Parameter estimation of feedback gain in a stochastic model of renal hemodynamics: differences between spontaneously hypertensive and Sprague-Dawley rats

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Departments of 1Biostatistics and 4Medical Physiology, University of Copenhagen, Copenhagen, Denmark; 2Department of Physiology and Biophysics, University of South Florida, Tampa, Florida; and 3Department of Molecular Pharmacology, Physiology, and Biotechnology, Brown University, Providence, Rhode Island

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Ditlevsen S, Yip KP, Marsh DJ, Holstein-Rathlou NH. Parameter estimation of feedback gain in a stochastic model of renal hemodynamics: differences between spontaneously hypertensive and Sprague-Dawley rats. Am J Physiol Renal Physiol 292: F607–F616, 2007. First published October 3, 2005; doi:10.1152/ajprenal.00263.2005.—Proximal tubular pressure shows periodic self-sustained oscillations in normotensive rats but highly irregular fluctuations in spontaneously hypertensive rats (SHR). Although we have suggested that the irregular fluctuations in SHR represent low-dimensional deterministic chaos in tubuloglomerular feedback (TGF), they could also arise from other mechanisms, such as intrinsic instabilities in preglomerular vessels or inputs from neighboring, coupled nephrons. To test this possibility, we applied a parameter estimation procedure to a model of TGF, where a stochastic process was added to represent mechanisms not included explicitly in the model. In its deterministic version, the model can have chaotic dynamics arising from TGF. The model introduces random fluctuations into a parameter that determines the gain of TGF. The model shows a rich variety of dynamics ranging from low-dimensional deterministic oscillations and chaos to high-dimensional random fluctuations. To fit the data from normotensive rats, the model must introduce only a small variation in the feedback gain, and its estimates of that gain agree well with experimental values. These results support the use of the deterministic model of nephron dynamics in normotensive rats. In contrast, the irregular tubular pressure fluctuations in SHR were best described by a model dominated by random parameter fluctuations. The results point to the failure of simple mathematical models of nephron dynamics: adequate to describe processes that are important for the irregular tubular pressure fluctuations and the need to consider other factors, such as differences in vascular function or nephron-nephron interactions, in further work on this problem.

stochastic processes; spectral density; nephron; tubuloglomerular feedback mechanism; deterministic chaos

AUTOREGULATION OF GLOMERULAR filtration rate (GFR) and renal blood flow is essential for proper functioning of the kidney. Two mechanisms mediate the autoregulatory adjustments of preglomerular resistance in response to changes in renal perfusion pressure: tubuloglomerular feedback (TGF) and the myogenic response. TGF operates in each nephron to stabilize nephron blood flow, single-nephron GFR, and the tubular flow rate. The anatomic basis for TGF is the juxtaglomerular apparatus, where the macula densa acts as the sensor mechanism and the afferent arteriole as the effector mechanism of the system. The myogenic mechanism is an intrinsic response of the preglomerular vessels, in which an increase in transmural pressure results in vasoconstriction (8).

Experimental studies have demonstrated the presence of self-sustained oscillations in proximal tubular pressure (P1), and experimental studies show that the phenomenon is caused by intrinsic delays in TGF signal transmission (16). In Sprague-Dawley (SprD) and Wistar rats, the oscillations are regular, with only one distinct peak in the frequency spectrum at ~20–40 mHz and an amplitude of 3–4 mmHg. In contrast, in spontaneously hypertensive rats (SHR), the oscillations appear as highly irregular fluctuations, with several frequency peaks within the range 10–50 mHz and a smaller amplitude of only 1.5–2 mmHg (14, 16, 35). Evidently, the dynamic behavior of P1 in SHR is not the same as in normotensive rats.

Why the rhythm in P1 is different in SHR and SprD rats is unknown. We suggested that the irregular fluctuations in the SHR could represent low-dimensional deterministic chaos in the TGF system (16, 34, 35). Low-dimensional deterministic chaos is characterized by its sensitive dependence on initial conditions, time series from SHR were subjected to a variety of tests (34). The correlation dimension of the phase space attractor was 2.3, a noninteger value consistent with low-dimensional deterministic chaos. The Lyapunov exponent was found to be positive, consistent with sensitive dependence on initial conditions. Both findings are characteristic of systems showing deterministic chaos but may also be found in systems with other types of dynamics (30).

