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Characterizing multimode interaction in renal autoregulation

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Abstract

The purpose of this paper is to demonstrate how modern statistical techniques of non-stationary time-series analysis can be used to characterize the mutual interaction among three coexisting rhythms in nephron pressure and flow regulation. Besides a relatively fast vasomotoric rhythm with a period of 5–8 s and a somewhat slower mode arising from an instability in the tubuloglomerular feedback mechanism, we also observe a very slow mode with a period of 100–200 s. Double-wavelet techniques are used to study how the very slow rhythm influences the two faster modes. In a broader perspective, the paper emphasizes the significance of complex dynamic phenomena in the normal and pathological function of physiological systems and discusses how simulation methods can help to understand the underlying biological mechanisms. At the present there is no causal explanation of the very slow mode. However, vascular oscillations with similar frequencies have been observed in other tissues.

Keywords: rhythmic dynamics, synchronization, nephrons, wavelet analysis

1. Introduction

Regulation and coordination of our normal physiological functions involve the interplay of a large variety of rhythmic processes that occur at all the different levels of the organism's structural hierarchy and cover a wide range of different time scales. Examples at the cellular level are the sustained metabolic oscillations and calcium dynamics observed, for instance, in

smooth muscle cells (Aalkjaer and Nilsson 2005), and the characteristic spiking and bursting phenomena observed in the membrane potential of cold and warm receptor cells (Schäfer *et al* 2004), insulin producing pancreatic β-cells (Sherman *et al* 1988), and many other types of nerve and gland cells. Rhythmic phenomena, although typically at significantly longer time scales, are also manifest in the regulation of many organs as well as in the overall hormonal coordination of the organism.

The coexistence of two or more modes in the functioning of a biological system often leads to different forms of interaction. A typical result of such an interaction is synchronization (or entrainment), where each of the interacting modes adjust their dynamics so as to attain a rational ratio between their frequencies. Synchronization plays an important role in the dynamics of many living system (Pikovsky *et al* 2001, Mosekilde *et al* 2002). The well-known examples are the entrainment between the heart rate and the breathing cycle (Schäfer *et al* 1999, Rzeczinski *et al* 2002), entrainment of the cell division cycle to the circadian rhythm and cooperative dynamics of neuronal ensembles (Kopell *et al* 2000). Pathological synchronization of oscillatory activity in the brain is supposed to lead to Parkinsonian tremor (Tass 2002, Tass *et al* 2006) and significant efforts are made to try to develop effective techniques to desynchronize the entrained cells through deep brain electrical stimulation. Tass (Tass *et al* 2000), for instance, has used models of a large number of globally coupled so-called phase oscillators to devise a phase-resetting technique that can be used in demand mode and seems to produce long-term beneficial effects. Altinok *et al* (2007) have used a model of the synchronization of the cell cycle with the circadian rhythm to design an improved chronotherapeutic treatment of male patients with intestinal cancer. Synchronization has also been observed among the mechanisms of renal autoregulation (Holstein-Rathlou *et al* 2001) where their manifestations are clearly distinguishable between normotensive and hypertensive rats (Sosnovtseva *et al* 2002, 2007a).

Modulation is another example of nonlinear interaction between the modes in a biological system. Here, the instantaneous amplitude or frequency of one (the fast) mode is forced to vary in step with the other (slower) process. A well-known phenomenon, observed in humans as well as animals, is the respiratory modulation of the heart rate, with the heart rate increasing during inspiration and decreasing during expiration (Bračič and Stefanovska, 2002). This phenomenon is clearly revealed in the pulse oximetry signal (Adison and Watson 2004). We have recently reported on mode-to-mode interaction in the form of frequency and amplitude modulation both for the mechanisms of kidney autoregulation (Sosnovtseva *et al* 2004, 2005b, Marsh *et al* 2005) and for coexisting rhythmic components of the intracellular processes (Sosnovtseva *et al* 2005a, Brazhe *et al* 2006).

Over the years we have developed a series of models of the processes involved in renal autoregulation (Mosekilde 1996, Barfred *et al* 1996, Sosnovtseva *et al* 2007b) and we have simulated in detail how neighboring nephrons synchronize their autoregulatory processes (Mosekilde *et al* 2002). The purpose of the present paper is to demonstrate how modern statistical techniques for nonstationary time-series analysis (wavelet and double wavelet techniques) can be used to identify several coexisting rhythms in nephron pressure and flow regulation. Besides a relatively fast myogenic mode with a period of 5–8 s and a somewhat slower mode associated with an instability in the tubuloglomerular feedback control with a period of about 35 s, we also identify a very slow mode with a period of 100–200 s. In the work (Sosnovtseva *et al* 2005b), we have examined interactions between the first two rhythmic components and we have shown that hypertensive rats typically display a higher strength of the frequency/amplitude modulation of the myogenic dynamics. Unlike the previous studies, we focus in this paper on the very slow rhythmic dynamics and on interactions between the revealed 100–200 s oscillatory process and the two known mechanisms of renal regulation.

