Hormonal regulation of salt and water excretion: a mathematical model of whole kidney function and pressure natriuresis

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Moss R, Thomas SR. Hormonal regulation of salt and water excretion: a mathematical model of whole kidney function and pressure natriuresis. Am J Physiol Renal Physiol 306: F224–F248, 2014. First published October 9, 2013; doi:10.1152/ajprenal.00089.2013.—We present a lumped-nephron model that explicitly represents the main features of the underlying physiology, incorporating the major hormonal regulatory effects on both tubular and vascular function, and that accurately simulates hormonal regulation of renal salt and water excretion. This is the first model to explicitly couple glomerulovascular and medullary dynamics, and it is much more detailed in structure than existing whole organ models and renal portions of multiorgan models. In contrast to previous medullary models, which have only considered the antidiuretic state, our model is able to regulate water and sodium excretion over a variety of experimental conditions in good agreement with data from experimental studies of the rat. Since the properties of the vasculature and epithelia are explicitly represented, they can be altered to simulate pathophysiological conditions and pharmacological interventions. The model serves as an appropriate starting point for simulations of physiological, pathophysiological, and pharmacological renal conditions and for exploring the relationship between the extrarenal environment and renal excretory function in physiological and pathophysiological contexts.

pressure natriuresis; urine concentration; mathematical model; whole kidney model

THE KIDNEY, BY VIRTUE OF REGULATING salt and water excretion, plays a key role in regulating arterial pressure (112–114, 224). Although the underlying causes of hypertension are not completely understood (31, 65, 211, 213, 215), it is clear that changes in renal function are observed in hypertensive persons and animals (64, 66, 111, 230). Thus, as physiological models attempt to simulate whole body physiology and to support personalized health care (143–145, 167), detailed mathematical models of renal function are needed (90). In the words of Montani and Van Vliet (223), “there is no doubt that modeling the regulation of long-term BP is worthy of much further study. However, to be useful, mathematical models must be well rooted in empirical data to confirm the behavior of the complete model and its components. With this in mind, it is our belief that mathematical models of long-term BP control should incorporate a pressure natriuresis mechanism that does not adapt to pressure itself, but is sensitive to modulation by neurohumoral systems.” Explicit representation of the dependence of homeostatic functions (e.g., pressure natriuresis) upon neural and hormonal stimuli is a necessary step towards predicting pathophysiological and pharmacological effects in a physiological model. At this time, no mathematical model both predicts whole kidney function and incorporates neural and/or hormonal regulation of the renal vasculature and tubular epithelia.

Mathematical models of renal physiology can be classified into two categories: intraorgan models of cellular, vascular, and tubular function that attempt to explain individual facets of internal renal physiology, and models that predict whole organ function (e.g., net urine excretion) but do not represent the underlying structure and function of the internal physiology. In turn, the intraorgan models can be classified as investigating distinct aspects of renal physiology. Vascular models have been used to investigate theafferent arteriolar myogenic response (50, 279, 345), the medullary regulation of blood flow and oxygen transport (47–49), and, more broadly, renal blood flow autoregulation (93, 158, 226). Glomerular models have been used to study the physical properties that govern glomerular ultrafiltration (46, 69, 78, 80, 81, 146). The dynamics of the tubuloglomerular feedback (TGF) mechanism has been investigated with models of the glomerulus and predistal tubule (16, 77, 138, 139, 178, 187). Multinephron versions of these models have explored the interactions and couplings that arise between neighboring nephrons (19, 20, 140, 181, 205, 206, 228, 229, 247, 286). Mathematical models of individual transporter kinetics have been used to explain the transport properties of epithelial cells (43–45, 203, 222, 335, 337), and multinephron models have been used to explain the resulting transport properties of entire tubule segments (303, 304, 327–329, 331–333, 336, 338, 339). Mathematical models have also been used to propose and evaluate hypotheses concerning the (as yet unexplained) ability to concentrate urine to levels observed (8, 10, 105, 122, 159, 166, 188, 189, 265, 266, 317, 318) in antidiuresis. Hargitay and Kuhn (127) proposed the first mathematical model of counter-current urine concentration in 1951. No hypothesis could explain how passive mechanisms in the inner medulla could increase urine concentration until Kokko and Rector (168) and Stephenson (293) simultaneously proposed that urea reabsorption from the collecting duct could drive salt reabsorption from the loop of Henle (25, 154, 268, 297). Stephenson then established universal limits on the concentrating power of any passive transport mechanism in the inner medulla (294–296, 298, 299). These models were flat (2-dimensional) and lumped the vasa recta and interstitium into a single compartment (central core). More recent models have continued to establish necessary properties and limits of such models (17, 18, 186, 202, 300, 307) and have expanded to explore the effects of epithelial properties (42, 149–151, 225, 308), three-dimensional structural features (179, 180, 183–185, 342, 343), and hypothetical mechanisms for increasing the
urine concentration (134, 162, 194, 241, 309, 310, 314, 353). A common feature of these urinary concentration models is that the medullary inflows [i.e., to the descending limbs of Henle (DLH) and the descending vasa recta (DVR)] are prescribed boundary conditions, rather than accounting for the variations in delivery that are induced by upstream regulatory mechanisms. Furthermore, these models have focused solely on antidiuresis and have not considered the regulation of urine production under other physiological conditions. Only a handful of modeling studies have investigated diuresis, representing the medulla at a very simplified level of detail (234–236).

In contrast to these detailed models of specific portions of renal physiology, existing whole organ and multiorgan models (1, 21, 26, 28, 35, 41, 110, 115–119, 135, 147, 157, 227, 311, 319, 340, 344) have almost completely ignored most of these structural and functional properties, either treating the kidney as a “black box” or representing only the most basic physiological details (e.g., treating the kidney as a single nephron). This approach has been taken due to factual, experimental, and computational limitations. For example, it remains unknown how the kidney is able to concentrate urine to the degree that is evident in antidiuresis, and so this cannot be modeled mechanistically without adding an extra, hypothetical, driving force [e.g., metabolic osmole production in the medullary interstitium (310, 314)] or some other hypothetical mechanism. Likewise, the details of medullary circulation and oxygenation, and the role of medullary tubular-vascular interactions in urine concentration, remain open areas of investigation (89, 91, 237, 242). Tubular transport details, which are necessary but not sufficient for modeling whole organ function, also remain incompletely elucidated (312, 334). Also, although detailed models exist for each segment of the nephron tubule and collecting duct, it is not trivial to connect these models together.

Our aim was to build a lumped-nephron model that accurately simulates regulation of renal salt and water excretion and that explicitly represents the main features of the underlying physiology, incorporating the major hormonal regulatory effects on both tubular and vascular function. The model that we present [2-dimensional, with explicit DVR but lumped ascending vasa recta (AVR) and interstitium] is simpler in medullary structure than recent medullary models but extends previous medullary and whole organ models with the inclusion of renal vasculature and glomeruli, cortical proximal and distal tubules, TGF-induced regulation of glomerular blood flow based on macula densa sodium concentration, and hormonal effects on both renal vasculature and epithelial transport properties. Accordingly, the inflows to the medullary vessels of this model vary naturally as a function of cortical and medullary tubular transport, glomerular filtration rate, and plasma hormone levels; this is the first model to explicitly couple glomerulovascular and medullary dynamics.

Since the properties of the vasculature and epithelia are explicitly represented (epithelia are treated as single-layer barriers), they can be altered to simulate pathophysiological conditions and pharmacological interventions. In addition to being a stand-alone model of whole kidney function, the purpose of this model is to be embedded in whole body physiological models to explore the relationship between the extrarenal environment and renal excretory function in physiological and pathophysiological contexts.

**MODEL STRUCTURE**

Rather than being structured around the nephrons that connect to a single exiting collecting duct (309, 314, 342) or the vessels arranged around a single medullary vascular bundle (179, 183, 184), this model is structured around a small number of distinct lumped nephrons that approximate the heterogeneity and relative distribution observed in vivo and that drain into a lumped aggregate outer medullary collecting duct (Fig. 1). Each lumped nephron receives filtrate from a distinct glo-

![Fig. 1. An overview of the vascular and tubular structure of the model (artificially separated to the left and right, for clarity), which contains 10 superficial nephrons (represented as one lumped short nephron) and 5 juxtamedullary nephrons whose loops of Henle descend to different depths in the inner medulla (IM); all nephrons drain into the outer medullary collecting duct. One third of the lumped descending vasa recta (DVR) vessels descend into the inner medulla, and the medullary interstitial fluid contains 100 mM of anonymous external osmoles. Plasma antidiuretic hormone (ADH) affects epithelial water permeability along the distal convoluted tubule (DCT) and the medullary collecting duct; plasma aldosterone and angiotensin II affect the rate of active sodium transport along the DCT; plasma ADH and aldosterone affect the rate of active sodium transport along the medullary collecting duct. Sodium concentration at the macula densa affects the resistance of the afferent arteriole [tubuloglomerular feedback (TGF)].](https://example.com)
merulo-vascular apparatus, whose TGF-induced afferent arteriolar vasoconstriction is driven by the sodium concentration at that lumped nephron’s macula densa. The intuition is that the model should not force nephrons that differ in structure (e.g., loop length) or behavior (e.g., epithelial properties) to autoregulate identically. An autoregulatory difference would affect filtrate and efferent plasma flows, which in turn would alter the cortical or medullary blood flow (depending on whether the nephron in question is superficial or juxtamedullary) and may also affect the degree to which the nephron is able to maintain the interstitial environment. Ultimately, differences in autoregulation between nephrons could affect pressure natriuresis.