It is well known from nonlinear dynamic systems theory that oscillations and chaos can be induced by varying the value of a single parameter, designated the bifurcation parameter (29). The gain of the TGF mechanism is central to the understanding of P1 dynamics. This parameter plays an important role in the stability of the pressure and flow regulation, and in previous analyses it has been found to be a main bifurcation parameter...
Free-flow pressure values were then estimated as the mean value of $P_i$ in a base run of the model incorporating the estimated parameters. Stop-flow pressure (SFP) values and the open-loop TGF gain were estimated from the model with the estimated parameters by setting GFR, the proximal tubular absorption rate (APR), and flow into the loop of Henle to zero in the model and then determining the SFP for different induced flows in the loop of Henle.

**MATERIALS AND METHODS**

*Experimental methods.* The experimental data are taken from a previous study, where $P_i$ in two superficial nephrons and $P_a$ were recorded simultaneously in 14 SprD rats and 21 SHR (35). Briefly, the experiments were performed in male SprD rats (250–300 g body wt) and in 10- to 12-wk-old male SHR (220–260 g body wt). Anesthesia was induced by 5% halothane in 25% oxygen and 75% nitrogen. The rats were placed on a heated operating table and connected to a small-animal respirator. Catheters were placed in the right external jugular vein for infusions and in the left carotid artery for measurement of $P_a$. The left kidney was exposed through a midline incision extended to the left flank, immobilized with a Lucite ring, and superfused with saline preheated to 37°C. Data collection started after a 45-min recovery period. $P_t$ was measured simultaneously with the servo-nulling method in two adjacent nephrons. The recording time varied from 5 to 21 min in the individual rats. $P_t$ was recorded on a cassette data recorder for offline analysis and replayed to a 12-bit analog-to-digital converter (Data Translation). To prevent aliasing and to reduce sampling noise, each signal was passed through an analog low-pass filter with a 1.5-Hz cutoff frequency. The sampling rate was 4.8 Hz, and each experiment was used to generate two 4,096-point data sets. Protocol approval was granted from The Danish Animal Experiments Inspectorate (No. 2004/561-914). Further details on recording, sampling, and experimental procedures are available elsewhere (35).

*Model.* The rat nephron model is described in the Appendix, and the model parameters and values are defined in Tables 2 and 3. Details of the deduction and physiological justification of the model have been published previously (2, 9, 13–15, 24). The model consists of three coupled parts: a glomerular-tubular model, a TGF-afferent arteriolar model, and a model of the delay in the TGF, representing the delay caused by the time taken by the signal to pass through the loop of Henle and the delay in the signaling process from the macula densa to the afferent arteriole. The model is shown in Fig. 1.

A key parameter in the understanding of the dynamics of renal pressure and flow regulation is the gain of the TGF mechanism (2). Previous analysis of the deterministic version of the present nephron model has shown that the parameter $Z$, which is one of the model parameters that determines the feedback gain, is a major bifurcation parameter of the system (2, 5, 24). For small values of $Z$, $P_t$ and nephron blood flow are stable without oscillations. As $Z$ is increased, a Hopf bifurcation occurs, and the system shows stable, regular oscillations. In normotensive rats, $P_t$ and flow show regular oscillations with a period of 25–35 s (Fig. 2), and experimental studies have found a value for $Z$ in the range where the models predict regular oscillations (5, 22). When $Z$ is further increased, the system exhibits deterministic chaos, which could resemble the dynamic behavior of SHR. However, the values of $Z$ are higher than those found experimentally, and the amplitude of the chaotic dynamics in the model is larger than that observed experimentally in SHR (5, 16).

In our earlier study (9), we proposed to model $Z$ as a time-varying stochastic process. Former models where $Z$ has been considered constant have failed to capture the fluctuations in period and amplitude that can be found in the experimental traces (cf. Fig. 2). Experimental studies have clearly shown that the feedback gain is subject to a variety of influences that may change over short time scales (e.g., fluctuations in blood pressure or angiotensin II levels) (3).
The main hypothesis of the present work is that more realistic simulations can be obtained by a stochastic approach, where the variance structure of the feedback amplification can be approximated by a stochastic process. This is an Ornstein-Uhlenbeck process (25a). We assume that the process $Z(t)$ is a one-dimensional standard Wiener process. This is an Ornstein-Uhlenbeck process (25a). We assume that $Z(0)$ is normally distributed with mean $\alpha$ and variance $\sigma^2/(2\beta)$ and is independent of $W(t)$. The process $Z$ is ergodic, with stationary distribution $N[\alpha, \sigma^2/(2\beta)]$ the normal distribution, provided that $\beta > 0$. It is assumed that, on average, $Z$ is positive, which requires that $\alpha > 0$. In this model, $Z$ can become negative for some time points. This can be justified by experimental evidence that vasodilator stimuli, such as NO, may originate from the macula densa (33). Thus it could be possible that, momentarily, TGF caused positive feedback, even though, on the average, over several minutes the negative feedback is the strongest, as observed in micropuncture experiments.

