Telemetry for Cardiovascular Monitoring in a Pharmacological Study
New Approaches to Data Analysis

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Abstract—Radio-telemetry systems offer the ability to measure blood pressure and heart rate in experimental models of hypertension without the stress artifacts induced by some other methods. We therefore aimed to develop improved, nonparametric regression methods for radio-telemetry data and to use these to assess the effects of pharmacological interventions on cardiac and vascular hypertrophy in the stroke-prone spontaneously hypertensive rat. One control group and 5 groups treated either with losartan (alone or in combination with \textit{N}-nitro-L-arginine methyl ester [L-NAME]), perindopril (also alone or in combination with L-NAME), or hydralazine plus hydrochlorothiazide were monitored for 4 weeks. Cardiac hypertrophy was assessed by the left ventricle plus septum weight to body weight ratio and vascular hypertrophy by flow-cytometry analysis of vascular smooth muscle cell polyploidy. Hemodynamic series were split into trend and cyclic components by the seasonal and trend decomposition procedure based on Loess and compared between groups by Loess regression modeling. Systolic and diastolic blood pressures were reduced systematically by losartan and perindopril (\(P<10^{-10}\)) but to a lesser extent by hydralazine plus hydrochlorothiazide (\(P<10^{-3}\)), and diurnal variation was reduced in the latter group (\(P<10^{-6}\)). L-NAME significantly reduced the hypotensive effect only of losartan. Vascular and cardiac hypertrophy were significantly attenuated with losartan or perindopril, but were unchanged with other treatments. The new analysis proposed here identifies differential effects on trends and cyclic variation and associations with regression of end-organ damage for losartan and perindopril compared with hydralazine plus hydrochlorothiazide. The method offers a powerful tool for detailed investigation of radio-telemetry data. (Hypertension. 1999;33[part II]:248-255.)

Key Words: telemetry ■ statistics ■ hypertension, genetic ■ hypertrophy, cardiac ■ rats

Monitoring of blood pressure and heart rate in experimental models of hypertension has played an important part in the study of cardiovascular physiology, pharmacology, and more recently in the genetics of hypertension. A wide variety of techniques have been used to obtain these measurements. Anesthesia is the simplest method but it affects normal physiological regulation and thus measurements from conscious animals are preferred. The most common techniques for monitoring conscious animals use tethers or restraining devices with measurements taken via indwelling exteriorized vascular catheters or blood pressure cuffs. This use of restraints and tethers introduces a significant stress artifact with well-documented elevations of plasma catecholamines and cortisol. In addition, the so called noninvasive tail-cuff measurements require both warming and restraint, thus producing several stressful stimuli.

Radio-telemetry provides an alternative means of obtaining blood pressure and heart rate measurements from freely moving conscious animals. It provides the ability to collect data around the clock without the stress from human contact. We have previously used a radio-telemetry system (Dataquest IV, Data Sciences International) to monitor blood pressure and heart rate in genetic crosses designed to map quantitative trait loci for blood pressure regulation. However, these studies used simple means and standard deviations of blood pressure and heart rate variables measured over 96 hours at baseline or after salt-loading. This type of analysis, although very successful for genetic studies, does not fully utilize all the data provided by the radio-telemetry system and provides no information on changes in blood pressure or heart rate during the course of intervention. Previous attempts to work at a finer level of detail have consisted of, for example, investigating circadian rhythms by contrasting mean daytime and nighttime blood pressures or sets of 1-hour averages for a 24-hour period, or they have examined the differences between maximum and minimum pressures in each of several

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24-hour periods as a measure of long-term variability. There may still be a substantial data reduction and consequent loss of information inherent in such approaches. If interest resides in features that are purely cyclic in nature, such as circadian rhythms, then 2 powerful methods exist for expressing telemetry observations in terms of sinusoidal functions. Rhythm or Cosinor analysis decomposes a signal into a sum of cosine functions of period 24 hours or less, with analysis being based on study of the constant, amplitude, and phase parameters of the model. The second method, spectral analysis, uses the Fourier transform to estimate the variance of a signal due to oscillations at each of a range of frequencies.

