_{A_o} L))$, $\tilde{K}_{M,i} = K_{M,i}/C_o$, $\tilde{\kappa}_i = \kappa_i/(Q_o/(c_{A_o} L))$, $\tilde{P}_i = P_i/P_o$, $\tilde{R}_i = R_i/R_o$, $\tilde{\mu} = \mu/(\pi P_o R_o^4/(Q_o L))$, where $T_o = \pi R_o^2 L/Q_o$. Then, expressing Eqs. (7) and (3) in terms of nondimensional variables, simplifying, and dropping the tildes, we obtain

$$\frac{\partial}{\partial t} P_i - \frac{R_i^2}{4\mu \alpha_i} \frac{\partial}{\partial x} R_i \frac{\partial}{\partial x} P_i = \frac{R_i^3}{16\mu \alpha_i} \frac{\partial^2}{\partial x^2} P_i, \quad (8)$$

$$R_i^2 \frac{\partial}{\partial t} C_i = -2R_i C_i \frac{\partial}{\partial t} R_i - Q_i \frac{\partial}{\partial x} C_i - \left(\frac{V_{max,i} C_i}{K_{M,i} + C_i} + \kappa_i (C_i - C_e) \right). \quad (9)$$

In the derivation of the characteristic equation, we make the simplifying assumption that, at steady state, $P_i(x)$ is linear and we chose the radius parameter $\beta_i(x)$ such that $R_i(x)$ is constant. We further assume that the nephrons share the same transport parameters (i.e., $V_{max,i}$, $K_{M,i}$, and κ_i are the same for all *i*); thus their steady-state Cl^- concentration profiles

are the same. Given these assumptions, we first linearize Eq. (8) by assuming infinitesimal perturbations in C_i , P_i , and R_i :

$$C_i(x, t) = C_{ss}(x) + \varepsilon D_i(x, t) \quad (10)$$

$$P_i(x, t) = P_{ss,i}(x) + \varepsilon G_i(x, t), \quad (11)$$

$$R_i(x, t) = 1 + \varepsilon r_i(x, t), \quad (12)$$

where $C_{ss}(x)$ and $P_{ss,i}(x)$ denote steady-state Cl^- concentration and pressure, respectively; and $\varepsilon D_i(x, t)$, $\varepsilon G_i(x, t)$, and $\varepsilon r_i(x, t)$ denote deviations of C_i , P_i , and R_i , respectively, from steady state, with $\varepsilon \ll 1$. Steady-state tubular radius is assumed to be normalized to 1. Note that from Eq. (1),

$$\frac{\partial}{\partial x} P_{ss,i}(x) = -8\mu, \quad (13)$$

in nondimensional form, with steady-state tubular flow rate normalized to 1. Note that the π factor does not appear in Eq. (13) because it has been incorporated in the nondimensionalization of μ . Also, from the pressure-radius relationship (Eq. (4)), one can show that

$$\varepsilon r_i(x, t) = \alpha_i (P_i(x, t) - P_{ss,i}(x)) = \varepsilon \alpha_i G_i(x, t). \quad (14)$$

Substituting Eqs. (11) and (12) into Eq. (8), we obtain

$$\frac{\partial}{\partial t} (P_{ss,i} + \varepsilon G_i) - \frac{(1 + \varepsilon r_i)^2}{4\mu\alpha_i} \left(\varepsilon \frac{\partial r_i}{\partial x} \right) \frac{\partial}{\partial x} (P_{ss,i} + \varepsilon G_i) = \frac{(1 + \varepsilon r_i)^3}{16\mu\alpha_i} \frac{\partial^2}{\partial x^2} (P_{ss,i} + \varepsilon G_i). \quad (15)$$

Keeping only the $\mathcal{O}(\varepsilon)$ terms and simplifying, we obtain a linear advection-diffusion equation for G_i . This linearization applies to each coupled nephron, with the subscript i used to represent nephron i . For each nephron i , we obtain the equation

$$\frac{\partial}{\partial t} G_i + 2 \frac{\partial}{\partial x} G_i = \frac{1}{16\mu\alpha_i} \frac{\partial^2}{\partial x^2} G_i, \quad (16)$$

subject to the boundary conditions:

$$G_i(0, t) = P'_i(C_{op}) D_i(1, t - \tau) + \sum_{j \neq i} \phi_{ij} P'_j(C_{op}) D_j(1, t - \tau_j), \quad (17)$$

$$G_i(L_0, t) = 0. \quad (18)$$

where $P'_i(C_{\text{op}}) \equiv \left. \frac{dP_i}{dC_i} \right|_{C_i=C_{\text{op}}}$. The boundary condition at $x = 0$ (i.e., Eq. (17)) specifies the change in $P_{i,0}$ in response to a deviation in macula densa Cl^- concentration; that response has a delay of τ_i . The other boundary condition (Eq. (18)) imposes a fixed pressure value at $x = L_0$.

As in previous studies [9,25,26,17], we assume that $D_i(x, t)$ can be written as $D_i(x, t) = f_i(x)e^{\lambda_i t}$, where f_i is a differentiable real function with $f_i(0) = 0$, and $\lambda_i \in \mathbb{C}$. We further assume that

$$D_j = D_i \equiv D, f_j = f_i \equiv f, \lambda_i = \lambda_j \equiv \lambda. \quad (19)$$

With this notation, the boundary condition (Eq. (17)) becomes

$$G_i(0, t) = P'_i(C_{\text{op}})f(1)e^{\lambda(t-\tau_i)} + \sum_{j \neq i} \phi_{ij} P'_j(C_{\text{op}})f(1)e^{\lambda(t-\tau_j)}, \quad (20)$$

or, if we define $\phi_{jj} \equiv 1$,

$$G_i(0, t) = \sum_{j=1}^N \phi_{ij} P'_j(C_{\text{op}})f(1)e^{\lambda(t-\tau_j)}.$$

Now, we assume that the solution takes the form $G_i(x, t) = G_i(0, t)g_i(x)$, or

$$G_i(x, t) = g_i(x) \left(\sum_{j=1}^N \phi_{ij} P'_j(C_{\text{op}})f(1)e^{\lambda(t-\tau_j)} \right), \quad (21)$$

The boundary conditions for g_i are set to $g_i(0) = 1$ and $g_i(L_0) = 0$, so that Eqs. (17) and (18) are satisfied. Substituting Eq. (21) into Eq. (16), we find

$$\frac{1}{16\mu\alpha_i} g_i''(x) - 2g_i'(x) - \lambda g_i(x) = 0. \quad (22)$$

Using the boundary conditions for g_i , we then find that,

$$g_i(x) = c_i^+ e^{k_i^+ x} + c_i^- e^{k_i^- x}, \quad (23)$$

where

$$k_i^\pm = 16\mu\alpha_i \pm \sqrt{(16\mu\alpha_i)^2 + 16\mu\alpha_i\lambda}, \quad (24)$$

$$c_i^{\pm} = \pm \frac{e^{k_i^{\mp} L_0}}{e^{k_i^{-} L_0} - e^{k_i^{+} L_0}}. \quad (25)$$

Note that in the rigid-tube limit, i.e., $\alpha_i \rightarrow 0$, $k_i^{+} - k_i^{-} \rightarrow 0$ and $g_i(x)$ approaches a linear function, thereby approximating the behavior of a rigid tube. Also, when the solution (Eq. (21)) is substituted into Eq. (16), the factor $f(1)$ cancels on both sides of the equation; thus, the value of $f(1)$ is not needed in the analysis.