To enable a comparison between the present results and previous published experimental results, the parameter estimates from the present study were used to simulate open-loop (stop-flow) and closed-loop (free-flow) micropuncture experiments. In a stop-flow experiment, a wax block is inserted into the proximal tubule, and distal flow rate is controlled through a micropump inserted into the last accessible convolution of the proximal tubule. The responses to variation in microperfusion rate are then measured as changes in SFP. To mimic the experimental setting, GFR, APR, and flow into the loop of Henle were set to zero in the model. Then the SFP was determined in the model for the estimated parameter values and for different induced flows in the loop of Henle. Since a steady state of the model was used to determine the SFP, $Z$ was kept constant at its mean value $\alpha$.

**Estimation method.** The adequacy of a mathematical model can be evaluated by simulating a time series from the model and then comparing the result with a time series from a physiological experiment. Model parameters are estimated by minimizing some distance function between the experimental and the simulated data over the parameter range of the model. The most important characteristics of the dynamics of Pt can be characterized by the spectral density, because period and amplitude and their fluctuations characterize the dynamics. In our previous study (9), we proposed to estimate the parameters by the least squares distance between the logarithmically transformed spectral densities estimated from simulated and experimental data.

The spectral density of the experimental data was estimated as follows. Simultaneous time series from two nephrons have been obtained for each rat. It appears reasonable to assume that the dynamics of the nephrons within the same kidney are subject to the same variability in factors affecting renal function. To gain precision in the estimation of the spectral density, the empirical periodograms from the two nephrons within the same kidney were averaged. Moreover, a moving average over three points [a Daniell window (28)] has been applied to further reduce the variance. The spectral density of the model as a function of the parameters was numerically approximated by averaging over 10 empirical periodograms calculated on simulated time series with the given parameter values. Details can be found in our previous study (9).

In our previous work (9), the bias of the estimation procedure was shown to be on the order of 1% for $\alpha$, 15% for $\beta$, and 10% for $\sigma$. The coefficients of variation of the estimates were shown to be on the...
order of 1.3% for $\alpha$, 33% for $\beta$, and 15% for $\sigma$. We would thus expect $\alpha$ to be the best determined and $\beta$ the least.

We estimated the delay in the loop of Henle and macula densa ($T$), together with the parameters $\alpha$, $\beta$, and $\sigma$. Since $T$ is the major determinant of the oscillatory period, which is clearly recognizable only in SprD rats, it was fixed at 12 s as estimated in previous studies (9, 14, 16). Previous studies have found the same GFR, tubular flow, and tubular length in SHR as in SprD or Wistar-Kyoto rats (1, 31). Since the delay in the loop of Henle is mainly determined by its structure and the flow rate of tubular fluid, it appears likely that there are no major differences in this parameter between the strains.

Statistical methods. Estimates from SprD rats and SHR were compared by Student’s $t$-test for unpaired data (Welch test). $P < 0.05$ was considered statistically significant. Since the estimates of $\alpha$, $\beta$, and $\sigma$ and the slopes of the TGF function showed skewed distributions, these estimates were logarithmically transformed before the analysis. Values are means $\pm$ SD.

RESULTS

Figure 2 shows $P_t$ from an SprD and an SHR nephron, together with simulated trajectories with the estimated values of the parameters from the observed series and the trajectories of $Z(t)$ for the same simulations. Qualitative differences between the two strains of rats are shown in Fig. 2 (top traces). The normotensive SprD rat shows a much more regular pattern, with larger amplitude, than the irregular small variations in the SHR. The time series simulated from the model incorporating the estimated values for the feedback gain parameters are in good agreement with the observed dynamics in the time domain but less so in the frequency domain. The underlying process of $Z(t)$ in the simulation varies less and more smoothly for SprD rats than for SHR.

Figure 3 is a box plot of the parameter estimates for the two strains of rats.