By means of double-wavelet techniques we investigate how the very slow mode modulates the two faster modes. The idea of such an analysis is twofold: (i) by measuring the degree of modulation of one mode by another we can characterize the magnitude and form of the nonlinear coupling mechanisms between the modes and (ii) by applying a similar approach to experimental data obtained during administration of, for instance, an antihypertensive drug, we can extract significant information about the drug's effects on the nephron autoregulation.

At the present we know of no explanation of the physiological mechanisms underlying the very slow mode. It might not be related to the nephron itself, but could easily arise in the larger vessels of the kidney. In fact, the considered experimental data does not even exclude that it may arise outside the kidney and be transmitted by the blood pressure into the tubular pressure. Let us note that vascular oscillations with similar frequencies have recently been observed in other tissues (Hill *et al* 2005), and the presence of a 'third', slow renal autoregulatory mechanism in the kidney was described in recent works by Just (2007) and by Gorbach *et al* (2007).

2. Wavelet analysis

Many physiological processes are highly nonstationary and inhomogeneous, which is why spectral analysis of corresponding time series are often based on the wavelet transform (Grossman and Morlet 1984, Daubechies 1992, Meyer 1992, Chui 1992, Mallat 1998). Wavelets provide a tool for detecting periodicities in short, nonstationary data and for following the temporal evolution of different rhythmic components in the case of multimode dynamics. The wavelet transform of a signal $x(t)$ is obtained as follows:

$$W_x(a,b) = \frac{1}{\sqrt{a}} \int_{-\infty}^{\infty} x(t)\psi^* \left(\frac{t-b}{a} \right) \mathrm{d}t, \tag{1}$$

where $\psi([t-b]/a)$ is a translated and scaled version of the wavelet 'mother' function $\psi(t)$, with a and b characterizing the time scale and temporal localization, respectively, the asterisk refers to the complex conjugate. $W_x(a,b)$ are the wavelet coefficients. The choice of $\psi(t)$ depends on the problem to be solved. In the spectral analysis of nonstationary data, the *Morlet* function is typically used,

$$\psi(t) = \pi^{-1/4} \exp(\mathrm{j}2\pi f_0 t) \exp\left[-\frac{t^2}{2} \right]. \tag{2}$$

The selection of f_0 allows us to search for a compromise between the localizations of the wavelet in the time and frequency domains. We use here $f_0 = 1$. The relation between the scale a and the central frequency for the 'mother' function f in this situation is $f = 1/a$.

In addition to the wavelet coefficients $W_x(a,b)$ or $W_x(f,b)$, the energy density of the signal $x(t)$ in the time-scale plane can be estimated: $E_x(a,b) = Ca^{-1}|W_x(a,b)|^2$, with C being a normalizing parameter that depends on the choice of $\psi(t)$. The time-averaged energy density is an analog to the Fourier power spectrum. $E_x(f,b)$ can be treated as a surface in three-dimensional space whose sections at fixed time moments correspond to the local energy spectrum. Considering the dynamics of local maxima of $E_x(f,b)$ it becomes possible to reveal the time evolution of spectral peaks. The latter gives us a possibility to extract time series of the instantaneous frequencies and amplitudes associated with the physiological rhythms being of interest.

In the case of interactions, the faster mode can be modulated by a slower physiological process. Aiming to study modulation properties, we proposed the *double-wavelet* technique (Sosnovtseva *et al* 2004, 2005a) that consists in the following. The instantaneous frequency

or amplitude of the fast mode is considered as input signal for the second wavelet transform (1). Again, the wavelet coefficients and the energy density are estimated and the simplified visualization of the energy density is considered (i.e., the dynamics of only local spectral peaks). The latter will contain information about all modes involved in the modulation process. The second wavelet transform allows us to characterize deviations of the frequency/amplitude of the fast mode associated with any slower processes. A detailed analysis of possibilities and limitations of the double-wavelet technique is described in our previous work (Sosnovtseva *et al* 2005b).

3. Multimode dynamics in renal autoregulation

Renal autoregulation is mediated by at least two different mechanisms, the tubuloglomerular feedback (TGF) and the myogenic response of the afferent arteriole (Leyssac and Holstein-Rathlou 1986, Holstein-Rathlou and Marsh 1994). The TGF mechanism produces a negative feedback control that regulates the nephronal blood flow and, hence, the single-nephron glomerular filtration rate and the tubular flow rate in dependence of the NaCl concentration of the fluid that leaves the ascending limb of the nephron. As shown in early works by Leyssac and Holstein-Rathlou (1986) and Holstein-Rathlou and Leyssac (1986), this feedback regulation can become unstable and generate self-sustained oscillations in the proximal tubular pressure with a typical period of 30–40 s. Similar oscillations can be detected in the distal tubular pressure and, with a time delay of about 15 s, in the chloride concentration near the macula densa (Holstein-Rathlou and Marsh 1994). While for normal rats the oscillations have the appearance of regular self-sustained oscillations, highly irregular oscillations are observed for spontaneously hypertensive rats (Holstein-Rathlou and Leyssac 1986). Thus, the development of hypertension seems to be accompanied by a transition of the regulatory dynamics into a state of deterministic chaos.