Currently, there are no available methods of analysis for a radio-telemetry series of blood pressure or heart rate measurements that allow a comprehensive analysis of a number of different features of the data within a common framework. We therefore decided to develop a new method to extract overall trend and cyclic features, to be robust to signal variability and missing data, to allow for autocorrelation within the time series of each experimental unit and to maintain a balance between compression of the data for simplicity and maintenance of the raw data to preserve information.

Our previous pharmacological studies on regression of cardiac and vascular hypertrophy in the stroke-prone spontaneously hypertensive rat (SHRSP) used tail-cuff methods for blood pressure and heart rate monitoring. These studies showed that 3 pharmacological interventions, namely perindopril, losartan, and a combination of hydralazine and hydrochlorothiazide produce comparable hypotensive effects but had differential effects on cardiac and vascular hypertrophy. In view of the deficiencies of the tail-cuff method, it has become essential to repeat these cardiac and vascular hypertrophy regression studies by the use of 4 weeks of continuous radio-telemetry monitoring.

The aims of the current study have been to develop a new nonparametric regression analysis of blood pressure and heart rate telemetry monitoring and to assess the effects of several pharmacological interventions on cardiac and vascular hypertrophy in the SHRSP.

Methods

Experimental Rats

The SHRSP colony in Glasgow was established from breeding pairs obtained from DF Bohr, Department of Physiology, University of Michigan in 1991, as previously described. Six groups of SHRSP were used: control rats (n = 10), rats treated with losartan 40 mg \( \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) (n = 6), rats treated with perindopril 2 mg \( \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) (n = 6), rats treated with a combination of hydralazine and hydrochlorothiazide 4 mg/d each (n = 6, H/H), rats treated with a combination of losartan 40 mg \( \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) and \( N^\circ \)-nitro-L-arginine methyl ester (L-NAME) 20 mg \( \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) (n = 6, L/L), and rats treated with a combination of perindopril 2 mg \( \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) and L-NAME 20 mg \( \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) (n = 6, P/L). Equal numbers of male and female animals were used in each group. All rats were housed under controlled conditions of temperature (21°C) and light (12-hour light/dark cycle, 7 AM to 7 PM) and were maintained on normal rat chow (rat and mouse No. 1 maintenance diet, Special Diet Services) and water ad libitum. At the end of the 4-week treatment period, the animals were killed by halothane overdose and tissues were removed as described in the following sections. The experiments were approved by the Home Office of Her Majesty’s Government according to regulations regarding experiments with animals in the United Kingdom. These regulations meet all the requirements of the American Physiological Society.

Radio-Telemetry Monitoring of Blood Pressure and Heart Rate

The Dataquest IV telemetry system (Data Sciences International) was used for measurement of systolic pressure, diastolic pressure, mean arterial pressure, heart rate, and motor activity, as previously described. Briefly, the monitoring system consists of a transmitter (radio frequency transducer model TA11PA), receiver panel, consolidation matrix, and personal computer with accompanying software. Before the device was implanted, calibrations were verified to be accurate within ±3 mm Hg. Rats at 15 weeks of age were anesthetized with halothane, and the flexible catheter of the transmitter was surgically secured in the abdominal aorta just below the renal arteries and pointing upstream (against the flow). The transmitter was sutured to the abdominal wall. Rats were housed in individual cages after the operation. Each cage was placed over the receiver panel that was connected to the personal computer for data acquisition. The rats were unrestrained and free to move within their cages. Hemodynamic data were sampled every 5 minutes for 10 seconds. Preliminary experiments showed that blood pressure and heart rate took up to 7 days to stabilize postoperatively. Therefore, each drug treatment was commenced 7 days after surgery and telemetry data were collected for 28 days on treatment.

Evaluation of Cardiac Hypertrophy

Immediately after exsanguination, the thorax was opened and the heart was removed, blotted with tissue paper, and weighed. The atria and right ventricle were then removed and the left ventricle and septum were weighed. Heart weight to body weight (HW BW) and left ventricle plus septum weight to body weight (LV+S BW) ratios were then determined.