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<tr>
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<th>slope at IP</th>
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<td>SHR</td>
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Fig. 3. Box plot of estimates of parameters in feedback gain for SprD and SHR. Box contains the middle 50% of the estimates. Dot in the box indicates the median. Ends of the vertical lines indicate minimum and maximum. ◦, Outliers.
Table 1. **Summaries of estimates.** Mean values are given ±1 S.D. *n* = number of rats, each rat has time series from two nephron tubules

| Parameter | SHR (*n* = 21) | SprD (*n* = 14) | *P*
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<tr>
<td><em>P</em>&lt;sub&gt;a&lt;/sub&gt;, mmHg</td>
<td>144.4±22.3</td>
<td>109.7±8.2</td>
<td>&lt;0.001</td>
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<td>FFP, mmHg</td>
<td>11.6±1.7</td>
<td>13.1±2.4</td>
<td>0.012</td>
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<tr>
<td>Mean from simulated time series</td>
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<tr>
<td>FFP, mmHg</td>
<td>14.0±0.9</td>
<td>13.1±0.4</td>
<td>0.0003</td>
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<td>Steady state from simulated time series</td>
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<tr>
<td>IP, nl/min</td>
<td>12.2±0.3</td>
<td>12.5±0.3</td>
<td>0.016</td>
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<td>Slope of TGF function at IP, mmHg&lt;sup&gt;*&lt;/sup&gt;·nl&lt;sup&gt;-1&lt;/sup&gt;·min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>3.1±1.6</td>
<td>2.7±0.9</td>
<td>NS</td>
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<tr>
<td>Mean of parameter estimates</td>
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<tr>
<td>α</td>
<td>21.6±1.7</td>
<td>12.5±4.2</td>
<td>0.02</td>
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<tr>
<td>β, s&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.29±0.87</td>
<td>0.10±0.18</td>
<td>NS</td>
</tr>
<tr>
<td>σ, s&lt;sup&gt;-1/2&lt;/sup&gt;</td>
<td>4.8±4.7</td>
<td>2.1±1.8</td>
<td>0.0095</td>
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Values are means ± SD; *n*, number of rats (each has time series from 2 nephron tubules). *P* values refer to statistical significance of differences between means. Estimates of α, β, σ, and slopes of tubuloglomerular feedback (TGF) function were logarithmically transformed before means were compared. SHR, spontaneously hypertensive rats; SprD, Sprague-Dawley; *P*<sub>a</sub>, arterial blood pressure; FFP, free-flow pressure; IP, inflection point; NS, not significant.

In Table 1, the estimates are summarized. The mean value of Z, α, is estimated to be 12.5 ± 4.2 for SprD rats and 21.6 ± 11.7 for SHR. The estimates of α from the two strains of rats were significantly different. The drift parameter, β, is estimated to be 0.10 ± 0.18 and 0.29 ± 0.87 s<sup>-1</sup> for SprD rats and SHR, respectively; the difference is not significant. These estimates correspond to a correlation time of the feedback gain variation, 1/β, of ~5–10 s. Finally, the standard deviation of the driving Wiener noise, σ, was estimated to be 2.1 ± 1.8 s<sup>-1/2</sup> for SprD rats but was much larger (4.8 ± 4.7 s<sup>-1/2</sup>) for SHR; the difference was significant. This result indicates that the contribution of random noise had to be substantially greater in the SHR than in the SprD rats to achieve an adequate fit of the model to the power spectra obtained from experimental data.

The value of α at which chaos is present depends on the value of T in the TGF (5). In the preceding estimations, we used *T* = 12 s, which is the value previously estimated in SprD rats (9). However, the lowest value of α where chaos is present in the model is *T* = 16 s (5). We therefore performed a second set of parameter estimations at this value of the delay, which resulted in nearly identical values of the estimates for α, β, and σ (results not shown).

The magnification, M, is a measure of the efficiency of a regulatory system, with values between 0 and 1. It is defined as the change in late flow rate per change in the inflow into the proximal tubule, and, correspondingly, the open-loop gain (OLG) is defined as (1 − M)/M (12). Simulating from our model with the mean values of the estimated parameters, we find M = 0.123 and 0.107 in SprD rats and SHR, respectively. This corresponds to an OLG of 7.1 for SprD rats and 8.3 for SHR.

Parameter estimates vary much less for SprD rats than for SHR (Fig. 3), perhaps because SHR represent a more inhomogeneous group than SprD rats and, also, because of the more noisy behavior of Pt in SHR, which makes parameter estimation less precise.

In Fig. 4, the logarithm of the spectral densities for two SprD rats and two SHR is plotted against frequency (in Hz), together with the logarithm of the spectral densities approximated from the mathematical model incorporating the estimated parameter values. The model captures the slow dynamics of TGF better than the dynamics of the myogenic mechanism, because the fitted parameters primarily affect the dynamics of the TGF mechanism, which is responsible for the slow components in the spectral density, and because the TGF oscillation is the most prominent peak in the experimental data. Because of the smoothing action of the proximal tubular compliance, the faster dynamics due to the myogenic response, which appear in the spectra from the experimental data as peaks at ~0.1 Hz, are much less prominent.