The myogenic mechanism represents the intrinsic response of the smooth muscle cells in the vascular wall to changes in the TGF-signal as well as to other stimuli. This mechanism operates in the 0.1–0.25 Hz frequency range. An increase of the transmural pressure elicits a contraction of the vascular smooth muscle cells in the arteriolar wall causing vasoconstriction and a reduction of the blood flow. Since both mechanisms act on the afferent arteriole to control its hemodynamic resistance, the activation of one of the mechanisms modifies the response of the other (Holstein-Rathlou *et al* 1991, Chon *et al* 1994, Feldberg *et al* 1995).

The individual unit of the kidney, the nephron, may thus be considered as a bimodal oscillator that displays oscillations in its pressure and flow regulation at two different time scales: a fast rhythm arising from the vasomotoric dynamics of the smooth muscle cells in the afferent arteriole, and a slower rhythm produced by the delayed tubuloglomerular feedback. However, analyses of tubular pressure recordings demonstrate that the nephron dynamics is more complicated than this and not explainable by only two oscillatory modes. In particular, wavelet analyses reveal the presence of additional very slow rhythmic components in the 0.002–0.01 Hz frequency range whose physiological interpretation is less obvious. Particularly for hypertensive rats, these very slow rhythmic components can be even more clearly expressed in the power spectrum of tubular pressure recordings than the other modes. As illustrated in figure 1(a), the spectral power associated with the rhythm at $\approx$0.005 Hz is larger than that of the TGF-dynamics ($\approx$0.035 Hz) and even larger in comparison with the power of the myogenic mode ($\approx$0.2 Hz). In the following, we will denote the very slow oscillations, the TGF-oscillations and the myogenic modes as very low-frequency (VLF), low-frequency (LF) and high-frequency (HF) dynamics, respectively. The presence of the VLF-rhythms can be

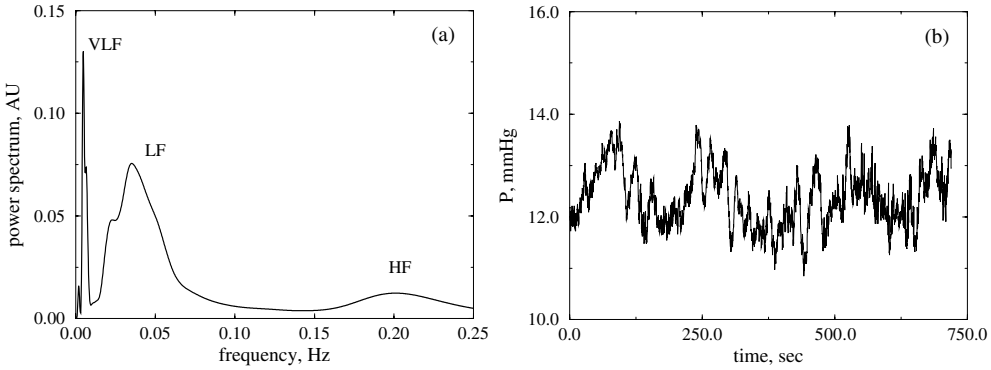

Figure 1. (a) Power spectrum of tubular pressure recording from a spontaneously hypertensive rat. The very slow oscillatory component (≈0.005 Hz) is even more pronounced than the other rhythms. (b) Experimental time series showing pronounced oscillations with a period of about 200 s.

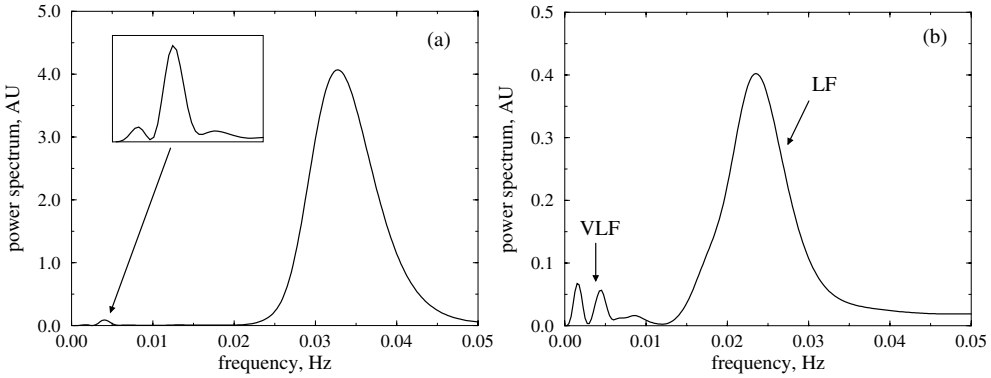

Figure 2. Examples of the power spectra obtained in the VLF- and LF-ranges for a normotensive (a), respectively, a hypertensive (b) rat.

visually observed in figure 1(b) where rather strong oscillations with a period of about 200 s are seen.