Preparation of Aortic Vascular Smooth Muscle Cell Nuclei and Flow Cytometric Analysis

The thoracic aorta was carefully dissected and vascular smooth muscle cells (VSMC) were prepared by enzymatic dissociation as previously described. Human peripheral blood lymphocytes were stained in parallel to provide a diploid profile for DNA peak standardization. The DNA flow cytometry was carried out by the use of a FACScan bench-top analyzer (Becton-Dickinson) with a 15-mW argon air-cooled laser with emission wavelength of 488 nm. Analysis of the DNA profiles obtained was carried out by the use of the LYSYS software package (Becton-Dickinson).

Statistical Analysis

Hemodynamic data were collated and analyzed with the statistical software system S-Plus 4.5 Professional (MathSoft, Inc). The measurements from the final 7 days of 1 male animal receiving the combination of perindopril and L-NAME were judged to be unreliable because of a fault with the implanted transmitter and were therefore coded as missing.

By applying the seasonal and trend decomposition procedure based on Loess (STL), each hemodynamic series was expressed as a sum of components representing overall trend, cyclic behavior with a 24-hour period, and residual variability. The STL algorithm proceeds by iterating between 2 steps. In stage 1, each animal’s data are split into 288 subseries, 1 for each individual time point recorded in 24 hours of readings at 5-minute intervals. Each subseries is then smoothed individually before being recombined into a single (stationary) estimate of cyclic behavior. In the second stage, the whole hemodynamic series is smoothed by the use of a large bandwidth to reveal broadband, nonstationary features. After convergence, the
trend and cyclic components are subtracted from the original series to obtain the residual component.

In both steps, the smoothing technique used was that of Loess, which is a robust, nonparametric local regression method. It is based on fitting quadratic models by weighted least squares, assuming only a symmetric error structure, within neighborhoods of prespecified sizes. Points are weighted inversely with distance from the center of the neighborhood, the size of which (known as the span or bandwidth) is a parameter under user control. Bandwidths for each step of the STL algorithm were chosen by eye to give acceptably smooth estimates, with the same value being used across all animals' series.

Mean profiles (for either trend or periodic behavior) were obtained for each treatment group by Loess with the use of bandwidths equivalent to 5% of the range of the data, and then compared by an approximate likelihood ratio test. The effective numbers of parameters for comparison of 2 groups in terms of their respective trend components were 59.6 and 119.2, whereas for periodic behavior they were 29.8 and 59.6. Probability values for very large F-statistics were approximated to an order of magnitude, but to allow for the influence of multiple testing, raw probability values were increased by a factor of 15 (the number of possible pair-wise comparisons for each phenotype).

In the comparisons between treatment groups of heart to body weight ratios and VSMC polyploidy the sample sizes involved were relatively small, and there was some evidence of variance heterogeneity. Accordingly, pair-wise differences between groups were estimated by Bonferroni-corrected Mann-Whitney CIs (significance levels of 0.003) after first screening with Kruskall-Wallis tests.

Results

The smoothed trend components for systolic blood pressure (SBP) for the 6 groups are displayed in Figure 1A. As would be expected, there was a marked hypotensive effect of losartan and perindopril; there was a pronounced drop in SBP of 50 mm Hg with respect to the control group over the first 12 days of treatment, followed by a relatively modest decrease of around 10 mm Hg over the remainder of the treatment period (control versus losartan, $F_{5,3455} = 10^{10}$). The decrease in SBP was slightly greater for perindopril (versus losartan, $F_{80,5} = 10^{-6}$). Under treatment with H/H, the initial fall in SBP was of a similar magnitude, but systolic pressure was then maintained at the same level during the rest of the experiment so that it was 20 mm Hg higher than with losartan ($F_{447,5} = 10^{-8}$) and perindopril ($F_{749,5} = 10^{-8}$) when telemetry recording was completed. The
addition of L-NAME to perindopril decreased the hypotensive effect of the latter by a small but significant amount ($F = 245, P < 10^{-5}$), whereas when added to losartan, L-NAME almost reversed the reduction in SBP ($F = 3710, P < 10^{-10}$).