Table 1 summarizes the results of the open- and closed-loop simulations of micropuncture experiments: IP is the inflection point of the feedback curve, i.e., the perfusion rate at which the half-maximum response is seen. IP also corresponds to the point of maximal slope of the open-loop feedback curve and, therefore, to the perfusion rate at which overall TGF gain attains its maximal value.

For comparison with the model without system noise, the model has been simulated for four different values of α: 10, 15, 25, and 40. In all simulations, the noise was set to zero: σ = 0, in which case β is irrelevant and *T* = 16 s. In Fig. 5, simulations of the Pt oscillations are plotted against time. Only for unrealistically high values of α did the dynamics resemble anything close to experimental results. However, in this case, the amplitude of the Pt oscillations was much larger in the simulations than is found experimentally.

**DISCUSSION**

Previous studies and theoretical considerations have suggested that differences in the dynamic behavior of the Pt oscillations in normal and hypertensive rats arise from differences in parameters of the TGF system. We suggested that the irregular fluctuations in the SHR represent what has become known as low-dimensional deterministic chaos (16, 34, 35). In a chaotic system, the irregular fluctuations are generated by a small set of deterministic ordinary differential equations. Thus,
Fig. 4. Spectral densities for 2 SprD rats (top) and 2 SHR (bottom), log(spectrum) against frequency in Hz. Solid line, spectral density estimated from observed data; dotted line, spectral density approximated from theoretical model with fitted parameter values.

Fig. 5. Simulations of Pt oscillations for 4 different values of $\alpha$: 10 (A), 15 (B), 25 (C), and 40 (D). In all simulations, noise was set to zero: $\sigma = 0$, in which case $\beta$ is irrelevant and delay ($T$) = 16 s.
Despite an apparently complex behavior, the structure of the system is relatively simple, with a limited number of state variables; i.e., it is a low-dimensional process. However, research during the last decade has shown that it is not possible to prove that an experimental time series actually arises from a chaotic system (17) because of the inevitable presence of measurement noise and/or system drift in an experimental setting. These confounding factors can lead to erroneous or biased conclusions when experimental data are analyzed by many of the available algorithms for calculating Lyapunov exponents or correlation dimensions or other measures characteristic of chaotic processes (17).

Because of the problems proving the presence of chaos in experimental time series, an alternative approach has been to rely on mathematical modeling of TGF. All the various dynamic models of TGF have, without exception, shown that regular oscillations in P\textsubscript{T} occur when the OLG in TGF exceeds a certain limit (2, 13, 16, 20, 24). Furthermore, the value at which the bifurcation to an oscillation occurs is within the experimentally determined range for the gain in TGF (2, 16). In contrast, several of the published models have failed to reproduce deterministic chaos, even at high values for TGF gain (16). In the mathematical models of TGF in which chaotic dynamics have been found, parameter values (in the case of the present model, for values of \( p \)) significantly greater than experimentally measured values have been required to achieve the bifurcation (5, 16). Because of these apparent discrepancies, it has been suggested that the transition to chaos in TGF could depend on additional factors, such as an increased nephron-nephron coupling in SHR (16, 27).

Consequently, it is necessary to consider the possibility that the irregular fluctuations in P\textsubscript{T} in SHR may arise from mechanisms other than deterministic chaos in the TGF system of a single nephron. A method for including unknown factors in a model is to introduce a stochastic component into the system. The stochastic fluctuations represent the influence of the many unmodeled (and possibly unknown) subsystems. The resulting model is known as a stochastic model and will correspond to a system with many degrees of freedom, i.e., a high-dimensional process.

To rigorously test whether the irregular fluctuations in SHR are best described by a low-dimensional deterministic model or a high-dimensional stochastic model, we have applied a recently developed procedure that allows parameter estimation in complex physiological models of TGF (9). By using a model that, in its deterministic version, can have chaotic dynamics (2) and combining it with a stochastic process that allows random fluctuations in one of the key parameters (Z) that determines the gain of TGF, we obtain a model that has a rich variety of dynamics ranging from deterministic to random fluctuations (see Appendix). Using experimental data as input, we can then estimate the parameters of the model. In most cases, the value of Z was well below the value (~25) where chaotic dynamics are present (cf. Figs. 3 and 5).