We would like to emphasize, however, that the presence of such a pronounced VLF-mode as seen in figure 1(a) does not reflect the typical situation. Most often the VLF-component, while observable in the dynamics of both normotensive and hypertensive rats (figure 2), is characterized by a rather low power relative to the other rhythms. In the spectrum displayed in figure 2(a), for example, the VLF oscillations are very small as compared to the strong TGF-mode. To make them more 'visible', a representation of this spectrum on a logarithmic scale may be useful. For hypertensive rats (figure 2(b)), the amplitude of the TGF-rhythm is usually significantly lower than in figure 2(a), and the VLF-dynamics is therefore more pronounced in the power spectrum.

4. Experiments and statistical analysis

In order to focus the discussion on the typical phenomena in nephron multimode dynamics we shall consider the average results of an extensive series of experiments. Animal preparation

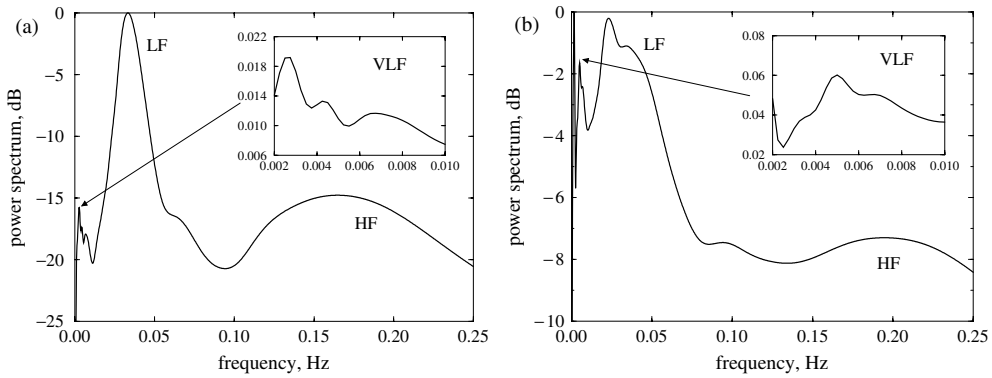

Figure 3. Averaged power spectra: over 32 recordings for normotensive rats (a), and over 42 recordings for hypertensive rats (b). Besides the slow (LF) and the fast (HF) dynamics there is a very slow component. The insets show more detailed spectra in the VLF-frequency range. Aiming to illustrate different details of multimode dynamics the full spectra are given in logarithmic frequency scale while inserts are shown in normal frequency scale.

and experimental procedure were described by Yip *et al* (1992). We use here the same data set as in the mentioned work (Yip *et al* 1992). These data have been the basis of several our studies devoted to interaction phenomena (Sosnovtseva *et al* 2002, 2007a, Marsh *et al* 2005). In the previous works we have analyzed, however, only two modes in nephron dynamics without the consideration of the VLF component and its influence on faster dynamics.

Experiments were performed in male Sprague-Dawley rats, 250–300 g BW, and in 10–12 week old spontaneously hypertensive rats (SHR). Anesthesia was induced with 5% and maintained with 1% Halothane in a gas mixture containing 25% oxygen and 75% nitrogen. Tubular pressure data from single nephrons of 13 normotensive rats and 18 SHR were measured. For this purpose the left kidney was exposed through a midline incision, immobilized in a Lucite ring and superfused with saline preheated to 37 °C. Paired measurements of tubular pressure were made with the servo-nulling technique. The 13 normotensive rats gave 16 pairs of time series (6 pairs from 3 rats and 10 pairs from 10 rats). The 18 SHR provided 21 pairs of time series (6 pairs from 3 rats and 15 pairs from 15 rats). Proximal tubular pressure was measured in two or three tubules simultaneously. Tubular pressure data were recorded through a low-pass Butterworth filter with a cutoff frequency of 1.5 Hz. The data were then digitized at 4.8 Hz. The respiratory signal was removed with a Kaiser–Bessel low-pass filter with a cutoff frequency of 0.5 Hz and attenuation of 50 dB. The calculations reported in this paper were performed on the output of the Kaiser–Bessel filter.

Figure 3 shows that all the above oscillatory modes are distinguishable in the averaged power spectra for the two strains. It is also seen that the VLF-mode is more pronounced for hypertensive than for normotensive rats. Comparison of the power spectra in figure 3 shows that the amplitude of the LF-mode for normotensive rats is significantly larger than the amplitude of the other rhythmic components with the VLF and HF dynamics displaying somewhat similar low amplitudes (figure 3(a)). A different situation is observed for hypertensive rats. Here, the VLF is much stronger (figure 3(b)) with amplitudes comparable to those of the LF-dynamics.