The model explicitly represents arterial and venous vasculature, six lumped nephrons of different lengths, the collecting duct, and the cortical and medullary interstitia. Each lumped nephron \(i\) represents an aggregate of \(N_i\) nephrons and consists of a proximal convoluted tubule (PCT), a proximal straight tubule, a thin descending limb, a thin ascending limb (juxtamedullary nephrons only), a thick ascending limb, and a distal convoluted tubule (DCT) that drains into the aggregate collecting duct. The vasculature encompasses an arcuate arterial segment into which the renal blood flow is delivered, afferent and efferent arterioles, glomeruli, cortical peritubular capillaries, DVR, and an arcuate vein that delivers the venous return back to the body. The AVR are lumped together with the medullary interstitium, on the grounds that they are highly permeable and have greater surface area and lower flow velocities than the DVR (241) and so essentially are in osmotic equilibrium with the interstitium.

The model equations are shown using the notation in Supplemental Tables S1 and S2, the equations are listed throughout the text, and parameter values are given in Supplemental Tables S3 to S5.

**Vascular structure.** Mean arterial pressure (\(P_{\text{Art}}\)) drives blood-flow along an arcuate artery, along which there is a basal resistance (\(R_{\text{Art}}\)). Afferent arterioles branch in parallel from the end of the arcuate artery, where the driving pressure is referred to as the cortical arterial pressure (\(P_{\text{CRA}}\)). This parallel branching necessarily precludes temporal coupling between neighboring nephrons (as observed experimentally in Refs. 51, 137, 207, 252, 284, 285 and modeled in Refs. 19, 20, 140, 181, 205, 206, 228, 229, 247, 286); such transient dynamics are assumed here to exert negligible influence on the steady-state behavior.

The afferent arteriolar structure of the autoregulatory model of Moore et al. (226) is used for each nephron, a series of resistances that represent the net resistance of the arteriole: a basal resistance (\(R_B\)); a descending myogenic resistance (\(R_{\text{MD}}\)), stimulated by upstream pressure; an ascending myogenic resistance (\(R_{\text{MA}}\)), stimulated by downstream pressure); a TGF-induced resistance (\(R_{\text{TGF}}\)); the resistance of the glomerular capillary bed (\(R_G\)); and the resistance of the efferent arteriole and subsequent vasculature (\(R_{\text{Eff}}\)).

Differences in afferent arteriolar basal resistance reflect the distance between the arcuate artery and the glomerulus, (superficial nephrons have higher basal resistances than juxtamedullary nephrons) and are used instead of an explicit interlobular (cortical radial) artery to simplify the model calculations.

The blood flow into the \(i^{th}\) afferent arteriole (\(B_{\text{Art}}^i\)) is solved by dividing the pressure drop across the arteriole by the net resistance of the arteriole, where \(P_G(0)\) is the pressure at the start of the glomerular capillary bed (Eq. 1).

If \(R_{\text{Art}}\) is zero, then \(P_{\text{CRA}} = P_{\text{Art}}\) and the afferent flows can be directly obtained from (Eq. 1). Otherwise, conservation of mass (i.e., flow) dictates that the interlobular artery flow equals the sum of the afferent flows (Eq. 2), which can be rearranged to solve for \(P_{\text{CRA}}\) (Eq. 3). A general solution is obtained for the case where each lumped nephron \(i\) represents an aggregate of \(N_i\) identical nephrons (Eq. 4).

\[
B_i^{\text{Art}} = \frac{P_{\text{CRA}} - P_G(0)}{R_B + R_{\text{MD}} + R_{\text{TGF}}} = \frac{P_{\text{CRA}} - P_G(0)}{R_{\text{Art}}} \quad \text{(1)}
\]

\[
\frac{P_{\text{Art}} - P_{\text{CRA}}}{R_{\text{Art}}} = \sum_i B_i^{\text{Art}} = \sum_i \frac{P_{\text{CRA}} - P_G(0)}{R_{\text{Art}}} \quad \text{(2)}
\]

\[
P_{\text{CRA}} = \frac{P_{\text{Art}}}{R_{\text{Art}}} + \sum_i \left(\frac{P_G(0)}{R_{\text{Art}}}\right) \quad \text{(3)}
\]

\[
P_{\text{CRA}} = \frac{P_{\text{Art}}}{R_{\text{Art}}} + \sum_i \left(\frac{N_i P_G(0)}{R_{\text{Art}}}\right) \quad \text{(4)}
\]

**Autoregulation.** Glomerular blood flow is regulated by the myogenic resistances \(R_{\text{MD}}\) and \(R_{\text{MA}}\) and by the TGF-induced resistance \(R_{\text{TGF}}\).

In the autoregulatory model of Moore et al. (226), two myogenic responses were proposed: a descending myogenic response, stimulated by upstream arterial pressure, and an ascending myogenic response, stimulated by downstream vascular resistance. Both responses were quantified by scaling coefficients, \(G_D\) and \(G_A\), for the descending and ascending responses, which represent the extent to which they could regulate afferent arteriolar pressure; at \(G_A = 1\) the response can keep the afferent arteriolar pressure constant, and at \(G_A = 0\) there is no response. This formulation was chosen because estimates of \(G_D\) can be obtained from in vitro studies and also for mathematical simplicity (226). The equations for these responses (Eqs. 5–7) can be rearranged by substituting Eq. 6 into Eq. 5 to solve for \(R_{\text{MA}}\) (Eq. 8) from which \(R_{\text{MD}}\) can then be solved (Eq. 6).

The TGF-induced resistance in the afferent arteriole (Eq. 9) is a piece-wise linear function of the macula densa sodium concentration (\(C_{\text{NaMD}}\)) (226) that approximates the sigmoidal TGF response (278). The model behavior remained essentially unchanged when we replaced this piece-wise linear function with a sigmoid (not shown).

Moore et al. (226) fitted the TGF parameters to experimental data and showed that it produced autoregulation consistent with the known physiology. In our model, the myogenic scaling coefficient \(G_D\) was set to 0.3 for the superficial nephrons and to 0.5 for the juxtamedullary nephrons (Moore et al. used a conservative value of 0.3); \(G_A\) was set to 0 for all nephrons, because values comparable to those for \(G_D\) resulted in overregulation and caused the single nephron glomerular filtration rate (SNGFR) curves to become less linear.

\[
R_{\text{MA}} = G_A R_{\text{TGF}} \frac{R_B + R_{\text{MD}}}{R_G + R_{\text{Eff}}} \quad \text{(5)}
\]

\[
R_{\text{MD}} = G_D \left(\frac{P_{\text{CRA}}}{P_0} - 1\right) \frac{R_B + R_{\text{MA}} + R_{\text{TGF}} + R_G + R_{\text{Eff}}}{R_B + R_{\text{MA}} + R_{\text{TGF}} + R_G + R_{\text{Eff}}} \quad \text{(6)}
\]
Glomerular filtration and efferent blood flow. The glomerular filtration rate is determined by the net hydrostatic and oncotic driving force across the glomerular capillary wall and the glomerular ultrafiltration coefficient \( K_t \). Given a glomerular capillary bed of unit length, the assumption that the oncotic pressure of the filtrate is zero, and the afferent arteriolar plasma flow (Eq. 11), the plasma flow, hydrostatic pressure, and protein concentration at distance \( x \) along the glomerular capillary bed are given by Eqs. 12, 13, and 14. The hydrostatic pressure in Bowman’s space is calculated as an empirical function of the filtration rate (Eq. 15) (146), and oncotic pressure is calculated with a standard empirical function of protein concentration (Eq. 16) (226). The single-nephron filtration rate \( F^i_{\text{SNGFR}} \) is the difference between the afferent and efferent plasma flow rates (Eq. 17) and the net filtration rate \( F^i_{\text{GFR}} \) is scaled by the size of the aggregate \( N_i \) (Eq. 18). The efferent flows are delivered to the peritubular capillaries (Eqs. 19–20) and the DVR (Eqs. 21–22), where CTX is the set of nephrons whose efferent arterioles are connected to the peritubular capillaries and MED is the set of nephrons whose efferent arterioles are connected to the DVR.