We then linearize the solute conservation equation for each nephron i by substituting Eqs. (10)–(12) and the normalized form of Eq. (1) into Eq. (9):

$$\begin{aligned} (1+\varepsilon r_i)^2 \frac{\partial}{\partial t} (C_{ss} + \varepsilon D) &= -2(1 + \varepsilon r_i)(C_{ss} + \varepsilon D) \frac{\partial}{\partial t} (1 + \varepsilon r_i) \\ &+ \frac{(1+\varepsilon r_i)^4}{8\mu} \frac{\partial}{\partial x} (P_{ss} + \varepsilon G_i) \frac{\partial}{\partial x} (C_{ss} + \varepsilon D) \\ &- \left(\frac{V_{\max}(C_{ss} + \varepsilon D)}{K_M + C_{ss} + \varepsilon D} + \kappa(C_{ss} + \varepsilon D - C_e) \right). \end{aligned} \quad (26)$$

To simplify notations, we denote the active transport term by $K(C) = V_{\max}C/(K_M + C)$. Thus, at steady state, where the time-derivatives vanish, one obtains that

$$\frac{1}{8\mu} \frac{\partial}{\partial x} P_{ss} \frac{\partial}{\partial x} C_{ss} = K(C_{ss}) + \kappa(C_{ss} - C_e). \quad (27)$$

Keeping only the $\mathcal{O}(\varepsilon)$ terms in Eq. (26), and using the relation $\partial r_i / \partial t = \alpha \partial G_i / \partial t$ from Eq. (14), we arrive at the evolution equation for D ,

$$\frac{\partial}{\partial t} D = -2\alpha_i C_{ss} \frac{\partial}{\partial t} G_i + \frac{1}{8\mu} \left(4r_i \frac{\partial}{\partial x} P_{ss} \frac{\partial}{\partial x} C_{ss} + \frac{\partial}{\partial x} P_{ss} \frac{\partial}{\partial x} D + \frac{\partial}{\partial x} G_i \frac{\partial}{\partial x} C_{ss} \right) - (K'(C_{ss}) + \kappa) D. \quad (28)$$

Substituting $D(x, t) = f(x)e^{\lambda t}$ and $r_i(x, t) = \alpha_i G_i(x, t)$ into the above equation, we obtain

$$\lambda f(x)e^{\lambda t} = \frac{1}{8\mu} (C'_{ss} g'_i(x) G_i(0, t) + P'_{ss} f'(x) e^{\lambda t} + 4\alpha_i C'_{ss} P'_{ss} g_i(x) G_i(0, t)) - 2\alpha C_{ss} \lambda g_i(x) G_i(0, t) - (K'(C_{ss}) + \kappa) f(x) e^{\lambda t}. \quad (29)$$

Applying Eq. (13), canceling out $e^{\lambda t}$, rearranging, and substituting in Eq. (21),

$$f'(x) + (\lambda + K'(C_{ss}) + \kappa) f(x) = \left(\sum_{j=1}^N \phi_{ij} e^{-\lambda \tau_j} P'_j(C_{op}) f(1) \right) \left(\frac{1}{8\mu} C'_{ss}(x) g'_i(x) - 2\alpha_i g_i(x) (\lambda C_{ss}(x) + 2C'_{ss}(x)) \right). \quad (30)$$

Recall that the Cl^- concentration at the loop bend (i.e., entrance to the TAL) is assumed fixed, so $D(0) = f(0)e^{\lambda t} = 0$, which implies that $f(0) = 0$. Hence, we obtain the following solution for Eq. (30):

$$f(s) = \exp \left(- \int_0^s (\lambda + K'(C_{ss}) + \kappa) dy \right) f(1) \times \int_0^s \sum_{j=1}^N \phi_{ij} P'_j(C_{op}) e^{-\lambda \tau_j} \left(\frac{1}{8\mu} C'_{ss} g'_i - 2\alpha_i g_i (\lambda C_{ss} + 2C'_{ss}) \right) \times \exp \left(\int_0^x (\lambda + K'(C_{ss}) + \kappa) dy \right) dx. \quad (31)$$

Setting $s = 1$ and canceling the factor $f(1)$,

$$1 = \sum_{j=1}^N \phi_{ij} e^{-\lambda \tau_j} P'_j(C_{op}) \int_0^1 \left(\frac{1}{8\mu} C'_{ss} g'_i - 2\alpha_i g_i (\lambda C_{ss} + 2C'_{ss}) \right) \times \exp \left(- \int_x^1 (\lambda + K'(C_{ss}) + \kappa) dy \right) dx. \quad (32)$$

To facilitate a comparison of Eq. (32) with the characteristic equation derived for a rigid TAL [9], we use the relation

$$\frac{d}{dx} C_{ss}(x) = -K(C_{ss}) - \kappa(C_{ss}(x) - C_e(x)), \quad (33)$$

which can be obtained from Eq. (9) by eliminating all the time-derivatives and setting $Q_{ss} = 1$. Upon differentiation, Eq. (33) yields

$$C''_{ss}(x) = -K'(C_{ss})C'_{ss}(x) - \kappa(C'_{ss}(x) - C'_e(x)) \quad (34)$$

$$\Rightarrow K'(C_{ss}) + \kappa = \frac{\kappa C'_e}{C'_{ss}} - \frac{C''_{ss}}{C'_{ss}}. \quad (35)$$