The finite time of the experimental recordings (usually about 1000 s) limits our spectral analysis to frequencies above 0.002 Hz. We used the restriction that the analyzed data series should contain more than two periods of very slow oscillations. The averaged frequency of

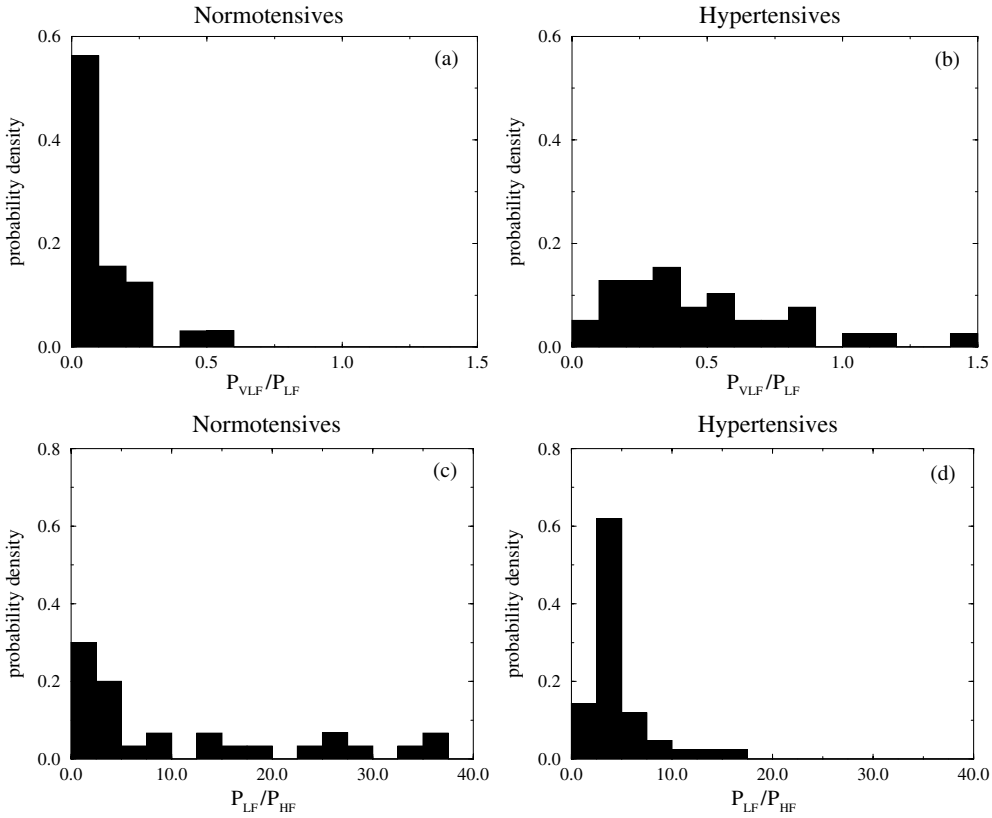

Figure 4. Fraction of time series showing power ratios between different frequency bands for normotensive (a, c) and hypertensive (b, d) rats, respectively. P_{VLF}, P_{LF} and P_{HF} were calculated as integrals of power spectra over the corresponding frequency intervals. The total number of recordings is 74 with 32 recordings from normotensive rats and 42 from hypertensive rats.

the VLF-mode in figure 3(b) takes a value of about 0.005 Hz, i.e. at least five periods of this mode can be distinguished in the tubular pressure data. This is enough to make reliable spectral estimations with the wavelet transform. It is known that wavelet analysis has some problems with a correct representation of both the amplitudes of the rhythmic components and the integrated power spectrum over some frequency range (Maraun and Kurths 2004). Thus, if the real amplitudes are estimated, the integrated power does not correspond to results of the Fourier analysis and vice versa. In the present paper, we aimed to obtain correct values of amplitudes and, hence, consider only ratios of powers in different frequency bands.

To illustrate the distinctions between the two strains of rats in more detail let us consider the ratio of the powers associated with the various spectral regions. Based on figure 3, we expect that the two strains should display significant differences in these power ratios. Figure 4 confirms this: the strongest differences are observed for the VLF and the LF ranges ($P_{\mathrm{VLF}}/P_{\mathrm{LF}}$ in figure 4, panels (a) and (b)). More than 55% of the normotensive rats and only 5% of the hypertensive rats are characterized by a ratio $P_{\mathrm{VLF}}/P_{\mathrm{LF}} < 0.1$. Nephrons from normotensive rats thus demonstrate low power in the VLF-range in comparison with nephrons from hypertensive rats. The opposite situation is true for the LF-mode. The hypertensive rats show only small amplitude of the TGF-based rhythms while the normotensive rats

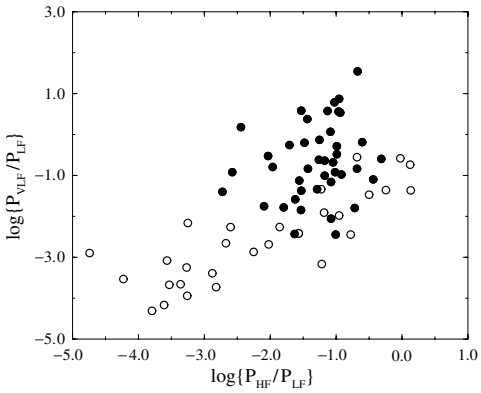

Figure 5. Distributions of powers in the tubular dynamics for normotensive (open circles) and hypertensive (filled circles) rats. Power ratios for individual time series are plotted on a logarithmic scale.