\[
P'_G(x) = P'_G(0) - \int_0^x R'_G Q'_G(x) dx
\]

\[
P'_G(x) = P'_G(0) - \int_0^x R'_G Q'_G(x) dx
\]

\[
C'_G(x) = C'_G(0) + \frac{Q'_G(x)}{Q'_G(0)} - 1
\]

\[
F^i_{\text{SNGFR}} = Q'_G(0) - Q'_G(1)
\]

\[
F^i_{\text{GFR}} = F^i_{\text{SNGFR}} \times N_i
\]

\[
F^V_{\text{PTC}}(0) = \sum_{i \in \text{CTX}} Q'_G(1)
\]

\[
F^S_{\text{PTC}}(0) = F^V_{\text{PTC}}(0) \times C^S_{\text{Plasma}}
\]

The ratio of net DVR flow to net descending limb flow is an important factor in establishing the upper limit on urine concentration and remains an experimentally inaccessible quantity. Mathematical models of the urine concentrating mechanism specify the flow rates at the corticomedullary boundary as boundary conditions and the DVR:DLH flow ratio varies from 0.2–3.0:1 in different regions of the medulla and in different medullary models (see Supplemental Table S9 for examples from 3 models). However, when two or more of the juxtamedullary efferent arterioles feed the DVR in our model, the DVR flow is significantly greater than the descending limb flow and “washes out” the medullary interstitial gradient, greatly reducing the maximal urine concentration. To maintain a DVR:DLH flow ratio similar to existing medullary models, only one-fifth of the juxtamedullary efferent arterioles in our model supply blood to the DVR; this is inconsistent with the known physiology (242) but is necessary in order for the DVR:DLH flow ratio to be sufficiently low that the model is capable of producing concentrated urine.

In the model, the filtration fraction is autoregulated over a wide range of arterial pressure by the TGF and myogenic responses. Outside of this range (i.e., when the arterial pressure is too low or too high) the filtration fraction is proportional to the arterial pressure. This changes the cortical colloid oncotic pressure, which is understood to affect fractional reabsorption in the proximal tubule (107, 329). However, the variation in efferent colloid oncotic pressure in our model is on the order of 2–5 mmHg, which is less than a mosmol/kg H\(_2\)O at 37°C (1 mosmol/kg H\(_2\)O contributes ~19.3 mmHg of oncotic pressure at 37°C; Ref. 170). Variations of <1 mosmol/kg H\(_2\)O have been observed in the experimental literature (259, 260) and have a negligible effect on passive (osmotic) reabsorption; accordingly, the effects of the cortical colloid oncotic pressure on passive reabsorption are ignored in our model.

Filtrate flow and tubular transport. Throughout this manuscript, “flow” refers to the axial flow of plasma or filtrate (i.e., in the vasculature or the nephron tubule) and “flux” refers to the transmembrane transport of water or solutes. Volume and solute fluxes (J\( V \) and J\( S \)) are calculated using centered finite differences across each discrete tubule slice of width \( \Delta x \) (Eqs. 23–24). The partial molar volume of water (\( V_W \)) at 37°C is taken to be 0.0181357 l/mol [given a density of 993.36093 kg/m\(^3\) (169) and a molar mass of 18.01528 g/mol (87)]. A nonreabsorbable solute (NRS) is used to represent solutes that are filtered into the nephron from the bloodstream and are neither reabsorbed from nor secreted into the nephron tubule and collecting duct and also to represent the external osmoles in the medullary interstitium (Eq. 23), plasma solute concentrations are listed in Supplemental Table S3.

Solute and volume reabsorption in the PCT are specified by fractional reabsorption values (Eqs. 26 and 27) since reabsorption in the PCT is flow-dependent (253) and is regulated by mechanisms not yet fully elucidated (106, 212, 330, 334). The fractional reabsorption is assumed to decrease in response to elevated arterial pressure to simulate the development of a proximal pressure diuresis (Eq. 28), as characterized by Chou and Marsh (54) and used by Moore et al. (226). Accordingly,
reabsorption in this segment is solely a function of the arterial pressure and is not influenced by plasma hormone levels. This pressure-natriuresis mechanism, which adapts to pressure, is exactly the type of mechanism Montani and Van Vliet argue against (223), but it remains unclear how this mechanism could be formulated solely as a function of hormonal and/or neural stimulation or by tuning the fractional reabsorption parameters. It has been suggested that inhibition of sodium reabsorption in the proximal tubule in response to elevated renal perfusion pressure is greater in juxtamedullary nephrons than in superficial nephrons (120), but this is not addressed in our model.

The cortical interstitium is assumed to be isotonic to plasma, and the cortical transmembrane fluxes (i.e., reabsorption and secretion along the PCT and DCT segments) are added to the net peritubular capillary flow, resulting in the net peritubular capillary outflow to the renal vein (Eq. 29). The medulla is divided into discrete slices, numbered 1-D (from the corticomedullary boundary to the papillary tip (Eq. 30), as are those of the descending and ascending limbs of Henle (Eq. 31), reflecting the connectivity of the vasa recta and the loops of Henle. The net fluxes out of the tubes in each slice (i.e., those of the descending and ascending limbs of Henle, and of the DVR) are added to the AVR flow, as is the shunted DVR flow at each slice (Eq. 32). The AVR outflow is defined to be the AVF flow at the corticomedullary boundary, and the combined cortical peritubular capillary and medullary AVR outflow form the net renal venous return (Eq. 33). The urine outflow is defined to be the collecting duct flow at the papillary tip (Eq. 34).

\[
J_{\text{Tube}}^x = 2\pi r \times \Delta x \times \left[ C_{\text{Int}}^{\text{GS}} + \sum S \sigma_S \phi_S \left[ C_{\text{Int}}^{\text{GS}} - C_{\text{Tube}}^{\text{GS}} \right] \right] \times P_t \times V_w \times 10^3 \tag{23}
\]

\[
J_{\text{Tube}}^x = 2\pi r \times \Delta x \times \left( \left[ C_{\text{Tube}}^{\text{GS}} - C_{\text{Int}}^{\text{GS}} \right] \times P_S + 10^6 \right)
+ V_{\text{Max}} \times \left( \frac{C_{\text{Tube}}^{\text{GS}}}{C_{\text{Tube}}^{\text{GS}} + K_{\text{SM}}^{\text{GS}}} \right) + (1 - \sigma_S) \times J_x \times \left( \frac{C_{\text{Tube}}^{\text{GS}} + C_{\text{Int}}^{\text{GS}}}{2} \right) \tag{24}
\]

\[
F_x^{\text{Tube}}(a) = F_x^{\text{Tube}}(0) - \int_0^a J_{\text{Tube}}^x(x) dx \tag{25}
\]

\[
J_x^{\text{PCT}} = F_{x}^{\text{PCT}} \times F_{x}^{\text{SNGFR}} \tag{26}
\]

\[
J_x^{\text{PCT}} = F_{x}^{\text{PCT}} \times F_{x}^{\text{SNGFR}} \times C_{\text{Plasma}} \tag{27}
\]

\[
F_{x}^{\text{PTC}}(1) = F_{x}^{\text{PTC}}(0) + \sum_j J_j^{\text{PCT}} + \sum_j J_j^{\text{DCT}} \tag{29}
\]

\[
F_x^{\text{AVR}}(D) = F_x^{\text{DVR}}(D) + \sum_j J_j^{\text{ALH}(d)}(D) + J_{\text{Shunt}}^{\text{AVR}}(d) \tag{30}
\]

\[
F_x^{\text{ALH}}(x)(D) = F_x^{\text{DHL}}(D) \tag{31}
\]

\[
J_{\text{Shunt}}^{\text{AVR}}(d) = F_x^{\text{DVR}}(d - 1) \times \frac{N_{\text{DVR}}(d - 1) - N_{\text{DVR}}(d)}{N_{\text{DVR}}(d - 1)} \tag{32}
\]

\[
F_x^{\text{AVR}}(d - 1) = F_x^{\text{AVR}}(d) + \sum_j J_j^{\text{DHL}(d)}(d) \tag{33}
\]

\[
F_x^{\text{VR}} = F_x^{\text{PTC}}(1) + F_x^{\text{AVR}}(0) \tag{34}
\]

\[
F_{x}^{\text{UR}} = F_{x}^{\text{CD}(D)} \tag{35}
\]

\[
F_x^{\text{AVR}}(d - 1) = F_x^{\text{AVR}}(d - 1) \times c_{\text{Int}}^{\text{IN}(d)} \tag{36}
\]

**Morphology and model parameters.** The model consists of six distinct lumped nephrons: one superficial nephron whose loop of Henle descends to the outer/inner medullary boundary, and five juxtamedullary nephrons whose loops of Henle descend to different depths into the inner medulla. Each lumped juxtamedullary nephron represents a single nephron, while the lumped superficial nephron represents an aggregate of 10 nephrons to ensure a 2:1 ratio of superficial to juxtamedullary nephrons, as reported for the rat kidney (160, 171, 184, 309, 342). The superficial nephrons are lumped together on the grounds that superficial nephrons are essentially homogeneous in structure and that their loops of Henle span the outer medulla in a uniform manner, so we assume that the macula densa sodium delivery is identical for all superficial nephrons and that they autoregulate uniformly.