Now substituting Eq. (35) into Eq. (32), we get

$$1 = \sum_j \phi_{ij} \gamma_j e^{-\lambda \tau_j} \int_0^1 e^{-\lambda(1-x)} \left(\frac{1}{8\mu} g'_i - 2\alpha_i g_i \left(\lambda \frac{C_{ss}}{C'_{ss}} + 2 \right) \right) \exp \left(- \int_x^1 \frac{\kappa C'_e}{C'_{ss}} dy \right) dx, \quad (36)$$

where $\gamma_j \equiv P'_j(C_{op})C'_{ss}(1)$ is the TGF gain. The gain γ_j can be related to the parameters $K_{1,j}$ and $K_{2,j}$ in the pressure response function (Eq. (5)). Differentiating Eq. (5) with respect to C_i and setting C_i to C_{op} , we obtain that $P'_j(C_{op}) = K_{1,j}K_{2,j}$; thus

$$\gamma_j = -K_{1,j}K_{2,j}C'_{ss}(1). \quad (37)$$

In the case of two coupled nephrons ($N = 2$) one may obtain for $i = 1$:

$$\frac{1}{\omega(\lambda)} = \gamma_1 e^{-\lambda\tau_1} + \phi_{12} \gamma_2 e^{-\lambda\tau_2}, \quad (38)$$

where

$$\omega(\lambda) \equiv \int_0^1 e^{-\lambda(1-x)} \left(\frac{1}{8\mu} g'_i - 2\alpha_i g_i \left(\lambda \frac{C_{ss}}{C'_{ss}} + 2 \right) \right) \exp \left(- \int_x^1 \frac{\kappa C'_e}{C'_{ss}} dy \right) dx. \quad (39)$$

Equation (38) can be rewritten as

$$1 - \frac{1}{\gamma_1 e^{-\lambda\tau_1} \omega(\lambda)} = -\phi_{12} \frac{\gamma_2}{\gamma_1} e^{-\lambda(\tau_2 - \tau_1)}. \quad (40)$$

An identical equation holds for $i = 2$, but with the indices reversed. If the coupling is symmetric, i.e. if $\phi_{12} = \phi_{21} = \phi$, then one obtains

$$\left(1 - \frac{1}{\gamma_1 e^{-\lambda\tau_1} \omega(\lambda)} \right) \left(1 - \frac{1}{\gamma_2 e^{-\lambda\tau_2} \omega(\lambda)} \right) = \phi^2. \quad (41)$$

In the rigid-tube limit, where $\alpha_i \rightarrow 0$, $g'_i \rightarrow -1/L_0$, Eq. (41) reduces to the characteristic equation found in [17] for a TGF model with two coupled nephrons with rigid TALs.

4. Model Results

To attain a more comprehensive understanding of the dynamics that may arise from the coupling of one nephron's TGF system to another, we used the model's characteristic equation (Eq. (41)) to predict parameter boundaries that separate qualitatively differing behaviors. For simplicity, we frequently refer to an individual model TGF system as a "nephron"; when a living nephron is intended, we make that meaning explicit. The two coupled nephrons are indexed by "A" and "B"; the nephron index is omitted in the uncoupled case. Throughout this study, we assume symmetric coupling, i.e., we assume that $\phi \equiv \phi_{AB} = \phi_{BA}$. Unless stated otherwise, we assume that $\phi = 0.2$ [13,27].

4.1. Two coupled nephrons having identical parameters: gain and delay

We first studied the effect of coupling on the behavior of two identical model nephrons; thus, we assumed that $\gamma \equiv \gamma_A = \gamma_B$ and $\tau \equiv \tau_A = \tau_B$. We examined model behavior in the γ - τ plane, a subset of the high-dimensional parameter space involving γ_A , γ_B , τ_A , τ_B , ϕ , α , etc.

We determined parameter regions that correspond to differing combinations of signs of the real parts of the $\lambda_{n,m} \equiv \rho_{n,m} + i\omega_{n,m}$ (i.e., $\rho_{n,m}$ positive, negative, or zero); the imaginary part $\omega_{n,m}$ corresponds to the frequency of the oscillations. To determine the parameter regions, we computed values of γ - τ pairs that are encompassed within the physiologic range and that correspond to $\rho_{n,1} = 0$ or $\rho_{n,2} = 0$, for $n = 1, 2$, or 3. These roots may indicate a solution

bifurcation or a transition between differing stable solution behaviors. These γ - τ pairs, which form curves in the γ - τ plane, were obtained for four cases: (1) an uncoupled system with base-case compliance, i.e., $\phi = 0$ and $\alpha = 1.33 \times 10^{-5} \text{ cm}\cdot\text{mmHg}^{-1}$; (2) a coupled system with base-case compliance, i.e., $\phi = 0.2$ and $\alpha = 1.33 \times 10^{-5} \text{ cm}\cdot\text{mmHg}^{-1}$; (3) a coupled system with double compliance, i.e., $\phi = 0.2$ and $\alpha = 2.67 \times 10^{-5} \text{ cm}\cdot\text{mmHg}^{-1}$; and (4) a coupled system with 1/5 compliance, i.e., $\phi = 0.2$ and $\alpha = 2.67 \times 10^{-6} \text{ cm}\cdot\text{mmHg}^{-1}$. The results are shown in Fig. 2, panels A, B, C, and D, respectively. The physiologic range was assumed to fall within the rectangular domain given by $(\gamma, \tau) \in [0, 10] \times [0, 0.5]$.

A detailed analysis of the uncoupled TGF system with base-case compliance can be found in Ref. [19]. Full-model simulations revealed that for sufficiently small values of γ or τ , i.e., for points (γ, τ) that roughly correspond to those that fall below all curves $\rho_n = 0$, any initial solution, or any transient perturbation of a steady-state solution, results in a solution that converges to the time-independent steady-state solution (where $Q \equiv Q_o$): that solution is the only stable solution. It is also noteworthy that, with a nonzero backleak permeability, the curves for $\rho_n = 0$, $n = 1, 2, 3$ cross each other; such crossings are not found when backleak permeability is set to zero [18]. As a result of these crossings, the system exhibits parameter regimes where $\rho_n > 0$ for some $n > 1$ and $\rho_1 < 0$. A consequence is that, as revealed by numerical solutions of the full model equations (see below), a LCO of frequency f_2 corresponding to the imaginary part of λ_2 can be elicited, as can a LCO of frequency f_3 . In addition, some parameter regions may exhibit multistability: an oscillatory solution may be either a stable f_1 -LCO or an f_2 -LCO, depending on the initiating perturbation; in some other region, an oscillatory solution may be either an f_2 -LCO or an f_3 -LCO.