It is worth noting that, in case of the SprD rats, the parameters estimated are in good agreement with the values that have been found experimentally with open- and closed-loop approaches (9, 16). For example, Lesyssac and Holstein-Rathlou (22) found a slope at \( IP = 2.3 \pm 0.7 \) and \( 2.2 \pm 1.0 \) (SE) mmHg·nl\(^{-1}\)·min in halothane-anesthetized Wistar-Kyoto and SprD rats, respectively. These values are not significantly different from the value found in the present study (2.6 mmHg·nl\(^{-1}\)·min). Also, \( M = 0.123 \) in SprD is within the range of values previously reported from our laboratory (12, 18). Maximal compensation values of 0.23 ± 0.02 and 0.07 ± 0.08 have been reported by Holstein-Rathlou (12) and Karlsen et al. (18), respectively.

Comparison of SprD rats and SHR revealed a much greater variability in Z in the SHR. Thus \( \sigma \) of the associated Ornstein-Uhlenbeck process was significantly higher in the SHR than in the SprD rats (cf. Table 1). The larger \( \sigma \) implies a greater variability in the Ornstein-Uhlenbeck process, which determines Z. Physiologically, this increased variability will correspond to greater fluctuations on a short time scale in the gain of TGF in SHR than in SprD rats. Since TGF is one of the key mechanisms involved in renal autoregulation of blood flow and GFR, an increased variability in feedback gain could cause a less precise and/or a less stable autoregulatory process in the SHR. Zhong and co-workers (36) recently showed that autoregulatory dynamics display greater nonstationarity in SHR than in SprD rats. This observation was based on an entirely different approach, where the spontaneous fluctuations in P\textsubscript{T} and renal blood flow were used to assess a time-dependent transfer function between blood pressure and blood flow. Using this approach, they found a greater variability in the time-dependent behavior of the transfer functions in the SHR than in the SprD rats (36). This analysis of experimental results is in excellent agreement with the results of the present study.

The random fluctuations in TGF gain in our model were assigned to the parameter Z in the transfer function of TGF representing the effect of the macula densa on the afferent arteriole. However, it should be noted that the total gain in TGF is dependent on many other factors, including the characteristics of the afferent arteriole. Assigning all the variability to one parameter is therefore only a convenient way to lump several potential sources of variability in TGF gain. The observed increased variability need therefore not necessarily reflect differences in macula densa function between the SHR and SprD rats but could also reflect an increased variability in the other subsystems that determines total feedback gain. In this connection, it is of interest that several studies have found a greater instability in vessels from SHR and stroke-prone SHR (4, 25). When vessels are exposed to a vasoconstrictor, such as noradrenaline, there are greater fluctuations in the developed force in the hypertensive than in the normotensive rats (4, 25). An increased vascular variability would be reflected in the present model by an increased variability in Z. Another source of increased variability could be an increased interaction between nephrons in SHR (7, 32). The interaction strength is increased two- to threefold in hypertension (7, 32). This change in interaction strength will inevitably increase the appearance of signals from adjacent nephrons in measurements from individual tubules and could well contribute to the observed behavior of P\textsubscript{T} signals in SHR.

It is apparent from Fig. 2B that, in SHR, Z dips below zero quite frequently. This indicates that, during brief periods, the feedback is actually positive. There are no experimental data that allow us to assess whether this is a reasonable result. As mentioned above, it is known that the macula densa produces vasoconstricting (e.g., adenosine) and vasodilating (e.g., NO) substances. At the level of the individual nephron, it cannot be excluded that random (molecular) fluctuations in the produc-
tion rates could cause this effect. However, again it has to be stressed that \( Z \) is a lumped parameter, and the positive feedback could also be caused by decreased TGF activity in neighboring nephrons, causing vasoconstriction in the measured nephron, or it could represent variability in local nervous activity. Clearly, this is an issue that warrants further investigation.

From the standpoint of model theory, the main conclusion of the present study is that the type of deterministic chaos generated by our simple model is insufficient to explain the experimentally observed fluctuations in \( P_t \) in SHR. Although some of the current models of single-nephron dynamics have shown more complex spectra (21), it seems fair to state that all other reduced single-nephron models in the literature fail to bifurcate beyond regular oscillations when an increase in TGF gain is applied. We suggest that all current models of this type, therefore, are not adequate to describe the irregular fluctuations in \( P_t \) in the SHR. In contrast, application of the model to data from SprD rats yields much lower values of \( \sigma \) than application of the model to data from SHR and excellent agreement between estimated and measured values of the OLG. This result supports the use of the deterministic model to describe nephron dynamics in normotensive animals.