It is assumed that one-third of the loops of Henle enter the inner medulla (124) and that the fraction of these loops w(x) that descend to a depth x into the inner medulla (with total depth L) is approximated by (Eq. 37) (185, 202).

As in previous medullary models (309, 342), there is a 2:1 ratio of the number of DVR vessels to descending limbs of Henle in the outer stripe and in the inner medulla; the short DVR vessels are assumed to decrease linearly in number (as a function of depth) over the inner stripe. Thus the lumped DVR represents 30 vessels in the outer stripe, 20 of which terminate in the inner stripe, and 2 long DVR vessels terminate at the same depth as each loop of Henle in the inner medulla.

The structure of the loops of Henle and the distal tubules is described in Supplemental Table S4, and the epithelial properties of each tubule segment are presented in Supplemental Table S5. The functionally distinct portions of the descending and ascending limbs are classified as per Layton (179), from which the majority of the epithelial parameter values are derived. The bottom 60% of the inner medullary descending limbs, which reach at least 1 mm into the inner medulla are water impermeable, while the inner medullary portion of the descending limbs that do not descend 1 mm into inner medulla have different epithelial properties (LDL2) from the water-permeable portion of the longer descending limbs (LDL3). Note that reducing the length of the water-impermeable LDL segment to the final 10% of the limb had a negligible effect on urine concentration and pressure natriuresis (not shown). The thick ascending limbs of the juxtamedullary nephrons terminate at the corticomedullary boundary and connect to the DCT. In contrast, the thick ascending limbs of the superficial nephrons include a cortical portion (ALT CX).
experiments (see Supplemental Table S11), under the assumption that the interstitium was isosmotic to plasma and with the following default perfusion concentrations (when unreported): [K] = 0 mM, [Urea] = 20 mM, [NRS] = 5 mM; [NaCl] was always reported. When the perfused length of distal tubule was not specified, values were fitted for both 1.5 mm (see Table 1 in Ref. 325) and 2.2 mm (see Table VI in Ref. 86). The active sodium transport was estimated to be 9 nmol/(cm²s); based on a different set of assumptions Moore and Marsh (225) estimated a value of 36 nmol/(cm²s). Both distal tubule segments are assumed to have very low urea permeabilities, since the bulk of the delivered urea is returned to the collecting duct despite large (>50 mM) transepithelial gradients (154). The DCTs drain into a single lumped outer medullary collecting duct.

The ratio of nephrons to collecting ducts in the rat is reported to be 6:1 (154, 171) and the radius of an individual collecting duct in the rat is reported to be ~15 μm (see Table VIII in Ref. 86), so the lumped collecting duct in the model represents an aggregate of 2.5 individual collecting ducts, each with a radius of 15 μm, and so the lumped collecting duct has an initial radius of 37.5 μm. Since collecting ducts aggregate in vivo, the in silico radius shrinks as a function of medullary depth (Eq. 38), in proportion to the fraction of remaining in vivo collecting ducts (Eq. 38). However, since 20% of the juxtamedullary nephrons descend to the papillary tip (due to our coarse nephron population), the model CD radius decreases at a slower rate in the terminal IM than in medullary models with shunted or with near-continuous long-loop populations (179, 182, 184). Similar to previous medullary models (134, 186), potassium is introduced at 20 mM in the outer medullary collecting duct inflow (the CD is impermeable to potassium along its entire length); this is used to prevent the urine osmotes from being exclusively sodium.

The model medulla is “flat” (2-dimensional); that is, it ignores the three-dimensional arrangement of the various tubules, vessels and pockets of interstitial fluid in the medulla (243, 244). It is well established (186, 341) that flat medullary models cannot mechanistically explain the steep inner medullary osmotic gradient observed in antidiuretic rodents while respecting measured permeability values. The main problem is the high measured urea permeability of long descending limbs in the IM (52, 134, 186). Recent studies have verified that the detailed structure can significantly affect the maximal urine concentration (68, 185, 245). Since this model should build a physiological osmotic gradient in the inner medulla but is not focused on explaining its origin (an unsolved problem), we adopted the strategy used by Thomas and Wexler (314) of including unidentified external osmotes in the inner medullary interstitium. Although this begs the question of the urine concentrating mechanism, it allows us to modulate the inner medullary gradient to simulate various degrees of diuresis and antidiuresis.

In an attempt to obtain increased (i.e., more realistic) urea accumulation in the inner medullary interstitium despite the flat medullary structure, the DVR endothelial parameters were varied between the inner and outer stripes and the upper and lower inner medulla, as in the WKM model (314, 342, 343). It has recently been suggested that active secretion of urea in the proximal straight tubule could increase urea delivery to the inner medulla and play a role in the urine concentrating mechanism (15), but we have not included active urea secretion in our model.

Hormonal effects on epithelial parameters. The hormonal effects included in this model are: increased sodium reabsorption in the distal tubule in response to aldosterone and angiotensin II (83, 196); increased sodium reabsorption in the collecting duct in response to aldosterone and ADH (132, 152, 199, 255); and increased water permeability in the late distal tubule and collecting duct in response to ADH (34, 82, 97, 152). ADH has also been shown to increase sodium reabsorption in the thick ascending limb (123, 133, 161, 163, 270). However, this effect may be very small in the rat (270) and was not incorporated into the model.

The inner medullary collecting duct (IMCD) exhibits non-uniform hormonal responses; ADH increases the water and urea permeability of the terminal IMCD (152, 267, 269), which has different epithelial properties from the initial IMCD and earlier portions of the collecting duct (289). Both the initial and terminal IMCD show similar increases of water permeability in response to ADH, but in the absence of ADH the water permeability of the terminal IMCD is higher than the ADH-induced water permeability of the initial IMCD (see Fig. 1 of Ref. 177). These effects are maximal at ADH concentrations of 10⁻⁸ M and above (see Figs. 5 and 6 of Ref. 289).

Studies in UT-A1/3⁻⁻ knockout mice have shown that without urea transporters in the terminal IMCD, the corticomedullary urea gradient is significantly diminished and the maximal urine concentration is reduced, but the corticomedullary sodium gradient is not affected (95). This confirms that the original “passive” hypothesis, as proposed by Kokko and Rector (168) and Stephenson (293), is not the chief mechanism by which sodium is concentrated in the inner medulla (95, 186, 341).

Thus the regulation of terminal IMCD urea permeability by ADH was not explored for two reasons: by virtue of having a “flat” (2-dimensional) medulla, the model is incapable of producing a large corticomedullary urea gradient; and the urea gradient does not play a role in the inner medullary sodium concentration in the model [metabolic osmole production (310, 314) is used to provide an osmotic driving force].

PCT transport is controlled in the model by a pressure-natriuresis mechanism that responds directly to arterial pressure (as explained above); the sensitivity of PCT sodium reabsorption to angiotensin II (59, 79) is implemented via the parameter F_TANG (see Eq. 28), which adjusts the response of the pressure-natriuresis mechanism. The effect of modulating this parameter in response to the plasma angiotensin II concentration was explored in the set of model simulations that we present in RESULTS.

Each effect is implemented as a function of the log of the hormone concentration; epithelial effects are presented in Supplemental Table S6. Plasma hormone concentrations are used throughout the model to determine the degree of the hormonal
effects. While tubular hormone concentrations can greatly exceed those in the plasma, and while hormone secretion takes place in the nephron, in nonpathological states the external hormone levels appear to be chiefly responsible for the intrarenal hormone levels (209); it remains unclear whether this also holds for pathological conditions (233). For simplicity, we ignore hormone secretion in the nephron tubule.