When coupling is introduced, model behavior appears to become significantly more complex. Even for the simple case of two identical nephrons, the number of root curves within the physiologic range doubles from three ($\rho_n = 0$, $n = 1, 2, 3$) to six ($\rho_{n,m} = 0$, $n = 1, 2, 3$; $m = 1, 2$). This is an instance of spectral splitting in which the number of eigenvalues corresponding to each n equals the number N of coupled nephrons. In the case of two identical nephrons, the root loci arising from spectral splitting give rise, e.g., to parameter regions where: $\rho_{1,1} > 0$ and $\rho_{1,2} < 0$, and where also $\rho_{n,1} < 0$ and $\rho_{n,2} < 0$ for $n > 1$ (marked “I” in Fig. 2B); where $\rho_{1,1} > 0$ and $\rho_{1,2} > 0$, and where also $\rho_{n,1} < 0$ and $\rho_{n,2} < 0$ for $n > 1$ (marked “I’” in Fig. 2B); where $\rho_{2,1} > 0$ and $\rho_{2,2} < 0$, and where also $\rho_{n,1} < 0$ and $\rho_{n,2} < 0$ for $n \neq 2$ (marked “II” in Fig. 2B); and where $\rho_{1,1} > 0$ and $\rho_{1,2} < 0$, $\rho_{2,1} > 0$ and $\rho_{2,2} < 0$, and $\rho_{n,1} < 0$ and $\rho_{n,2} < 0$ for $n > 3$ (marked “III” in Fig. 2B). Other regions where some $\rho_{n,m}$ ’s are positive and others are negative can be similarly identified.

We computed numerical solutions for the full model equations for coupled identical nephrons, for selected values of gain γ and delay τ . Four example points are labeled in Fig. 3A: V ($\gamma = 0.8$, $\tau = 0.2$), W ($\gamma = 3.0$, $\tau = 0.4$), X ($\gamma = 3.0$, $\tau = 0.2$), and Y ($\gamma = 5.0$, $\tau = 0.1$). Solutions corresponding to these points, and the solutions’ power spectra, are shown in Fig. 3. Point V is located in the “ $\rho_n < 0$ ” region, where a transient perturbation results in an asymptotic convergence to a time-independent steady state. For the points W, X, and Y, the LCO frequencies are $f_1 = 39 \text{ mHz}$, $f_2 = 96 \text{ mHz}$, and $f_3 = 168 \text{ mHz}$, respectively.

In our previous TGF models [9,17], in which the TAL is represented by a rigid tube, the LCO frequencies have been found in good (although not precise) agreement with the frequencies predicted by an approximate formula (derived, in part, on the assumption that

the TAL backleak permeability is zero): $f_n \approx \frac{n}{T_o(1+2\tau)}$, where τ is in nondimensional form and T_o is the dimensional steady-state transit time. Thus, in those rigid-TAL models, we have, approximately, that $f_2 \approx 2f_1$ and $f_3 \approx 3f_1$. In contrast, the compliant coupled TAL used in this study predicted, for the points considered above, ratios of $f_2/f_1 = 2.46$ and $f_3/f_1 = 4.31$,

which deviate substantially from the theoretical ratios of 2 and 3, respectively. These results are consistent with our previous uncoupled compliant-TAL TGF model [19]. The differing frequency relationships between the rigid and compliant TAL cases is attributable, in most part, to the compliance of the model TAL tubular walls, inasmuch as the TAL was assumed to be rigid in the derivation of the formula for f_n .

Where TAL compliance is double that of base-case value, i.e., $\alpha = 2.67 \times 10^{-5} \text{ cm}\cdot\text{mmHg}^{-1}$, the gain values at which oscillatory states are attainable were significantly lowered; compare Fig. 2, panels B and C. *In vivo* effective TAL compliance may be lower than isolated tubule measurements [28] (see Discussion). Thus, we also computed root curves using a TAL compliance that is 1/5 of base-case value, i.e., $\alpha = 2.67 \times 10^{-6} \text{ cm}\cdot\text{mmHg}^{-1}$. The results are summarized in Fig. 2D. With this lower compliance value, substantially higher gain values are needed to produce oscillatory solutions (compare with Fig. 2B).

4.2. Two coupled nephrons with only one nephron having varying gain and delay

The case analyzed above in Section 4.1, where both nephrons had identical model parameters (in particular, identical gains and delays), revealed six qualitatively differing dynamic solution behaviors. However, these results may not be directly relevant to the physiologic context, where gains and delays likely differ significantly from nephron to nephron. To attain a more complete understanding of the impact of parameter variability, we investigated cases where the gain and delay were fixed in one model nephron but were varied in the other model nephron.

In one nephron, called nephron A, the gain and delay were fixed at $\gamma_A = 6.8$ and $\tau_A = 0.18$. Nephron A was coupled to a second nephron, nephron B, having variable gain and delay. As was the case with identical nephrons, coupling the nephrons complicates the model nephron behavior significantly. As before, there are two eigenvalues that are important within the physiological range (given by $(\gamma, \tau) \in [0, 10] \times [0, 0.5]$). Similar to [17], there is a natural correspondence between each nephron and a specific set of eigenvalues, i.e. nephrons A and B are associated with eigenvalues, $\lambda_{n,A}$ and $\lambda_{n,B}$, respectively, for $n = 1, 2, 3, \dots$. We analyzed the root loci for three different values of the compliance: the base-case compliance ($\alpha = 1.33 \times 10^{-5} \text{ cm}\cdot\text{mmHg}^{-1}$), double the base-case value, and one-fifth the base-case value.

Figure 4 shows the curves on which $\rho_{n,m} = 0$ for some $n = 1, 2, 3$ and $m = A, B$ under the different compliance values. Under the base-case and double compliance, $\rho_{1,A}$, $\rho_{2,A}$, and $\rho_{3,A}$ are always positive for the portion of the parameter space displayed, with the notable exception of the closed loop present in Fig. 4A for base-case compliance. Within that loop, $\rho_{1,A} < 0$. Below the black curve corresponding to $\rho_{1,B} = 0$, $\rho_{1,B}$ is negative, whereas above that curve $\rho_{1,B}$ is positive. Similarly, above the red curve corresponding to $\rho_2 = 0$ and above the blue curve corresponding to $\rho_3 = 0$, $\rho_{2,B}$ and $\rho_{3,B}$, respectively, are positive, and below the curves, those ρ 's, respectively, are negative.

With a TAL tubular compliance that is one-fifth of the base-case, Fig. 4C shows a qualitative difference relative to the previous compliance values considered. With this lower compliance, $\rho_{2,A}$, and $\rho_{3,A}$ are always positive for the portion of the parameter space displayed, whereas $\rho_{1,A}$ is negative in a region, which is indicated by the presence of a second curve corresponding to $\rho_{1,A} = 0$, which is not present in the previous cases. That curve, labeled “ $\rho_{1,A} = 0$,” delineates the boundary across which $\rho_{1,A}$ changes sign. The remaining curves separate parameter space in a way that is identical to that of previous compliance values.