Figure 1B displays estimates of the average periodic behavior over 24 hours of SBP for the 6 groups. These curves may be regarded as a set of correction factors (on the original scale of measurement) for different times of day that would be added to the trend function to recover an estimate of SBP for a specific point in time. The average magnitude of the effect of 24-hour periodicity was between 2 and 15 mm Hg. All 5 active treatment groups showed significant differences to control (perindopril, $F = 41$; losartan, $F = 40$; H/H, $F = 86$; P/L, $F = 61$; L/L, $F = 46$; all $P < 10^{-5}$), although the actual sizes of the differences were only on the order of 2 to 3 mm Hg for a given time of day. The animals receiving H/H showed the most alteration in periodic changes in SBP, with reduced amplitude of variability throughout the 24-hour cycle.

Qualitatively, diastolic blood pressure (DBP) behaved in a similar fashion to SBP. The losartan and perindopril treatments had similar hypotensive effects (versus control, $F = 5142$ and $F = 6766$, respectively; both $P < 10^{-10}$) of up to 40 mm Hg (Figure 2A). As before, the H/H group displayed a noticeable reduction (versus control, $F = 81, P < 10^{-7}$) in amplitude of 24-hour variation (Figure 2B).

Heart rates in the L/L group were lower than control ($F = 161, P < 10^{-5}$), by between 10 and 20 beats per minute (Figure 3A). Heart rates in the other treatment groups were higher than control, by the same margin (perindopril, $F = 309, P < 10^{-5}$; losartan, $F = 73, P < 10^{-6}$; H/H, $F = 237, P < 10^{-8}$; P/L, $F = 182, P < 10^{-8}$). In other respects, heart rates remained relatively unchanged over the duration of treatment. In terms of cyclic behavior, shown in Figure 3B, the 6 groups displayed patterns consisting of a period of relatively stable, positive periodic indices corresponding to the animals’ period of wakefulness during the night, followed by a period of stable, negative indices during the daytime, sleep period.
Again, all active treatment groups were significantly different to control (perindopril, $F=28, P<10^{-6}$; losartan, $F=119, P<10^{-6}$; H/H, $F=59, P<10^{-6}$; P/L, $F=55, P<10^{-6}$; and L/L, $F=126, P<10^{-6}$).

As demonstrated in Figure 4A, cardiac hypertrophy, as measured by LV+S:BW ratio (mg/g), was significantly reduced with respect to control in the animals that received perindopril (99.7% CI for group 2 control medians: $-1.13, -0.45$) or losartan (99.7% CI $-0.87, -0.39$). There was no reduction for the H/H group (99.7% CI $-0.52, 0.07$), for P/L (99.7% CI $-0.97, 0.73$) or for L/L (99.7% CI $-0.50, 0.74$) with respect to control. A similar finding was obtained for HW:BW (data not shown). In addition, the same treatments were associated with significant reductions of VSMC polyploidy (percentage, Figure 4B) in comparison to the control group (99.7% CI for perindopril–control $-15.6, -0.1$; and losartan $-14.4, -2.0$). Once again, there was no difference compared to control with H/H (99.7% CI $-10.5, 9.6$) or the 2 L-NAME groups (P/L $-13.9, 2.1$; L/L $-10.4, 0.8$).

Discussion

The STL decomposition and Loess regression method presented in this study is a novel graphical and inferential tool for the analysis of radio-telemetry monitoring data that draws on modern statistical modeling techniques that have only recently begun to find applications in the biological and biomedical sciences. It offers a number of advantages to previous approaches for the type of investigation described here. The STL method considers hemodynamic measurements as time series, rather than unrelated observations, thus allowing for autocorrelation between successive time points, whereas approaches based on comparison of summary measures or time-by-time analysis of variance do not. In the context of pharmacological intervention, many hypotheses of interest relate to trends in blood pressure or heart rate in the first instance, and the STL method allows model fitting and testing of such hypotheses in a particularly intuitive way, analogous to a standard analysis of covariance.