demonstrate larger oscillations. Figures 4(a), (b) display relatively strong distinctions between the normotensive and the hypertensive rats. Differences in the functioning of individual nephrons of the two strains occur also for other oscillatory components. Figures 4(c), (d) show that the ratio $P_{\mathrm{LF}}/P_{\mathrm{HF}}$ takes rather low values for hypertensive rats as compared with normotensive rats. Thus the highly irregular dynamics observed in hypertensive rats occurs at relatively low amplitudes of the TGF-mediated oscillations. On the other hand, normotensive rats demonstrate a higher variability in the amplitude of the TGF-mode and, as a consequence, in the ratio $P_{\mathrm{LF}}/P_{\mathrm{HF}}$. Figure 5 illustrates these distinctions in another way. It is clearly seen that characteristics of normotensive (open circles) and hypertensive (filled circles) rats are well separated in this figure.

5. Modulation properties of TGF-dynamics

In nonlinear systems, the instantaneous amplitude and frequency of one oscillatory mode can be modified by the presence of a slower mode. As mentioned in the introduction, a well-known example is the way the beating of the heart is modulated by the respiratory cycle (Schäfer *et al* 1999). Examination of this phenomenon can help us characterize the degree of coupling between the two systems (Rosenblum and Pikovsky 2001, Smirnov and Bezruchko 2003) and, at least qualitatively, predict situations in which the two systems will synchronize. Investigations of this type have recently been used, for instance, to characterize anesthetic depths (Musizza *et al* 2007).

The purpose of this section is to examine to what extent the presence of the very low-frequency oscillations modulate the instantaneous frequency and amplitude of the TGF-mediated oscillations for the two strains of rats. Figure 6 illustrates examples of how the characteristics of the TGF-dynamics can change in accordance with the slower processes. The instantaneous frequency of the VLF-rhythm can show significant variations (≈0.0025–0.01 Hz) (figure 6(a)). This means that the VLF-oscillations can have a very complicated effect on the individual nephron dynamics and this effect cannot be removed by a simple high-pass filtering of the experimental data as is typically used to detrend experimental recordings. Variation of the instantaneous frequency of the TGF-mode results in the absence of a sharp peak in the power spectrum computed from the tubular pressure data (see, for instance, the

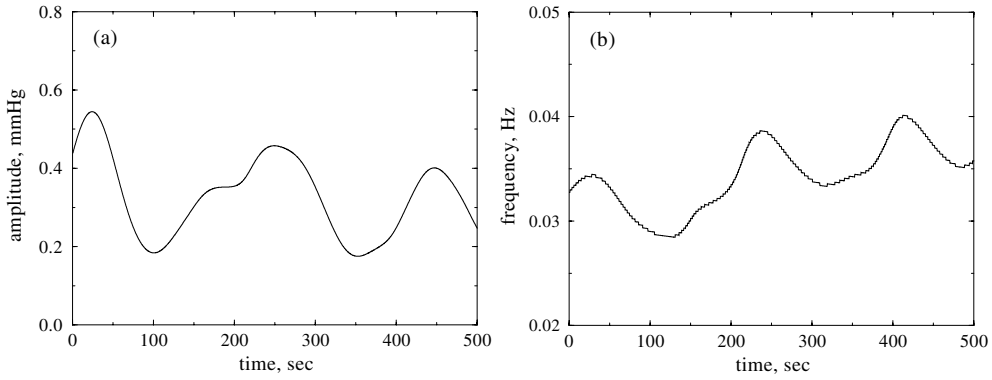

Figure 6. The instantaneous amplitude (a) and instantaneous frequency (b) of the TGF-mode vary slowly as these variables are modified by the presence of a very slow oscillation in the nephron dynamics. The two curves were computed from the same time series.

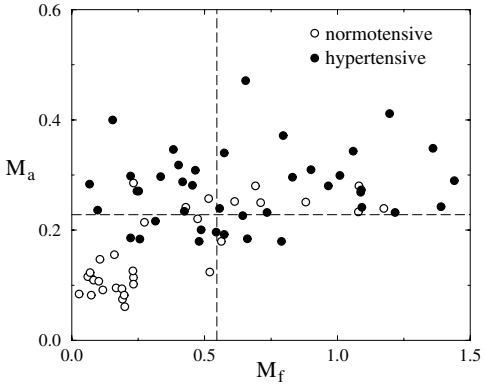

Figure 7. Distribution of depths of frequency and amplitude modulation of the TGF-mediated mode by the VLF-dynamics. Dashed lines represent the average values of the two depths of modulation. Hypertensive rats clearly display the higher amplitude and frequency modulations.

inset in figure 3(a)). This variation can presumably even lead to the disappearance of the VLF-rhythms in the averaged power spectrum for a large group of nephrons. Nevertheless, this dynamics has an effect on the two mechanisms of renal autoregulation, the tubuloglomerular feedback (figure 6) and (as we shall show in the next section) the myogenic response of the afferent arteriole.