4.3. Two coupled nephrons with identical gains, varying delays

In the next set of studies, we investigated model behavior in a different two-dimensional plane in the high-dimensional parameter space: the τ_A – τ_B plane. The two model nephrons were assumed to have identical gains: $\gamma \equiv \gamma_A = \gamma_B$. Figures 5 and 6, for $\gamma = 1.5$ and 3, respectively, show the roots $\rho_{n,1} = 0$ or $\rho_{n,2} = 0$ of the characteristic equation (Eq. (41)) for $n = 1, 2, 3$. In each figure, root loci were obtained for TAL compliance at the base-case value (panel A), two times of base-case value (panel B), and 1/5 of base-case value (panel C).

We first consider Fig. 5C, for $\gamma = 1.5$ and a low compliance ($\alpha = 2.67 \times 10^{-6}$ cm·mmHg⁻¹). In that figure, one observes three qualitatively distinct parameter regions: (1) the left-lower region, where the real parts of all eigenvalues are negative; (2) the left-upper and right-lower regions, where one, and only one, of $\rho_{1,1}$ and $\rho_{1,2}$ is positive, and all $\rho_{n,m} < 0$ for all other cases; and (3) the right-upper regions, where both $\rho_{1,1} > 0$ and $\rho_{1,2} > 0$, and all $\rho_{n,m} < 0$ for $n > 1$. These results indicate that the full model equations (Eqs. (1)–(5)) may have only two stable solutions: a time-independent steady-state solution and an f_1 -LCO, consistent with previous findings for the rigid-TAL case [15,16,18,17].

The above results can be related to results obtained for the γ – τ plane. Consider the line $\tau_A = \tau_B$ along the diagonal of Fig. 5C. That line intercepts the root curves twice, at $\tau_A = \tau_B = 0.338$ and at $\tau_A = \tau_B = 0.596$ (latter not shown). Then consider the same line in the γ – τ plane, which corresponds to the $\gamma = 1.5$ line in Fig. 2D: that line also intercepts the root curves twice, at the same points: $\gamma = 1.5$, $\tau = 0.338$, and at $\gamma = 1.5$, $\tau = 0.596$. In both Fig. 2D and Fig. 5C, only the interception $\gamma = 1.5$, $\tau = 0.338$ corresponds to a fundamental change in model solution behavior, from a stable steady state to an f_1 -LCO.

As TAL wall compliance increases, model behavior in the τ_A – τ_B plane becomes more complex. At base-case compliance, new root curves emerge across which one of $\rho_{2,1}$, $\rho_{2,2}$, $\rho_{3,1}$, or $\rho_{3,2}$ changes sign, introducing parameter regions where stable solutions having a frequency corresponding to the associated eigenvalues $\lambda_{n,1}$ or $\lambda_{n,2}$, $n = 2$ or 3, may be elicited; see Fig. 5A. The diagonal line $\tau_A = \tau_B$ intercepts the root curves eight times: first the closed curve for $\rho_3 = 0$, then twice the oval for $\rho_2 = 0$, then again the closed curve for $\rho_3 = 0$, then twice one of the ovals for $\rho_1 = 0$, and finally twice the other oval for $\rho_2 = 0$. These same interceptions can be observed by considering the line corresponding to $\gamma = 1.5$ in the γ – τ plane in the region shown in Fig. 2B. The complexity of model behaviors increases further when compliance is doubled; see Fig. 2C.

Even more complex model behaviors were obtained as gain γ increases. At $\gamma = 3$, root curves were obtained for all three compliance values across which one of ρ_1 , ρ_2 , or ρ_3 changes sign, introducing parameter regions where stable solutions having frequencies corresponding to the eigenvalues λ_1 , λ_2 , or λ_3 may be elicited; see Fig. 6.

4.4. Nephron-to-nephron coupling may introduce spectral complexity

Single nephron proximal tubule pressure in spontaneously hypertensive rats can exhibit highly irregular oscillations similar to deterministic chaos. In a previous modeling study [18], we used a model of coupled nephrons having rigid TAL walls to investigate potential sources of the irregular oscillations and the associated complex power spectra in spontaneously hypertensive rats. Our results suggest that the complex power spectra in spontaneously hypertensive rats may be explained by the inherent complexity of TGF dynamics, which may include solution bifurcations, modest time-variation in TGF parameters, and coupling between small numbers of neighboring nephrons. A goal of this study is to examine the extent to which similarly complex power spectra can be reproduced in a coupled-TGF model that represents the impacts of TAL tubular wall compliance on TGF-mediated flow dynamics.

We conducted a simulation using the full model (Eqs. (1)–(5)). The parameters of the two nephrons, designated A and B, were set to $(\gamma_A, \tau_A) = (3.35, 0.2)$ and $(\gamma_B, \tau_B) = (2.5, 0.4)$. The coupling coefficient was set to 0.2. Figures 7A–7C show oscillations in tubular fluid pressure, SNGFR, and Cl^- concentration at the macula densa for nephron A. Figures 8A and 8B show the power spectra corresponding to the tubular fluid pressure, in linear and logarithmic scales. In this nephron, the frequencies of ~ 40.3 and ~ 96.5 mHz were most excited. Respectively, these frequencies correspond to the fundamental frequency of nephron B, which is in the single-frequency region, and the double-frequency of nephron A, which is in the bistable region. The fundamental frequency of nephron A (~ 56.2 mHz) and the first harmonic of nephron B (~ 80.0 mHz) were also excited. Power spectra corresponding to the SNGFR and Cl^- concentration exhibit similar complexity (results not shown). These results show that irregular oscillations in tubular fluid flow and complexities in corresponding power spectra, similar to those observed in spontaneously hypertensive rats, can be obtained via internephron coupling, via the broadening of peaks, multiple peaks, and their harmonics.