From a physiological standpoint, the results of the present study and the study by Zhong and co-workers (36) suggest a greater variability in the autoregulatory mechanisms in kidneys of hypertensive animals. It is well known that the kidneys play a central role in determining the long-term level of \( P_t \) (10) and that this is associated with the kidney’s ability to eliminate sodium from the body (11). Clearly, this issue needs further experimental consideration.

In conclusion, the dynamics of the \( P_t \) fluctuations in SHR appear to be better described by a model that incorporates random fluctuations in the system parameters than by a model that operates in a chaotic domain. We argue that the results point to the failure of the present models to adequately describe processes that are important for the observed irregular \( P_t \) fluctuations and the need to consider the importance of other factors, such as differences in vascular function and/or nephron-nephron interactions in further work on this problem.

**APPENDIX**

**Mathematical model.** The mathematical model consists of the glomerular-tubular model, the TGF-afferent arteriolar model, and the model for the delay. By coupling these, a dynamic model of the integrated function of the nephron is obtained. The model is illustrated in Fig. 1.

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The variables are categorized as follows: constants, the values of which are unchanged during model study; system variables, with evolution in time that is defined through differential equations; auxiliary variables, which change in time as functions of the system variables; a random variable modeled as an Ornstein-Uhlenbeck process (25a); and parameters that are supposed constant and with values that are estimated individually for each rat from the data.

All model variables are listed and values for the constants are given in Tables 2 and 3.

**Glomerular-tubular model.** The transit time for a volume element in the glomerular capillaries is several orders of magnitude smaller than the period length of the oscillations in \( P_t \), so we assume that GFR equilibrates instantaneously and is defined by the instantaneous plasma flow (GPF), the hydrostatic glomerular capillary pressure \( (P_g) \), and \( P_t \).

GPF is given by the conventional flow equation, where GBF is blood flow, Hct is the arterial hematocrit, and \( R_a \) is the flow resistance in the afferent arteriole

\[
\text{GPF} = \frac{\text{GBF}}{(1 - \text{Hct})} \left( (P_g - P_{av})/R_a \right)
\]

such that, by assuming that the glomerular capillaries are impermeable to protein, mass conservation yields

\[
\text{GFR} = (1 - C_e/C_a)(1 - \text{Hct})(P_g - P_{av})/R_a
\]

where \( C_a \) and \( C_e \) are afferent and efferent plasma protein concentrations, respectively. The hemodynamic resistance of the preglomerular vessels is assumed constant in the first part of the afferent arteriole, whereas close to the glomerulus the TGF signal from the macula densa causes the normalized radius of the arteriole, \( r \), to react to the activation level, such that the resistance is proportional to \( r^{-4} \) and is given by the expression

\[
R_a = R_a0(\lambda + (1 - \lambda) \cdot r^{-4})
\]

where \( \lambda \) is the fraction of the afferent arteriole having a fixed resistance.

An expression for \( C_e \) is obtained by assuming that filtration equilibrium is established before the blood leaves the glomerular capillaries and idealizing the glomerular capillary bed as a single tube of equivalent surface area. This leads to a third-order polynomial in \( C_e \) with coefficients depending on \( P_t \) and \( R_a \). The value for \( C_e \) is the
positive root of the third-order polynomial, which can be shown to have a unique solution. 

\( P_e \) is determined by mass conservation, such that GFR equals the sum of GFR and the flow through the efferent arteriole 

\[
\text{GBF} = \text{GFR} + \text{efferent arteriolar blood flow}
\]

APR is considered constant. The flow into the loop of Henle is given by the difference between \( P_e \) and \( P_a \), the last considered constant, divided by the flow resistance in the loop of Henle, \( R_n \). \( P_e \) changes in response to differences between the in- and outgoing flows 

\[
\frac{dP_e}{dt} = \frac{1}{C_{\text{mab}}} (\text{GFR} - \text{APR} - \text{flow in loop of Henle})
\]

where \( C_{\text{mab}} \) is the compliance of the proximal tubule.