The modulation properties are expected to be different for normotensive and hypertensive rats. As illustrated in figure 7, the first group is characterized by lower values of the modulation depth for both, the frequency (M_f) and the amplitude (M_a) modulation. For amplitude modulation, $M_a = \Delta A/A$ where $\Delta A = (A_{\max} - A_{\min})/2$ and A is the mean value. For frequency modulation, $M_f = \Delta\omega/\Omega$, where $\Delta\omega = (\omega_{\max} - \omega_{\min})/2$ and Ω is the mean frequency. For nonstationary processes, $A(t)$ and $\Omega(t)$ are determined via a single-wavelet technique while $\Delta A(t)$ and $\Delta\Omega(t)$ are determined via the double-wavelet technique. Inspection of the figure clearly shows that there is a well-defined distinction between the two strains in the case of the amplitude and the frequency modulation. The mean values of

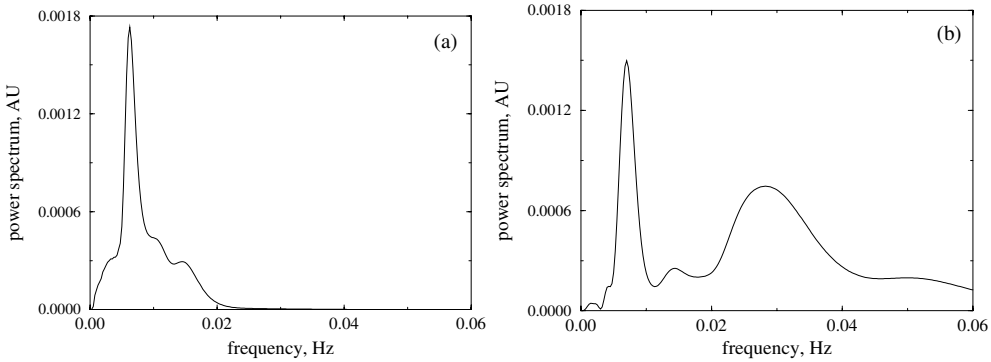

Figure 8. Characteristic power spectra of the modulation signal for the TGF (a) and the myogenic (b) dynamics.

the modulation depth over all data are indicated by the dashed lines in figure 7. Inspection of the figure also reveals that the depth of modulation and, hence, the nonlinear interaction between the involved mechanisms, is stronger for hypertensive than for normotensive rats. The number of nephrons with a frequency modulation that exceeds the average value is higher for hypertensive rats (60%) than for normotensive rats (26%). For the amplitude variations we obtained 71% and 32%, respectively.

6. Modulation properties of myogenic dynamics

Compared with the TGF-mode, the modulation properties of the myogenic dynamics are even more complicated. In a couple of recent papers (Sosnovtseva *et al* 2002) we have discussed the features of mode-to-mode interaction considering the nephron as a bimodal oscillator. However, the HF-dynamics will be influenced simultaneously by several rhythmic components. For the LF-dynamics considered in section 4, the spectrum of the modulation signal was rather simple (see, for instance, figure 8(a), where it contains only a single oscillatory component) and there were no obvious problems in estimating the instantaneous modulating frequency. For the HF-mode, the same spectrum becomes more complicated (figure 8(b)) since both the VLF and the LF dynamics contribute to the modulation of the myogenic mechanism. To detect the influence of each of these modes separately we need to extract the temporal variations of the instantaneous frequencies and amplitudes for these oscillatory components. Such an extraction can be performed by means of double-wavelet technique, and the contribution of each mode to the modulation process can thus be obtained. The double-wavelet method is more effective in the case of nonstationary multimode dynamics than simple estimations of the modulation depth that use, e.g., the minimal and the maximal values of the amplitude of the modulated oscillations. The double-wavelet approach accounts, for instance, for the fact that the estimated characteristics can vary strongly in time.

Figure 9 illustrates the distribution of depths of the frequency and the amplitude modulation of myogenic dynamics by the VLF-mode for hypertensive (filled circles) and normotensive (open circles) rats. In line with the results in the previous section, hypertensive rats are characterized by a strong mode-to-mode interaction. This is reflected in both, the amplitude and the frequency variations of the HF rhythmic components. Here, the relative number of nephrons with an amplitude modulation exceeding the average value is 69% for

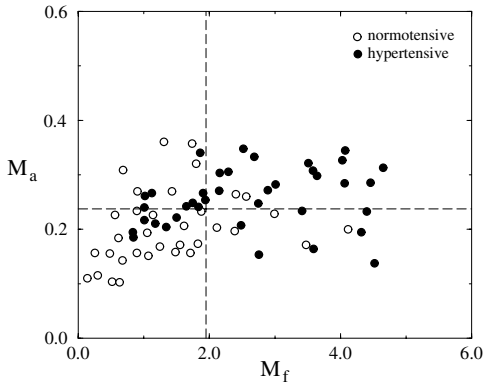

Figure 9. Distribution of depths of frequency and amplitude modulation of the myogenic mode by the VLF-dynamics.