5. Discussion

In a series of modeling studies [9,15,16,17,18,25,29,30,31,32], we have used a mathematical model of the TGF loop to propose plausible explanations for phenomena that have been reported in experimental studies: the emergence of LCO and irregular oscillations in TGF-mediated flow in rats in differing physiologic states. In these previous studies, the TAL was represented as a rigid tube, and the impacts of TAL tubular wall compliance on TGF-mediated dynamics *in vivo* were not incorporated. In the present study, we extended a TGF model, recently developed by us [19], that represents pressure-driven flow in conjunction with compliant TAL walls, to two nephrons coupled through their TGF systems. The principal goal is to investigate the impact of TAL wall compliance, vascular coupling, and multistability on model behaviors. To achieve that goal, we derived a characteristic equation for two symmetrically-coupled nephrons having compliant TAL walls, and we solved that characteristic equation numerically. The results demonstrate that differing dynamic behaviors, and parameter regions in which they occur, can be systematically identified based on the information provided by the characteristic equation for the coupled nephron system. The precise knowledge of the solution behaviors as a function of parameter values allows one to produce, within the context of the model, a variety of dynamic behaviors.

Impacts of TAL wall compliance

We have previously used a model that represents a TAL with compliant walls to study the system's flow dynamics [19,33]. That model exhibited essentially the same nonlinear phenomena as the rigid-tubule model. Furthermore, those results indicate that the high degree of nonlinearity exhibited by our previous rigid-tubule model is not an artifact of the rigid-tubule formulation. In fact, some of those phenomena are more marked in the more inclusive model than in the rigid-tubule model [33].

Consistent with the single-nephron results reported in [19], the two-coupled-nephron system studied herein predicts that TAL compliance increases the tendency of the TGF system to oscillate; i.e., oscillatory states are attainable in the compliant-TAL model at gain values that are substantially lower than in the rigid-TAL model; compare the panels, obtained for different degrees of tubular compliance, in each of Figs. 2, 4, 5, and 6.

The present model predicts oscillatory states for very low TGF gain values. For example, for a system of two coupled identical nephrons, the model predicts a stable oscillatory state for a typical normalized delay value of $\tau = 0.23$ at a feedback gain as low as $\gamma = 0.92$. Thus, for the TGF gain of a normotensive rat, which is ~ 3.5 [31], the coupled compliant-tube TGF

model predicts that nephron flow is always in an oscillatory state. That prediction appears to be inconsistent with experiments that reported that nephron flow and related variables may be in an approximate steady state, or may exhibit regular oscillations at the fundamental frequency. Also, although not shown in the results, the model also predicts that oscillations with frequencies as high as five times that of the fundamental frequency can be attained at physiologic delay and gain values. To our knowledge, oscillations more than twice the fundamental frequency have not been reported. To reconcile the above discrepancy, we note that the *in situ* compliance of the TAL walls depends on a number of factors: the inherent elasticity of the epithelium; possible tethering to other tubules via the interstitial matrix; possible synchronization of the oscillations in tubular fluid pressure and flow, and the resulting synchronization of the distension of the tubular walls, among neighboring tubules; and the renal capsule, which contains the kidney and may limit the distension of tubules and vessels. In addition, the base-case TAL compliance of our model was based on measurements in isolated tubules [8], which, owing to the above factors, may be substantially larger than the *in situ* compliance of the TAL. Indeed, the agreement of the predictions of our previous rigid-TAL model with experimental measurements [9,18] suggests that the *in situ* compliance of the TAL may be sufficiently low that the rigid-tube formulation is an adequate approximation.

Impacts of coupling

Our previous rigid-TAL TGF models predicted that coupling decreases the parameter region that supports a stable time-independent steady-state solution and increases the size of the region that supports stable oscillations [16,17]. The results in the present study, for two identical nephrons that are symmetrically coupled, extend this finding to the case of compliant TALs, as can be seen by comparing panels A and B of Fig. 2: the steady-state region marked " $p_n < 0$ " is diminished in size by coupling. Furthermore, coupling substantially increases the sizes of regions where $p_2 > 0$ or $p_3 > 0$, and it also increases the sizes of regions supporting the multistable LCO with more than one positive p_n .

Irregular flow oscillations

The present study is a sequel to our previous model studies [17,18] in which we developed a hypothesis for the mechanisms that result in the emergence of irregular flow oscillations that are mediated by TGF. These irregular flow oscillations, observed in spontaneously hypertensive rats, appear to exhibit characteristics of deterministic chaos [10,11]. We have hypothesized that these irregular oscillations arise from the interaction of several mechanisms, including (but perhaps not limited to) the excitation of multiple stable oscillatory modes, lability in key physiologic parameters, and interactions between the TGF systems of nearby nephrons, i.e., nephron-to-nephron coupling [17,18]. Indeed, internephron coupling has been shown to be stronger in spontaneously hypertensive rats than in normotensive rats [27,34].

Based on information provided by the characteristic equation, we identified a set of parameters of two coupled TGF systems that generate irregular oscillations in nephron flows and related variables; see Figs. 7 and 8. Indeed, owing to the substantial size of the parameter region that supports multistable LCO, a large class of parameter combinations will produce irregular flow oscillations. Thus, the present study is consistent with, and provides additional support for, the relevance of the model results concerning the effects of coupling presented in Ref. [17,18], and the present study supports a role for coupling in the emergence of irregular oscillations in spontaneously hypertensive rats.

Acknowledgments

This research was supported by the National Science Foundation, through grant DMS-0715021 (to A. T. Layton) and the EMSW21 Research Training Groups grant DMS-0943760 to the Department of Mathematics at Duke University; and by the National Institutes of Health, through National Institute of Diabetes and Digestive and Kidney Diseases grants DK-89066 (to A. T. Layton) and DK-42091 (to H. E. Layton).

Glossary

Parameters

α	TAL compliance ($\text{cm}\cdot\text{mmHg}^{-1}$)
C_o	$[\text{Cl}^-]$ at TAL entrance (mM)
κ	TAL chloride permeability (cm/s)
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 ($\text{cm}\cdot\text{s}^{-1}$)
P_{out}	pressure at end of nephron (mmHg)
T_o	base-case steady-state TAL transit time (s)
V_{max}	maximum transport rate of chloride ($\text{nmole}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$)

Independent Variables

t	time (s)
x	axial position along nephron (cm)

Specified Functions

$\beta(x)$	unpressurized TAL radius (μm)
$C_e(x)$	extratubular $[\text{Cl}^-]$ (mM)
$P_{\text{in}}(t)$	pressure at loop bend (mmHg)
$P_e(x)$	extratubular pressure (mmHg)

Dependent Variables

$C(x, t)$	TAL $[\text{Cl}^-]$ (mM)
$P(x, t)$	Tubular fluid pressure (mmHg)
$Q(x, t)$	Tubular fluid flow ($\text{nl}\cdot\text{min}^{-1}$)
$R(x, t)$	luminal radius (μm)