Vasoconstrictive effects of angiotensin II. In vivo and in vitro studies of Sprague-Dawley rats have found the afferent and efferent arterioles to have similar diameters and similar responses to angiotensin II (36, 37, 94, 195, 351), while studies in rabbits have shown differences in both diameter and response to angiotensin II (70, 71). Since the model parameters are derived from studies on and models of the rat kidney, we assume that the afferent and efferent arterioles have identical basal resistances and responses to angiotensin II and that their resistance is inversely proportional to the fourth power of their radius (Poiseuille’s law). The afferent and efferent arteriolar diameters are assumed to decrease by up to 15–17% in response to angiotensin II (36, 37) [i.e., arteriolar resistance doubles at the maximal response (29)]. More recent studies have shown the afferent and efferent diameters to decrease by one-third in vitro (195) and in isolated vessels (94) (an ~500% increase in resistance), but this resulted in significant overconstriction in our model.

The vasoconstrictive increase in resistance depends on the blood viscosity, which is a nonlinear function of both diameter and hematocrit (248, 249). We ignore this complication since the afferent and efferent arterioles are modeled without regard to their diameters [which vary depending on the depth of the glomerulus (176, 292)]. Since the efferent hematocrit depends on the filtration fraction, which itself depends on the efferent resistance (via the cortical radial artery pressure PCRA and the afferent plasma flow), this would have incurred an order of magnitude increase in computational cost.

Studies in the rat have also shown that angiotensin II affects the TGF gain (3, 4, 142, 219, 231, 275–277, 326), and the strength of the TGF response (R_{TGF}) was therefore calculated as a function of the angiotensin II concentration (Eq. 10), so as to remain proportional to the basal resistance of the afferent and efferent arterioles and preserve glomerular autoregulation (see Supplemental Table S6).

Numerical solution. The algorithm is documented in Supplemental Table S7. The system is solved by refining initial estimates for the TGF-induced afferent resistances R_{TGF} until the estimates are sufficiently close to the fixed point of the system.

For a given set of estimates for all R_{TGF}, the cortical radial artery pressure is solved (Eqs. 2–4) and the updated myogenic responses are obtained (Eqs. 5–8). The afferent flow rates are calculated (Eq. 1) and the initial glomerular pressures P_{G}(0) and filtration rates F_{SNFG} are iteratively solved (Eqs. 11–21) to achieve conservation of energy and flow. The cortical radial artery pressure is updated to account for the new myogenic responses and initial glomerular pressures, and this is repeated until the cortical radial artery pressure converges.

Once the filtration rates are known, PCT fluxes are calculated (Eqs. 26–28) and the inflows for the medullary portion of the model (i.e., the descending limb and DVR inflows) are known. The medullary interstitial solute concentrations are solved with a modified Newton-Raphson method, as described by Stephenson et al. (299), to achieve mass-balance (Eq. 36), which is realized when the sum of the axial flows (volume flow and each solute flow) across the boundaries of each medullary slice equal the net urine outflow (within the tolerance \( \varepsilon = 10^{-9} \)). By calculating the fluxes in the medullary tubule segments and the DCTs (Eqs. 23–24), the macula densa sodium concentrations are obtained. The estimates for the TGF-induced afferent resistances are then updated (Eq. 9) and the algorithm terminates when the estimates converge within the tolerance \( \varepsilon = 10^{-9} \).

The iterative solution for calculating the fluxes in a single tubule slice is solved with an analytic Jacobian matrix. The remaining iterative solutions, the nephron filtration rates F_{SNFG} and initial glomerular pressures P_{G}(0), the cortical radial artery pressure PCRA, and the medullary interstitial solute concentrations, are obtained with Jacobian matrices calculated numerically at each iteration. In all cases, the Jacobian matrix and error vector are used to solve for the corrections to the estimated quantities via LU factorization, as implemented by the dgetrs function in LAPACK (2).

The mesh size was 0.1 mm (i.e., 20 slices in the outer medulla and 50 slices in the inner medulla). A fivefold increase in spatial resolution had negligible effect on the steady-state solution. A single pressure-natriuresis curve (90–180 mmHg in 1-mmHg increments, comprising 91 simulations) typically took 2 h on a desktop computer (Intel i5–3210M).

The model can also be solved in a single iterative loop, accounting for all of the unknown quantities simultaneously, but this approach was found to be significantly slower than the algorithm outlined above.

Readers interested in running this model can contact the authors. We will provide the source code and also the model parameters and results from the tables and figures in this article as a benchmark.

RESULTS

To validate the model across a range of physiological states and to determine the model sensitivity to several key physiological parameters, we present the results of six in silico experiments:

1) An analysis of the antidiuretic model, both across a wide range of renal perfusion pressures and at a reference pressure of 90 mmHg.

2) An analysis of the effects that reducing plasma ADH concentration has on the model fractional excretion of water and sodium, the urine osmolality and the interstitial osmolality at the papillary tip; these results are compared with a number of studies of diuresis in male Wistar rats.

3) An analysis of the model sensitivity to the rate of DVR inflow, as determined by the number of juxtamedullary nephrons whose efferent arterioles supply the DVR.

4) An analysis of how the simulated administration of thiazide and amiloride affect sodium excretion in the model; the increases in fractional sodium excretion are compared with reference values from the literature.

5) A sensitivity analysis of the model with respect to the plasma concentrations of all three hormones, assessed at several perfusion pressures to identify the relative contributions.
that the three hormones make towards the regulation of water and sodium excretion in the model.

6) A comparison of the model excretion rates to data from many experimental studies of acute pressure natriuresis in the rat to evaluate how the range of model excretion rates compares to the variations observed in vivo.

The figures associated with each experiment are listed in Table 1. These experiments are provided to validate the model and also to demonstrate example uses of the model. The interested reader is invited to download the model and explore its behavior in response to different sets of conditions.

Antidiuretic model. The behavior of the antidiuretic model (i.e., with maximum hormonal responses) is summarized in Fig. 2. Whole kidney GFR is regulated when \( P_{\text{Art}} \) is between 100 and 145 mmHg (equivalent to \( \sim 2 \text{ ml/min} \) when scaled to an entire rat kidney, discussed below); superficial nephron SNGFR is maintained at 28 nl/min and juxtamedullary nephron SNGFR varies from 30–38 nl/min. Urine/plasma inulin is maximal at \( P_{\text{Art}} = 90 \text{ mmHg} \) but rapidly decreases between 100 and 110 mmHg, dropping to 20 at 120 mmHg, and ultimately to 2.2 at 180 mmHg. Sodium excretion is minimal (0.1–0.4 mmol/min) when \( P_{\text{Art}} \leq 110 \text{ mmHg} \).

In the model, glomerular capillary pressure \( P_{G}^i(0) \) remains 44 mmHg in the superficial nephrons and 35 mmHg in the juxtamedullary nephrons; the superficial pressure is consistent with micropuncture studies in normotensive rats (see Table 2 in Ref. 165, Ref. 316), and both the glomerular capillary pressure and net driving pressure are comparable to results discussed by Wright and Giebisch (350). The efferent peritubular oncotic pressure (23–25 mmHg) is similar to measurements in rats of 26 mmHg (see Table 14 in Ref. 165) and the efferent arteriolar resistance is 50% lower than afferent arteriolar resistance, consistent with in vivo measurements (29; see Table 2 in Ref. 291).

Experimental studies in a variety of adult rats have observed GFRs of \( \sim 1.0 \text{ ml·min}^{-1}·g \text{ kwt}^{-1} \) (kwt = kidney weight) and whole body GFRs in the range of 2.3–3.0 ml/min (Supplemental Table S8). In smaller Sprague-Dawley rats (170–230 g), whole body GFRs of 1.0–1.4 ml/min have been observed (324). Within the autoregulatory range \( (100 \leq P_{\text{Art}} \leq 145 \text{ mmHg}) \),

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**Table 1. Summary of the in silico experiments presented in this article**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Figures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basecase: the antidiuretic model</td>
<td>Figs. 2–4</td>
</tr>
<tr>
<td>Effects of plasma ADH concentration</td>
<td>Fig. 5</td>
</tr>
<tr>
<td>Vascular anatomy and the DVR</td>
<td>Figs. 6–7</td>
</tr>
<tr>
<td>Simulated thiazide and amiloride administration</td>
<td>Fig. 8</td>
</tr>
<tr>
<td>Model sensitivity to hormones</td>
<td>Figs. 9–11</td>
</tr>
<tr>
<td>Comparison to acute RFCs</td>
<td>Fig. 12</td>
</tr>
</tbody>
</table>

ADH, antidiuretic hormone; DVR, descending vasa recta; RFCs, renal function curves.
mmHg), the model whole body GFR is 1.92–2.43 ml/min [assuming that the rat kidney contains 32–38 × 103 nephrons (27, 301)], suggesting the model is equivalent to a kidney weight of around 1.9–2.4 g.