**TGF-afferent arteriolar model.** The TGF function, \( \Psi \), is assumed to be mediated only through the afferent arteriole, where NaCl concentration at the macula densa causes a signal to be transmitted to the afferent arteriole, which, in response, will constrict or dilate (3). In the model, the delayed flow into the loop of Henle, instead of the NaCl concentration at the macula densa, has been used as the signal to TGF. This simplification is motivated by the fact that the two are directly proportional and by the fact that it is the flow into the loop of Henle that has been the independent variable in the vast majority of published experiments on TGF (3). It is well established empirically that the activation level of the vascular smooth muscle cells in the wall of the afferent arteriole, \( \Psi \), can be described by a logistic equation (16)

\[
\Psi = \Psi_{\text{max}} - \frac{\Psi_{\text{max}} - \Psi_{\text{min}}}{1 + \exp(Z (F_3/F_{h0} - IP))}
\]

where \( \Psi_{\text{min}} \) and \( \Psi_{\text{max}} \) are the lower and upper activation limits, respectively; \( Z \) is a randomly varying parameter that determines the slope of the S-shaped TGF function and, thereby, the gain of the TGF mechanism; \( F_3/F_{h0} \) is flow at the macula densa normalized with respect to the equilibrium flow in the loop of Henle; and \( IP \), the inflection point of the curve, is the flow at which the feedback response is half-maximal and is given by

\[
IP = 1 - \frac{1}{Z} \log \left( \frac{\Psi_{eq} - \Psi_{\text{min}}}{\Psi_{\text{max}} - \Psi_{eq}} \right)
\]

where \( \Psi_{eq} \) is the equilibrium activation level.

The dynamics of the afferent arteriole are modeled by a second-order differential equation to mimic the tendency of the arterioles to perform damped, oscillatory contractions in response to stimuli 

\[
\frac{d^2\gamma}{dt^2} + \frac{d\gamma}{dt} = \frac{P_{eq} - P_{eq}}{\gamma}
\]

where \( \gamma \) is the radius of the afferent arteriole normalized with respect to its resting value, \( P_{eq} \), and \( P_{eq} \) represent the average and the equilibrium pressure in the variable part of the afferent arteriole, respectively, \( \gamma \) is the mass-to-elasticity ratio for the arteriolar wall and controls the natural frequency of the oscillations, and \( \omega \) is the damping of the arteriolar dynamics. 

\( P_{eq} \) is determined by assuming a linear pressure drop across the variable part of the arteriolar resistance 

\[
P_{eq} = \frac{1}{2} \left[ (P_a - (P_e - P_g)) + \frac{R_n}{R_a} + P_g \right]
\]

\( P_{eq} \) is determined by an empirical relation (2)

\[
P_{eq} = 0.006 e^{10(r-0.3)} + 1.6(r-1) + 3 \left[ 1 + e^{10.4(r-1)} + 7.2(r+0.9) \right]
\]

where the first two terms represent the passive, elastic response of the arteriolar wall to changes in the transmural pressure and the rest represent the active, muscular response to the activation by the TGF mechanism, \( \Psi \).

**Delay.** Flow at the macula densa does not change immediately after a change in \( P_e \), but only after a delay. Furthermore, it seems likely that an additional delay occurs in the transmission of the signal from the macula densa to the afferent arteriole. We have conflated these two delays into one and modeled it as a third-order lag, where the input is the flow into the loop of Henle and the output is the delayed flow, which serves as the input to the TGF function, \( F_3 \). The delay is defined by the equations 

\[
\frac{dF_1}{dt} = \frac{3}{T} (P_e - P_a - F_1)\]

\[
\frac{dF_2}{dt} = \frac{3}{T} (F_1 - F_2)\]

\[
\frac{dF_3}{dt} = \frac{3}{T} (F_2 - F_3)\]

where \( T > 0 \) determines the duration of the delay.

\( P_e \) In previous applications of the present model, \( P_e \) has been fixed to 13.33 kPa (100 mmHg) (2, 5, 9, 24). Inasmuch as \( P_e \) was measured in the present data set and we are looking at differences between SprD rats and SHR that are characterized by their increased \( P_a \), it is natural to use the actual measured value for each rat when estimating the parameters. It has been shown previously (6) that, despite the increased \( P_a \) in SHR, afferent arteriolar blood flow and \( P_e \) are the same in SHR and SprD.

Therefore, \( R_{h0} (= 2.44 \text{ kPa} \cdot \text{s} \cdot \text{nl}^{-1} \text{ in the previous models}) \) has been adjusted accordingly, such that the glomerular flow 

\[
\text{glomerular flow} = \frac{P_g - P_a}{R_a} = \frac{13.33 \text{ kPa} - P_g}{2.44 \text{ kPa} \cdot \text{s} \cdot \text{nl}^{-1}}
\]

A base run of the model gives \( P_g = 6 \text{ kPa} \), such that \( R_{h0} = P_g/3 - 2 \).

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A STOCHASTIC MODEL OF RENAL HEMODYNAMICS