References

1. Knepper M, Danielson R, Saidel G, Post R. Quantitative analysis of renal medullary anatomy in rats and rabbits. *Kidney Int.* 1977; 12:313–323. [PubMed: 604620]
2. Nyengaard J, Bendtsen T. Glomerular number and size in relation to age, kidney weight, and body surface in normal man. *Anat Rec.* 1992; 232:194–201. [PubMed: 1546799]

3. Schnermann, J.; Briggs, J. Function of the juxtaglomerular apparatus: Control of glomerular hemodynamics and renin secretion. In: DW, S.; G, G., editors. *The Kidney: Physiology and Pathophysiology*. Vol. 3rd Edition. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 945-980.
4. Schnermann, J.; Briggs, J. Function of the juxtaglomerular apparatus: Control of glomerular hemodynamics and renin secretion. In: RJ, A.; SC, H., editors. *Seldin and Giebisch's The Kidney: Physiology and Pathophysiology*. 4th Edition. Boston: Elsevier Academic Press, Amsterdam; 2008. p. 589-626.
5. Casellas D, Moore L. Autoregulation and tubuloglomerular feedback in juxtamedullary glomerular arterioles. *Am J Physiol (Renal Fluid Electrolyte Physiol)* 27. 1990; 258:F660–F669.
6. Holstein-Rathlou N, Marsh D. Oscillations of tubular pressure, flow, and distal chloride concentration in rats. *Am J Physiol (Renal Fluid Electrolyte Physiol)* 25. 1989; 256:F1007–F1014.
7. Holstein-Rathlou N, Marsh D. Renal blood flow regulation and arterial pressure fluctuations: a case study in nonlinear dynamics. *Physiol Rev*. 1994; 74:637–681. [PubMed: 8036249]
8. Holstein-Rathlou N, Marsh D. A dynamic model of the tubuloglomerular feedback mechanism. *Am J Physiol (Renal Fluid Electrolyte Physiol)* 27. 1990; 258:F1448–F1459.
9. Layton H, Pitman E, Moore L. Bifurcation analysis of TGF-mediated oscillations in SNGFR. *Am J Physiol (Renal Fluid Electrolyte Physiol)* 30. 1991; 261:F904–F919.
10. Holstein-Rathlou N, Leyssac P. TGF-mediated oscillations in proximal intratubular pressure: Differences between spontaneously hypertensive rats and Wistar-Kyoto rats. *Acta Physiol Scand*. 1986; 126:333–339. [PubMed: 3962682]
11. Yip K, Holstein-Rathlou N, Marsh D. Chaos in blood flow control in genetic and renovascular hypertensive rats. *Am J Physiol (Renal Fluid Electrolyte Physiol)* 30. 1991; 261:F400–F408.
12. Holstein-Rathlou N. Synchronization of proximal intratubular pressure oscillations: evidence for interaction between nephrons. *Pflügers Arch*. 1987; 408:438–443.
13. Källskog Ö, Marsh D. TGF-initiated vascular interactions between adjacent nephrons in the rat kidney. *Am J Physiol (Renal Fluid Electrolyte Physiol)* 28. 1990; 259:F60–F64.
14. Yip K, Holstein-Rathlou N, Marsh D. Dynamics of TGF-initiated nephron-nephron interactions in normotensive rats and SHR. *Am J Physiol (Renal Fluid Electrolyte Physiol)* 31. 1992; 262:F980–F988.
15. Kessler, K. Ph.D. diss. Duke University; Analysis of feedback-mediated oscillations in two coupled nephrons.
16. Pitman E, Zaritski R, Kessler K, Moore L, Layton H. Feedback-mediated dynamics in two coupled nephrons. *Bull Math Biol*. 2004; 66(6):1463–1492. [PubMed: 15522342]
17. Layton A, Moore L, Layton H. Multistable dynamics mediated by tubuloglomerular feedback in a model of coupled nephrons. *Bull Math Biol*. 2009; 71:515–555. [PubMed: 19205808]
18. Layton A, Moore L, Layton H. Multistability in tubuloglomerular feedback and spectral complexity in spontaneously hypertensive rats. *Am J Physiol Renal Physiol*. 2006; 291:F79–F97. [PubMed: 16204416]
19. Layton A. Feedback-mediated dynamics in a model of a compliant thick ascending limb. *Math Biosci*.
20. Angell S, Pruthi R, Shortliffe L. The urodynamic relationship of renal pelvic and bladder pressures, and urinary flow rate in rats with congenital vesicoureteral reflux. *J Urology*. 1998; 160:150–156.
21. Gottschalk C. A comparable study of renal interstitial pressure. *Am J Physiol*. 1952; 169:180–187. [PubMed: 14923878]
22. Gottschalk C, Mylle M. Micropuncture study of pressures in proximal and distal tubules and peritubular capillaries of the rat kidney during osmotic diuresis. *Am J Physiol*. 1957; 189:323–328. [PubMed: 13435366]
23. Layton A, Layton H. A region-based model framework for the rat urine concentrating mechanism. *Bull Math Biol*. 2003; 65(5):859–901. [PubMed: 12909254]
24. Layton H, Pitman E. A dynamic numerical method for models of renal tubules. *Bull Math Biol*. 1994; 56(3):547–565. [PubMed: 8087081]
25. Layton H, Pitman E, Moore L. Spectral properties of the tubuloglomerular feedback system. *Am J Physiol (Renal Fluid Electrolyte Physiol)* 42. 1997; 273:F635–F649.

26. Pitman E, Layton H, Moore L. Dynamic flow in the nephron: filtered delay in the TGF pathway. *Contemp Math*. 1993; 114:317–336.
27. Chen Y, Yip K, Marsh D, Rathlou NH. Magnitude of TGF-initiated nephron-nephron interaction is increased in SHR. *Am J Physiol (Renal Fluid Electrolyte Physiol 38)*. 1995; 269:F198–F204.
28. Young D, Marsh D. Pulse wave propagation in rat renal tubules: implications for GFR autoregulation. *Am J Physiol (Renal Fluid Electrolyte Physiol 9)*. 1981; 240:F446–F458.
29. Layton H, Pitman E, Moore L. Instantaneous and steady-state gains in the tubuloglomerular feedback system. *Am J Physiol Renal Physiol*. 1995; 268:F163–F174.
30. Layton H, Pitman E, Moore L. Nonlinear filter properties of the thick ascending limb. *Am J Physiol (Renal Fluid Electrolyte Physiol 42)*. 1997; 273:F625–F634.
31. Layton H, Pitman E, Moore L. Limit-cycle oscillations and tubuloglomerular feedback regulation of distal sodium delivery. *Am J Physiol Renal Physiol*. 2000; 278:F287–F301. [PubMed: 10662733]
32. Oldson D, Layton H, Moore L. Effect of sustained flow perturbations on stability and compensation of tubuloglomerular feedback. *Am J Physiol Renal Physiol*. 2003; 285:F972–F989. [PubMed: 12837687]
33. Layton A, Moore L, Layton H. Waveform distortion in TGF-mediated limit-cycle oscillations: Effects of TAL flow. *FASEB J*. 2009; 23:804–814.
34. Wagner A, Holstein-Rathou N, Marsh D. Internephron coupling by conducted vasomotor responses in normotensive and spontaneously hypertensive rats. *Am J Physiol (Renal Physiol 41)*. 1997; 272:F372–F379.
35. Mason J, Gutsche H, Moore L, Müller-Suur R. The early phase of experimental acute renal failure. IV. The diluting ability of the short loops of Henle. *Pflügers Arch*. 1979; 379:11–18.
36. MacPhee P, Michel C. Fluid uptake from the renal medulla into the ascending vasa recta in anaesthetized rats. *J Physiol*. 1995; 487:169–183. [PubMed: 7473246]
37. Garg L, Mackie S, Tischer C. Effects of low potassium diet on Na-K-ATPase in rat nephron segments. *Pflügers Arch*. 1982; 394:113–117.