It is understood that juxtamedullary nephrons have SNGFRs significantly larger than those of the superficial nephrons (153, 263, 264), due in part to anatomical and physiological differences (12, 176, 280, 292). However, extreme differences between experimental measurements of superficial and juxtamedullary SNGFR may be an artifact of the experimental technique (350), since stop-flow pressure measurements cause maximal vasodilation of the afferent arteriole (88). Under free-flow conditions, superficial and juxtamedullary SNGFRs have been observed to be very similar (see Table 12 in Ref. 88). Indeed, the model SNGFRs are relatively low compared with in vivo stop-flow measurements in the rat [24–40 and 35–70 nl/min in superficial and juxtamedullary nephrons, respectively (see Table 8 in Ref. 165, 176). However, the model SNGFRs are similar to results from free-flow micropuncture and microdissection studies, where representative SNGFRs for superficial and juxtamedullary nephrons in the rat kidney are 30 and 38 nl/min, respectively, with a ratio of superficial to juxtamedullary SNGFR of ~0.80 (see Table 7 in Ref. 165, Table 2 in Ref. 176, 263, 316, 350). Combined with the assumption that the PCT reabsorbs approximately two-thirds of the filtered load, this leads to flows of 10–12 nl/min entering the individual descending limbs of Henle, which matches the prescribed boundary conditions in existing medullary models (179, 309, 342).

Flow rate, solute concentration, and fractional delivery profiles for each nephron tubule segment are shown in Fig. 3 for $P_{\text{Art}} = 90$ mmHg. The descending limbs receive 40% of the filtered water and sodium and 60% of the filtered urea; three quarters of the water load and one-third of the urea load are reabsorbed in the descending limbs; seven-eighths of the sodium load and one-sixth of the urea load are reabsorbed in the ascending limbs. Accordingly, at low arterial pressures (i.e., when the model produces the highest urine concentrations) the fractional deliveries of water, sodium and urea to the distal tubule are 10, 4–5, and 29–35% respectively, and the fractional deliveries to the collecting duct are 0.6, 0.1, and 23% respectively. These model values are at the lower end of experimentally measured values in the rat (see Fig 5 in Ref. 101, Fig 2 in Ref. 200), as is to be expected due to the maximal hormone responses in the antidiuretic model. In fact, it is not until fractional sodium delivery to the collecting duct is at least 1% that there is net sodium reabsorption along the entirety of the collecting duct in our model; this is consistent with suggestions that the DCT and connecting tubule play a more significant role in sodium handling than previously thought and that the collecting duct might only play a significant role when the upstream tubule segments are overloaded (214).

Osmolality profiles along the tubule segments, DVR and the medullary interstitium are shown in Fig. 4 for different arterial pressures. The interstitial osmolality at the outer/inner medullary boundary ($x = 0.2$ cm) is 870 mosmol/kgH$_2$O when $P_{\text{Art}} = 90$ mmHg and falls to 730, 580, and 535 mosmol/kgH$_2$O as $P_{\text{Art}} \rightarrow 120$ mmHg (including metabolic osmolal production in the medullary interstitium, which maintains a driving force of 100 mosmol/l). As $P_{\text{Art}}$ increases, the osmolality in the terminal collecting duct falls below the interstitial osmolality at the papillary tip. This pressure-induced decrease in IMCD osmolality is a result of the increased tubular flow rates, caused by decreased fractional reabsorption in PCT. The medullary osmolality profile is similar to measurements made in antidiuretic rabbits (159) and male Wistar rats (6–9).

Regulatory effects of ADH. The role of ADH in the regulation of water and sodium excretion is illustrated in Fig. 5. At maximal concentration, ADH keeps fractional volume excretion is <1% when $P_{\text{Art}} \leq 110$ mmHg; fractional volume excretion is >10 times higher with minimal ADH. ADH also exerts some control over fractional sodium excretion when $P_{\text{Art}} \leq 110$ mmHg, but this effect is comparatively smaller than the regulation of volume excretion, and the effect is negligible when $P_{\text{Art}} \approx 120$ mmHg.

Studies of water diuresis in the rat have shown changes in urine osmolality and medullary interstitial osmolality that closely resemble the variations produced by our model in response to different levels of plasma ADH. Interstitial and urine osmolality profiles for the model are shown in Fig. 5, C and D. In male Wistar rats infused intravenously with dextrose, urine osmolality decreased from 1,361 to 59 mosmol/kgH$_2$O (the initial and final interstitial osmolality profiles closely match those of the model under maximal and minimal ADH responses, respectively) and urine flow-rate increased almost 40-fold (9). Intravenous infusion of lysine-vasopressin in male Wistar rats produced variations in urine flow and osmolality similar to those produced by our model (6, see Fig 1 in Ref. 7), a near 50-fold difference between minimal and maximal urine flow rates and urine osmolalities ranging from <100 to ~1,300 mosmol/kgH$_2$O; variations in papillary interstitial osmolality also closely resembled our model (see Fig 5 in Ref. 8). However, the model is unable to reproduce the significantly higher osmolalities of urine (>2,400 mosmol/kgH$_2$O) and papillary interstitium (~1,800 mosmol/kgH$_2$O) that have been measured in hydropenic male Wistar rats (121).

Vascular anatomy and the DVR. In the model, pressure natriuresis in the PCT causes flow rates in the downstream tubule segments to increase when arterial pressure increases, and so the ratio of DVR flow to descending limb flow (DVR:DLH) decreases (shown in Fig. 6). This change in ratio does not increase urine concentration, because the elevated net flow rates in both the loops of Henle and the vasa recta “wash out” the axial osmolality gradient. The interstitial osmotic gradient is maximal when arterial pressure is low ($P_{\text{Art}} < 100$ mmHg) despite the maximal DVR:DLH flow ratio, because the net flow rates are minimal.

The flow ratio in this model is not dissimilar to those of existing medullary models (Supplemental Table S9), although the ratio at the papillary tip is somewhat higher, but in this model only one of the five juxtamedullary efferent arterioles is connected to the DVR; the four remaining juxtamedullary efferent arterioles are connected to the cortical peritubular capillaries. This is not consistent with the known physiology (239) but is required for the model to produce concentrated urine in the antidiuretic state. If more than one of the five juxtamedullary efferent arterioles are connected to the DVR, the increase in net DVR flow rate greatly reduces the axial osmolality gradient and concentrating power of the model (Fig. 7). The “washed out” interstitial osmolality gradient results in decreased filtrate sodium concentration at the macula densa,
reducing the regulatory TGF response and resulting in elevated GFR (Fig. 7).

Papillary DVR blood flow in individual vasa recta has been measured at 8.83 ± 0.96 nl/min with a hematocrit of 9–21% (136); in our model, the papillary per vessel DVR plasma flow is 6.0–6.2 nl/min when P<sub>art</sub> ≤ 120 mmHg and increases to a maximum of 7.5 nl·min<sup>−1</sup>·vessel<sup>−1</sup> when P<sub>art</sub> = 180 mmHg. These values correspond to per-vessel DVR blood flows of 6.7–7.8 nl/min and 8.3–9.4 nl/min, respectively, for a hematocrit of 9–21%.

Simulation of thiazide and amiloride administration. Diuretics that act on the early and late distal tubule segments are understood to increase fractional sodium excretion by up to 5 and 10%, respectively (see Table 1 in Ref. 251), without observable effects on renal hemodynamics (72, 73, 174, 323).

Inhibition of sodium transport in the early and/or late distal tubule segments of the model, to simulate the effects of thiazide on the Na-Cl cotransporter (NCC; early DCT) and of amiloride (late DCT), results in a systematic increase of solute transport through the distal nephron segments.
Hormonal regulation in the model. The sensitivity of sodium and water excretion in the model to hormonal regulation was demonstrated by varying the concentration of each hormone independently to stimulate responses from 0 to 100%, in 25% increments. The results are summarized in Figs. 9 and 10. In response to different ADH concentrations, net volume excretion varies by >100-fold (0.33–42 nl/min) while net sodium excretion only varies 20-fold (0.06–1.37 mmol/min), illustrating that in the model sodium excretion is regulated most strongly by ADH, while sodium excretion is regulated to a similar degree by all three hormones.

The effects of ADH, aldosterone, and angiotensin II on the model urine osmolality are compared with clearance data from male Wistar rats at various stages of water diuresis (5) in Fig. 11. The model predicts a steeper relationship between urine osmolality and urine/plasma inulin (UPI) when UPI < 150 than was observed experimentally and cannot concentrate urine above 1,100 mosmol/kgH2O; in contrast, model UPI can exceed 1,200 (Fig. 2).