Cosinor or spectral methods are designed to identify the major frequency components of oscillatory behavior, and
are therefore extremely powerful for studies in which this is the main feature of interest. Lemmer et al\textsuperscript{11} used the former method to demonstrate differences between the circadian rhythms of Wistar-Kyoto (WKY), Sprague-Dawley, spontaneously hypertensive (SHR) and TGR[mRen-2]\textsuperscript{27} transgenic rats. The Cosinor model contains a rhythm-adjusted mean parameter that may be compared between groups, but this is insufficient to reveal anything other than overall differences in average hemodynamic measurements. Because the animals were not under drug treatment, systematic changes in pressures would not necessarily be expected, but the authors did use an ANOVA of 30-minute averages over the 4 day experimental period to show time-dependent variation in blood pressures and heart rate. Clearly, this approach would confound diurnal variation and systematic trends if applied to the current study. Calhoun et al\textsuperscript{20} also used Cosinor analysis, to examine the effects of basal and high salt diets on diurnal variation in WKY and SHR rats. However, because treatment regimens were involved, trends over the duration of the experiment were also of interest. The authors attempted to investigate this question by comparing initial and final hemodynamic measurements and ANOVA of 24-hour average measurements. The latter approach, in particular, removed much of the confounding effect mentioned above, but in doing so it discarded a substantial proportion of the information contained in the data and made no allowance for autocorrelation effects.

The STL method provides a solution for the problems identified above while also allowing periodic behavior to be modeled. The current study revealed a significant alteration in the pattern of diurnal variation for animals treated with the combination of H/H. In addition, because the STL/Loess

Figure 4. Cardiac and vascular hypertrophy for control animals and 5 treatment groups. A, Ratio of left ventricle plus septum weight to body weight, expressed in mg/g. Mann-Whitney 99.7% CI for group-control median differences were perindopril (−1.13, −0.45), losartan (−0.87, −0.39), H/H (−0.52, 0.07), P/L (−0.97, 0.73), and L/L (−0.50, 0.74). B, VSMC polyploidy, expressed as a percentage of cells in G\textsubscript{2}+M phase. Mann-Whitney intervals, as above, were perindopril (−15.6, −0.1), losartan (−14.4, −2.0), H/H (−10.5, 9.6), P/L (−13.9, 2.1), and L/L (−10.4, 0.8). Data displayed as mean±SE.
method is based on a nonparametric smoothing technique, it offers robustness to outlying observations and deals conveniently with missing data.

Previous studies from our laboratory and from other groups13–15,21 demonstrated that pharmacological interventions that block the renin-angiotensin system result in regression of cardiac and vascular hypertrophy. Moreover, the same studies showed that hypotensive treatments that resulted in a stimulation of the renin-angiotensin system frequently failed to cause regression of end-organ damage despite an equivalent blood-pressure–lowering effect. These data led to a hypothesis that angiotensin II plays a major role in cardiac and vascular hypertrophy, particularly in the aortic smooth muscle polyploidy observed in several experimental models of genetic and secondary hypertension.15,16,21,22 However, all the previous experimental studies used tail-cuff measurements for blood pressure monitoring. The current study is the first to use continuous radio-telemetry monitoring to verify these conclusions. In contrast to the previous tail-cuff blood pressure results, we have now demonstrated that the hypotensive effects of a combination of H/H were significantly different than those of perindopril or losartan. These differences are present for systolic and diastolic pressures, both for the smoothed trend components and for periodic behavior. It appears that the H/H regimen resulted in a similar initial fall of blood pressure to that observed with perindopril or losartan over the first 5 to 7 days of treatment. However, there was no further fall of blood pressure during the remaining 3 weeks while on the hydralazine-based regimen. Similarly, the rats receiving H/H showed the largest alterations in periodic blood pressure behavior as compared with the control group. This resulted in reduced amplitude of blood pressure variability with lower nighttime readings and higher daytime readings than in controls and in the other treatment groups.