Figure 1.

Panel A: A schematic drawing of two short-looped nephrons and their afferent arterioles (AA). The arterioles branch from a small connecting artery (unlabeled), which arises from a cortical radial artery (CRA). The nephron consists of the glomerulus (G) and a tubule having several segments, including: the proximal tubule (PT), the descending limb (DL), the thick ascending limb (TAL), and the distal convoluted tubule (DCT). Each nephron has its glomerulus in the renal cortex, and each short-looped rat nephron has a loop that that extends into the outer medulla of the kidney. The axis on the TAL of the lower nephron corresponds to the spatial axis used in the model (vide infra, Section 2.1); in this figure distance is indicated in terms of fractional (nondimensional) TAL length. Tubular fluid from the DL flows into the TAL lumen at $x = 0$; the chloride concentration of TAL luminal fluid is sensed by the macula densa (MD) at $x = 1$. The MD, a localized plaque of specialized cells, forms a portion of the TAL wall that is separated from the AA by a few layers of

extraglomerular mesangial cells; in this figure, the MD is part of the short TAL segment that passes behind the AA. Fluid from the DCT enters the collecting duct system (not shown), from which urine ultimately emerges. Structures labeled on one nephron apply to both nephrons. (Figure and legend adapted from Ref. [16].)

Panel B: Schematic representation of the i -th TAL. Hydrodynamic pressure $P_i(0, t)$ drives flow into TAL entrance ($x = 0$) at time t . Oscillations in pressure result in oscillations in TAL flow $Q_i(x, t)$, radius $R_i(x, t)$, and tubular fluid chloride concentration $C_i(x, t)$.

Figure 2.

Panel A: root loci for an uncoupled nephron having base-case TAL wall compliance. Panels B–D: root loci for two identical coupled nephrons, with TAL wall compliance at base-case value (B), twice of base case (C), and 1/5 of base case (D). Black, red, blue curves correspond to $\rho_1 = 0$, $\rho_2 = 0$, and $\rho_3 = 0$, respectively. The delay τ is expressed in nondimensional form in this figure and in subsequent figures.

Figure 3.

Sample solutions (which are identical in the two nephrons) at the points **V**, **W**, **X**, and **Y**, marked in panel A. Black, red, blue curves correspond to $\rho_1 = 0$, $\rho_2 = 0$, and $\rho_3 = 0$, respectively. Panels W1, X1, and Y1 show LCO of the f_1, f_2 , and f_3 modes, respectively; panels W2, X2, and Y2 show corresponding power spectra. Peaks in these spectra, and in the spectra of subsequent figures, have frequency labels that are in mHz. (Point **V**: $\gamma = 0.8$, $\tau = 0.2$; **W**: $\gamma = 3.0$, $\tau = 0.4$; **X**: $\gamma = 3.0$, $\tau = 0.2$; **Y**: $\gamma = 5.0$, $\tau = 0.1$)

Figure 4.

Root loci corresponding to nephron B ($\rho_{n,B} = 0$, denoted in figure by black, red, blue curves, corresponding to $n = 1, 2, 3$, respectively). Nephron A had fixed gain and delay ($\gamma_A = 6.8$, $\tau_A = 0.18$), while the gain and delay of nephron B were varied; η_A and η_B were fixed, and set to 1. A, base-case compliance; B, double compliance; C, 1/5 compliance.

Figure 5.

Root loci for two coupled nephrons with equal gains $\gamma = 1.5$ as a function of delays τ_A and τ_B . A, base-case compliance; B, double compliance; C, 1/5 compliance. Black, red, blue curves correspond to $\rho_1 = 0$, $\rho_2 = 0$, and $\rho_3 = 0$, respectively.

Figure 6.

Root loci for two coupled nephrons with equal gains $\gamma = 3$ as a function of delays τ_A and τ_B ; A, base-case compliance; B, double compliance; C, 1/5 compliance. Black, red, blue curves correspond to $\rho_1 = 0$, $\rho_2 = 0$, and $\rho_3 = 0$, respectively.

Figure 7. Oscillations in tubular fluid pressure (A), volume flow (B), and [Cl⁻] (C) at the macula densa in nephron A of a two coupled nephrons.

Figure 8. Power spectra corresponding to oscillations in tubular fluid pressure for two coupled nephrons (Fig. 7A). A, linear ordinates; B, logarithmic ordinates.

Table 1

Base-case parameter values.

Parameter	Dimensional Value	Reference
α	$1.33 \times 10^{-5} \text{ cm} \cdot \text{mmHg}^{-1}$	[8]
β_0	$9.38 \text{ } \mu\text{m}$	[†]
β_2	$5.69 \text{ } \mu\text{m}$	[†]
C_o	275 mM	[9]
κ	$1.50 \times 10^{-5} \text{ cm/s}$	[35]
K_M	70.0 mM	[9]
L_0	2.00 cm	[1]
L	0.500 cm	[1]
μ	$7.20 \times 10^{-3} \text{ g}/(\text{cm s})$	[28]
P_e	5.00 mmHg	[8,36]
P_{in}	10.0 mmHg	[28]
P_{out}	2.00 mmHg	[20,21,22]
Q_o	6.00 nl/min	[28]
R_o	$10.0 \text{ } \mu\text{m}$	[1]
V_{max}	$14.5 \text{ nmole} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$	[37,9]

[†] See text.