Pressure natriuresis in the rat. Acute sodium excretion is strongly regulated by the renin-angiotensin system (see Fig. 2 in Ref. 230) and angiotensin II (129, 217). Thus the hormonal effects incorporated into the model must necessarily produce similar regulation of sodium excretion. If we compare minimal and maximal sodium excretion rates observed in vivo in single studies of the rat, the variation in whole kidney pressure natriuresis appears to be ∼1:6 (e.g., Refs. 100, 208, 256, 258, 260, 324). Assuming that the variation in the PCT pressure-natriuresis response is of a similar magnitude, we can express FrANG as a function of the plasma angiotensin II concentration (see Supplemental Table S6) with a maximal value of 1 (minimal angiotensin II) and a minimal value of 1/6 (maximal angiotensin II). Experimental measurements of sodium and volume excretion in the rat are typically reported relative to the weight of the kidneys. Net GFR in the rat is consistently observed to be around 1.0 ml·min⁻¹·g kwt⁻¹ (Supplemental Table S8). The model sodium and volume excretion rates, for
both fixed and variable degrees of PCT pressure natriuresis, are compared against experimental measurements from 15 studies of sodium and volume excretion in response to acute changes in renal perfusion pressure in the rat (99, 100, 109, 148, 191–193, 198, 204, 208, 210, 246, 261, 306, 324), shown in Fig. 12.

The potential use of this model to simulate long-term (chronic) data bears some explanation. This is not a time-dependent, transient model; it predicts quasi-steady-state renal function under the assumption that both the extrarenal environment (represented here as the arterial pressure and the plasma solute and hormone concentrations) and the defining renal parameters remain stable for a sufficient amount of time for the kidney to reach such a quasi-steady state. Since the kidney can respond relatively rapidly (over the course of minutes to hours), this is sufficient for predicting renal function “acutely” (i.e., over a short period with a stable systemic environment and unchanging renal parameters). Since chronic conditions provoke gradual, long-term changes in the renal and extrarenal environment, the model would have to be embedded inside a whole body model [e.g., the Guyton model (115)] that is able to simulate the much slower progression of chronic conditions. Alternatively, the extrarenal environment and renal parameters could be modified in accordance with chronic data (e.g., obtained from longitudinal studies) and a whole body model would not be needed. In such a system, the kidney model presented here should be seen as a predictor of acute renal function at any given moment, and the whole body state (and perhaps certain renal parameters) would evolve via the interplay between the extrarenal environment and the acute changes in renal function.
DISCUSSION

This is the first model to explicitly couple glomerulovascular and medullary dynamics; the model includes arterial and venous vasculature (including cortical peritubular capillaries and DVR), glomeruli, PCTs, descending and ascending limbs of Henle, DCTs, the collecting duct, and cortical and medullary interstitia. Glomerular blood flow is regulated by a TGF response, as determined by the macula densa sodium concentration, and a myogenic response; plasma hormone concentrations affect the renal vasculature and epithelial transport properties.

We presented the results of six in silico experiments, as summarized in Table 1. The antidiuretic model yielded reasonable predictions of SNGFR and GFR in the rat kidney, produced a small volume of concentrated urine at low perfusion pressures, and demonstrated progressive washout of the axial osmolality gradient and elevated water and solute excretion rates in response to elevated perfusion pressure. In response to decreased levels of ADH, model fractional excretion rates were raised and both urine osmolality and the axial osmolality gradient were significantly reduced; the model predictions were in good agreement with data from several studies of diuresis in male Wistar rats. The model DVR:DLH flow ratio was shown to be comparable to those of existing models of the urine concentrating mechanism; increasing the number of efferent arterioles that supply blood to the DVR significantly elevated this ratio and nullified the concentrating power of the model. The effects of thiazide and amiloride administration were simulated by inhibiting active sodium transport along the model DCT, and the increases in fractional sodium excretion were shown to agree with experimental data. The model sensitivity to the plasma concentrations of all three regulatory hormones was then evaluated; ADH exerted the greatest control over volume excretion, while all three hormones exerted similar degrees of control over sodium excretion. Finally, the model excretion rates (at minimal, half-maximal and maximal hormone levels) were compared with experimental measurements in the rat; model water and sodium excretion rates were regulated in a manner that closely resembled acute renal function curves from in vivo studies of the rat, especially when the PCT pressure-natriuresis response was modulated by angiotensin II.

Moreover, the SNGFRs of the juxtamedullary nephrons reveal an interesting interaction between the concentrating power of the medullary environment and the TGF-induced regulation of glomerular blood flow. Between $P_{\text{Art}} = 105$ mmHg and $P_{\text{Art}} = 120$ mmHg, the juxtamedullary SNGFRs in the model rise sharply without inducing a stronger TGF response. Over this range of arterial pressure the medullary interstitial osmolality gradient is being washed out (e.g., consider the steep drop in urine/plasma inulin over this pressure range as shown in Fig. 4) and the intratubular sodium concentration in the long ascending limbs is reduced. Accordingly, the sodium concentration at the juxtamedullary macula densa does not increase in response to increased TGF, leading to a reduced concentrating power of the model.

Fig. 7. Effects of increasing the number of efferent arterioles connected to the descending vasa recta, which “washes out” the axial osmolality gradient in the medullary interstitium, greatly reducing the concentrating power of the model. A: GFR; B: papillary osmolality; C: urine/plasma inulin; D: sodium excretion.
not increase when the filtration rate is elevated, and so the TGF-induced vasoconstriction does not increase. It remains an open question whether or not the regulation of SNGFR may also differ between cortical and juxtamedullary nephrons (176, 350); experimental studies in a variety of species have shown evidence supporting (13, 108, 141, 155, 262, 281, 282, 305) and opposing (38, 57, 58, 92, 216, 346) the redistribution of SNGFR between these populations. It is conceivable that an interaction of this nature might explain why redistribution of SNGFR is not universally observed. The model is not sufficiently detailed to investigate this hypothesis but merely to stimulate speculation.

An alternative explanation for the inconsistent regulation of the juxtamedullary SNGFRs is that the chosen model myogenic response may provide insufficient regulation. In particular, while Moore et al. (226) demonstrated that their model provided strong autoregulation from 100 to 160 mmHg, they did so only for a superficial nephron under the assumption of a fixed axial osmolality gradient. While the outer medullary osmolality gradient is robust due to the large amount of active transport, the inner medullary gradient is much more sensitive to washout; this will affect sodium reabsorption in the ascending thin limbs and subsequently affect the TGF response, which will in turn alter the resistance required of the myogenic response to provide strong autoregulation. It is conceivable that the myogenic response should not have a constant gain factor over the range of renal perfusion pressures considered in the model simulations and that the gain factor should rather be a function of the perfusion pressure. Alternatively the myogenic response could be formulated as an explicit relationship between perfusion pressure and arteriolar diameter, but since the induced resistance is exceptionally sensitive to the diameter (assuming Poiseuille flow), the myogenic response would essentially be fine-tuned to provide exactly the desired regulation.

Beyond the inherent shortcomings of any model of a detailed and highly complex biological system, two weaknesses of this model are especially apparent. Firstly, the urine concentrating mechanism, whose details remain unknown and cause for much conjecture, is represented in this model as metabolic osmole production in the medullary interstitium [as first suggested by Thomas and Wexler (313) and Jen and Stephenson (156)], which is incapable in this model of maintaining a sufficient corticomedullary osmolality gradient at high arterial pressures (evident in Fig. 7B). This may be due, in part, to the magnitude of the pressure-natriuresis mechanism in the PCT (Eq. 28). Secondly, the model is only capable of producing a concentrated urine in the antidiuretic state when just one of the five juxtamedullary efferent arterioles is connected to the DVR (Fig. 6), which violates the known physiology; most, if not all, juxtamedullary efferent arterioles supply blood to the DVR (239). We now address these weaknesses.

Since the details of the urine concentrating mechanism are not understood, no phenomenological model can concentrate...
urine to antidiuretic levels without imposing an additional driving force for concentration [in this model, the metabolic production of medullary osmoles and inner-medullary anatomical features revealed by recent studies (68, 245)]. However, this model does not include several features of the in vivo kidney that are known or suspected to affect the urine concentration: regulation of the medullary blood flow; peristalsis of the renal pelvis and the functional effects of hyaluronan in the medullary interstitium; the three-dimensional arrangement of nephron tubules and vasa recta; the “patchy” variations in epithelial permeability characteristics (particularly in the long descending limb); the heterogeneity of the nephron population; the precise regulation of volume and sodium excretion in the PCT (glomerulotubular balance); and the regulatory effects of the renal sympathetic nerves.