Is it conceivable that these differences in blood pressure trends and circadian patterns could explain the magnitude of regression of the end-organ damage on various pharmacological treatments? The hemodynamic changes described above result in a considerably increased blood pressure load over time and an insufficient blood pressure dip during the day that might be comparable to the phenomenon of “nondipping” described in human hypertension and might be accompanied by more severe end-organ damage.23 One could conclude that hemodynamic changes alone or in conjunction with the measurable blockade of the renin-angiotensin system may explain the significant regression of cardiac and vascular hypertrophy in the SHRSP treated with perindopril or losartan and the lack of similar effect on treatment with H/H.

We also wished to assess the relative contributions of the renin-angiotensin system and/or the L-arginine/NO pathway to the beneficial effects of the angiotensin-converting enzyme (ACE) inhibitor and AT1 receptor antagonist in our model. We therefore challenged the beneficial effects of perindopril or losartan with L-NAME and found, in both cases, a reversal of their protective effects on vascular and cardiac hypertrophy. This is in line with our previous study that demonstrated that treatment of WKY rats with L-NAME induced hypertension, VSMC polyploidy, cardiomyocyte hypertrophy, and increased plasma renin activity.16 The effects of L-NAME might be because of the hemodynamic effects of blood pressure increase, but one would expect differential results with the combination of losartan and L-NAME compared with perindopril and L-NAME because the achieved blood pressures were significantly different. It is possible that a higher dose of losartan may have been as effective as perindopril in controlling blood pressure in the presence of L-NAME, since previous workers used 60 mg · kg⁻¹ · d⁻¹ losartan to reduce blood pressure in the SHR.24 Conversely, despite effective blood pressure control in the presence of L-NAME, the ACE inhibitor perindopril failed to prevent vascular and cardiac hypertrophy in the present study. Thus, the change in systolic arterial pressure per se was not entirely related to the hypertrophy observed in our experimental model. However, a previous study by Takemoto et al25 has also shown a divergence between systolic arterial pressure and coronary vascular remodeling in response to different doses of the AT1 subtype receptor antagonist CS-866 in L-NAME treated WKY rats. In addition, a study conducted by the same group26 showed that the aortic wall to lumen ratio was blunted in L-NAME–treated WKY, but only after 8 weeks of antihypertensive treatment, in contrast to the present study, which was of 4 weeks duration.

In the present study, we have shown relative differences in the role or contribution of NO to the antihypertensive actions of losartan and perindopril. The underlying mechanisms of action were studied by Michel et al,27 who found that ACE inhibition in L-NAME hypertension does not affect arterial wall cGMP, but does reverse the hypertension and prevents associated vascular damage. In addition, recent literature has suggested the existence of a link between angiotensin II (Ang II) and the kinin/NO system in various organs including the blood vessel wall, possibly mediated by the AT2 receptor.28,29 Indeed, Gohlke et al30 have recently reported that activation of AT2 receptors due to enhanced endogenous Ang II in response to losartan causes a bradycin-independant stimulation of aortic NO release with subsequent generation of cGMP, which is important in the mechanism of action of losartan. In several separate studies at the cellular level,31,32 counter-regulatory interactions have been demonstrated between Ang II and NO. It has recently been reported that NO can directly mediate down-regulation of AT1 receptor expression in VSMC from SHR,32 thus demonstrating a further direct mechanism whereby NO mediates antiproliferation in vascular tissue.

Such cellular mechanisms described above have to remain hypothetical at present. However, a firm conclusion can be reached regarding significant differences in achieved blood pressures between rats treated with H/H compared with those treated with losartan or perindopril. This is because of the STL/Loess method, which is utilized here for the first time. This new method of data analysis allowed for the assessment of trends and periodicity of direct intra-arterial blood pressure measurements and for accurate prediction of regression of end-organ damage in genetic hypertension.

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