Table 1. Depths of amplitude (M_a) and frequency (M_f) modulation (mean values ± standard deviations).

	M_a		M_f	
	Normotensive	Hypertensive	Normotensive	Hypertensive
LF-mode by VLF	0.16 ± 0.07	0.27 ± 0.07	0.36 ± 0.32	0.68 ± 0.39
HF-mode by VLF	0.19 ± 0.05	0.27 ± 0.06	1.41 ± 0.94	2.52 ± 1.15
HF-mode by LF	0.40 ± 0.14	0.56 ± 0.11	0.77 ± 0.33	1.40 ± 0.41

hypertensive rats and only 21% for normotensive rats. The corresponding results for the frequency modulation are 57% and 24%, respectively.

A study of interaction between the TGF and myogenic mechanisms in the form of the frequency and the amplitude modulation reveals a similar distinction between normotensive and hypertensive rats as in the case of the VLF-dynamics. As shown in our recent work (Sosnovtseva *et al* 2005b), the spontaneously hypertensive rats demonstrate a higher depth of modulation and therefore a stronger interaction between the two mechanisms in renal autoregulation. Table 1 consolidates all the discussed statistics of nephrons for the two rat strains according to their depth of frequency and amplitude modulation estimated from tubular pressure recordings.

Amplitude modulation of the myogenic mode by the TGF-mechanism has previously been discussed by Chon *et al* (1994). The effect of frequency modulation appears to be less well examined both in renal and in other vascular beds. Preliminary studies of this phenomenon have been reported in our recent publications (Sosnovtseva *et al* 2004, Marsh *et al* 2005).

7. Discussion

Interpretation of biological time series is often hampered by the nonstationarity of the available data. In most cases the effect of nonstationarity cannot be fully removed by means of trend correction procedures because of the continuous adjustments that take place as the living system adapts to changing internal or external conditions. Besides, it may be rather difficult

to reveal entrainment phenomena that occur only for short time intervals with the standard time series techniques. Application of standard methods based, e.g., on the Fourier transform to nonstationary data can lead to a number of misinterpretations of the obtained results as a simple consequence of limitations of the statistical tools. Observation of two peaks in the power spectrum of a physiological process, for instance, can correspond to two essentially different situations: the system may display two independent modes, or there is only one mode whose instantaneous frequency changes in time from one value to another.

During the last years, a variety of new tools have become available to the study of nonstationary dynamics of biological systems (Buldyrev *et al* 1993, Peng *et al* 1995, Ivanov *et al* 1999, Stanley *et al* 1999), with wavelet-analysis representing one of the most powerful approaches (Grossman and Morlet 1984, Daubechies 1992, Meyer 1992, Chui 1992, Mallat 1998). In order to study synchronization and modulation phenomena, some extensions of the wavelet analysis are needed. Adison and Watson (2004) recently proposed the so-called 'secondary wavelet feature decoupling method' that considers two computations of the wavelet transform followed one by the other. Independently, a similar idea ('double wavelet analysis') was proposed in our work (Sosnovtseva *et al* 2004) with the aim of characterizing the modulation phenomena in renal autoregulation. This new approach allows us to study mode-to-mode interaction even in the case of fairly rapid changes of the instantaneous frequencies of the rhythmic components. The double-wavelet technique has shown its effectiveness both in analysis of mathematical models of biomedical systems (where it was possible to confirm the obtained results) (Sosnovtseva *et al* 2005b) and in data analysis (Marsh *et al* 2005). In the present paper, the double-wavelet approach was used to study interaction of three modes in the dynamics of individual units of the kidney (the nephrons).

The concrete results we have obtained from the analysis of a total of 74 recordings may be summarized as:

- Normotensive rats display a regular and strong TGF-mediated rhythm, often with clear evidence of nonlinear dynamic phenomena in the form of harmonics and subharmonics.
- Hypertensive rats generally display less pronounced TGF-oscillations. On the other hand, the presence of strong components at very low frequencies is more common in hypertensive rats.
- Modulation of the TGF-rhythm by the very low-frequency modes is relatively weak in normotensive rats and much stronger in hypertensive rats.
- For the myogenic oscillations, the frequency modulation produced by the VLF mode is also much stronger in hypertensive rats than in the normotensive rats.

The obtained results show essential differences in the mode-to-mode interaction between normotensive and hypertensive rats for all the oscillatory components that we can detect in the individual nephron functioning. They are in clear agreement and show that hypertension is associated with an increased strength of interaction between most of the mechanisms of renal regulation. The observed interactions between the VLF-dynamics and the two mechanisms of renal autoregulation are new, and it seems important to reveal their different manifestations between the normal and pathological state.

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