Hyaluronan is one of the major nonstructural elements of the extracellular matrix and its medullary interstitial concentration is rapidly regulated by the body hydration status (104, 126); evidence suggests it is involved in the regulation of medullary fluid and solute transport, and in whole body fluid homeostasis (162, 302). Peristalsis of the renal pelvis and the functional effects of hyaluronan in the medullary interstitium; the three-dimensional arrangement of nephron tubules and vasa recta; the “patchy” variations in epithelial permeability characteristics (particularly in the long descending limb); the heterogeneity of the nephron population; the precise regulation of volume and sodium excretion in the PCT (glomerulotubular balance); and the regulatory effects of the renal sympathetic nerves.

Concerning the relationship between the DVR and the efferent arterioles, it is generally accepted that blood flow to the renal medulla is almost exclusively supplied through efferent arterioles of juxtamedullary glomeruli (22, 172, 221). A minor fraction might also be derived from periglomerular shunt pathways (39, 98, 237). The converse, that juxtamedullary glomerular efferent arterioles supply blood only to the medulla and not to the cortex, is uncertain (239); efferent arterioles in the inner cortex may give rise to a capillary plexus that supplies deep cortical regions (23).

All mathematical models of the urine concentrating mechanism show high sensitivity of the corticopapillary gradients to the relative flow rates into the descending limbs and DVR, which are manifest as prescribed boundary conditions. In previous medullary models, the ratio of net DVR flow to net DLH flow at the corticomedullary boundary has been between 0.5 and 1.5 (Supplemental Table S9). In our model, when one-fifth of the juxtamedullary efferent arterioles supply blood to the DVR, the ratio is 1.25 (Fig. 6, $P_{\text{Art}} = 90 \text{ mmHg}$); when all five juxtamedullary efferent arterioles supply blood to the DVR, the ratio increases to 6.25, which is much closer to the likely in vivo ratio (Supplemental Table S10). However, when the DVR flow exceeds the DLH flow to this degree the model is unable to produce concentrated urine (Fig. 7). This is due, in part, to the endothelial parameters of the DVR and descending
limbs; the DVR are water permeable but are also highly sodium permeable, while the descending limbs are highly water permeable and are sodium impermeable. In the outer medulla there is significant volume reabsorption in the descending limbs but only moderate volume reabsorption in the DVR, due to sodium secretion, and the ratio of net DVR flow to net DLH flow is amplified in the inner medulla (see Fig. 6).

Despite suggestions to the contrary (63), there is experimental evidence that the DVR are vasoactive (241) and that medullary blood flow is regulated (60, 67, 131, 354) [although not to the same degree as cortical blood flow (see Fig. 2 in Ref. 240)] and that this may play a role in pressure natriuresis (63, 242). While the mechanisms that regulate medullary blood flow remain incompletely understood (63), many factors that are involved in the regulation of medullary blood flow have been identified (65), including the renal nerves (discussed later), ADH and angiotensin II, atrial natriuretic peptide, and nitric oxide. Models of the urine concentrating mechanism have not, to date, incorporated these regulatory mechanisms; one possible explanation for this is a shortage of experimental data from which explicit models of these mechanisms can be derived.

The model DVR water permeability is identical to the values used in the WKM model [100–400 µm/s (342)] and is much lower than experimental measurements in the rat [>1,000 µm/s (see Fig. 13 in Ref. 237, 238)]. However, the model has a 2:1 ratio of DVR to descending limbs [as per the WKM model (342)] and this serves to double the effective water permeability to 200–800 µm/s, compared with more recent medullary models (179, 182) that have a 67:71 ratio (171, 173).

At low arterial pressures, the model DVR is sufficiently permeable that the DVR fluid at a given depth is almost isosmotic to the AVR fluid at the same depth (±25 mosmol/kgH2O near the OM/IM boundary, and even more similar towards the papilla). Increasing the model DVR water permeability to 1,000 µm/s would modestly enhance DVR water reabsorption and reduce the interstitial washout to a limited degree. A change in the model DVR:DLH number ratio would also affect the available DVR surface area for water and solute reabsorption and therefore have a modest effect on the DVR:DLH flow ratio in the medulla. The DVR:DLH flow ratio at the corticomedullary boundary would remain fixed since the net DVR inflow is determined by the juxtamedullary efferent arteriolar flow and is not determined on a per-DVR-vessel basis. It is also true that assuming sodium and urea reflection coefficients of 0.5 along the DVR is too simple an approximation to account for the combined dynamics of paracellular and transcellular fluxes across the DVR endothelium, but such considerations are beyond the scope of this model.
Incorporating fine-structure details into the model, such as the vascular bundles in the outer medulla, should also reduce the sensitivity of the model urine concentration mechanism to arterial pressure. Indeed, previous medullary models have shown that the three-dimensional arrangement of the medulla can affect the urine concentration (179, 180, 183–185, 342, 343). Another refinement to the model structure that could improve the resilience of the urine concentrating mechanism to elevated arterial pressure is to increase the distribution of loops of Henle in the inner medulla, providing a closer approximation to the smooth decrease in the number of loops as a function of medullary depth.

These observations highlight the differences between what is known about in vivo flow rates and the anatomy of the renal vasculature on one hand and the ability of current models of the urine concentrating mechanism to reproduce experimental observations of antidiuretic urine production. Unfortunately, many significant properties remain unknown and experimentally difficult or impossible to investigate. For example, the ratio of medullary blood flow to filtrate flow in the loops of Henle is a critical determinant of the interstitial osmotic gradient and urine concentration but remains experimentally inaccessible.

Reabsorption in the model PCT is solely a function of the arterial pressure, due to the limited understanding of the regulatory mechanisms (106, 212, 330, 334). Thus it is not clear how to refine the flux equations for this segment so that reabsorption is dependent on neurohumoral stimulation and maintains glomerulotubular balance (32) without resorting to a cellular model of the epithelium (e.g., Ref. 329). There is a large amount of experimental data concerning the regulation of sodium reabsorption in the PCT in response to changes in arterial pressure (14, 33, 53, 55, 102, 164, 175) and in response to angiotensin II (40, 59, 79, 190, 217, 218, 220, 232, 347, 348). Such studies generally report an insufficient number of data points from which to derive an explicit formulation for the PCT pressure-natriuresis response but can be used to help validate such a response. Certainly the response is nonlinear, unlike the model response, and the shape of this response will have significant repercussions for the predicted net sodium excretion rate; this bears further investigation.

Proximal tubule reabsorption is influenced by the peritubular oncotic pressure (107, 329), but the variation in efferent oncotic pressure in our model is negligible and would thus have no effect on proximal tubule reabsorption. The net filtration driving force at the end of the glomerular capillary bed is ~1 mmHg in the juxtamedullary nephrons and ~8 mmHg in the superficial nephrons, indicating that filtration equilibrium is almost reached. Larger variations in efferent oncotic pressure would arise in the model if angiotensin II, in addition to its vasoconstrictive effect on the afferent arteriole, also reduced the effective glomerular capillary surface area (and hence also reduced $K_i$). The filtration fraction is ~15% over the autoregulatory range of the model, which is lower than typical values of ~20% and may be caused by the choice of an empirical equation for hydrostatic pressure in Bowman’s space (Eq. 15 (146). Adjusting the vascular parameters of the model (e.g., $R_b$) did not affect the filtration fraction, but instead changed the afferent plasma flow.

Given our focus on regulation of water and sodium excretion, the most significant factor not included in the model is the activity of the renal sympathetic nerves, which affects the renal vasomotor control.
circulation, tubule function and renal hormonal secretion (74, 76); abnormal regulation of renal sympathetic nerve activity also has significant implications on renal function and pathophysiological states (11, 30, 75, 201). Omission of the renal nerves from this initial model was motivated by a desire for model simplicity; renal sympathetic nerve activity could be added in a manner similar to that of Karaaslan et al. for the Guyton model (157).

In conclusion, our model is much more detailed in structure than existing whole organ models and renal portions of multiorgan models and, in contrast to previous medullary models that have only considered the antiuretic state, is able to regulate water and sodium excretion over a variety of experimental conditions in good agreement with data from experimental studies of the rat. It is also the first model to explicitly couple glomerulovascular and medullary dynamics. Further refinement of the model in the future, as discussed above, should improve the model’s concentrating ability at high arterial pressures. The model serves as an appropriate starting point for simulations of physiological, pathophysiological and pathological renal conditions, and for exploring the relationship between the extrarenal environment and renal excre- 
tory function in physiological and pathophysiological contexts.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: R.M. and S.R.T. conception and design of research; R.M. performed experiments; R.M. and S.R.T. analyzed data; R.M. and S.R.T. approved final version of manuscript.

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F242 HORMONAL REGULATION OF SALT AND WATER EXCRETION: A MODEL

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