Circadian Blood Pressure Variation in Transgenic Hypertensive Rats

Björn Lemmer, Andreas Mattes, Manfred Böhm, Detlev Ganten

Automatic, around-the-clock blood pressure measurements have increased our understanding of hypertension in humans. Patients with essential hypertension display patterns similar to those observed in normotensive subjects, whereas those with secondary hypertension frequently show abnormal circadian rhythms characterized by a failure to reduce blood pressure at night. We have modeled this situation in rats. Normotensive Wistar-Kyoto and Sprague-Dawley rats, spontaneously hypertensive rats, and rats made hypertensive by transgenic implantation of the mouse salivary gland renin gene (TGR[mRen-2]27) underwent chronic implantation of a device that telemetrically monitored their blood pressures, heart rates, and motor activities. In either normotensive or hypertensive rats, motor activity peaked during the dark phase, indicating that animals from all strains were nocturnal. In both normotensive and spontaneously hypertensive rats, the 24-hour blood pressure and heart rate profiles showed peak values during the rats' active phase at night, ie, between midnight and 3 AM. In the transgenic rats, on the other hand, blood pressure values were at maximum during the day around noon, when the rats were in their resting phase. The heart rate of the transgenic rats nevertheless still peaked around midnight. These data suggest that normotensive rats and those with primary and secondary hypertension display circadian rhythms of blood pressure and heart rate analogous to those observed in normotensive and primary or secondary hypertensive humans, respectively. The TGR(mRen-2)27 strain may be a useful model with which to investigate the mechanisms responsible for alterations in circadian rhythms of blood pressure and heart rate in forms of secondary hypertension. (Hypertension 1993;22:97-101)

KEY WORDS • blood pressure • heart rate • circadian rhythms • telemetry • rats, inbred SHR • animals, transgenic

Ambulatory 24-hour blood pressure measurements show promise in supplanting casual readings in the diagnosis of arterial hypertension. In secondary forms of hypertension, the normal pattern of circadian variation is frequently disturbed, in that the nocturnal decrease in blood pressure fails to occur.1,3 Circadian changes in heart rate (HR), on the other hand, remain unperturbed. In Wistar-Kyoto (WKY) control rats and spontaneously hypertensive rats (SHR) initially developed from WKY rats by Okamoto and Aoki,4 the circadian rhythms in blood pressure and HR exhibit peak values during the rats' activity phase,5,6 similar to the situation in normotensive and essentially hypertensive humans.1,2 Recently, a novel model of hypertension (TGR[mRen-2]27) was developed by the introduction of the mouse Ren-2 salivary gland renin gene into the genome of the rat.7 However, no data were available as to whether these transgenic rats exhibited a similar circadian profile in blood pressure and HR as described for SHR. New telemetric methods permit the monitoring of blood pressure and HR in awake, freely moving animals on a continuous basis. We applied such techniques to test the hypothesis that circadian blood pressure variation differs in hypertensive rats of the SHR and TGR[mRen-2]27 strains.

Methods

Rat Strains and Environmental Conditions

Male WKY rats (strain, WKYCrIrBR) and SHR (strain, SHRNCrIrBR) were obtained from Charles Rivers Wiga, Sulzfeld, Germany, and Sprague-Dawley rats (SDR) (strain, HAN:SPRD) were from the Central Institute for Laboratory Animal Breeding, Hannover, Germany. The TGR(mRen-2)27 rats were developed originally in Heidelberg, Germany, by methods described in detail elsewhere.2 They were bred from WKY rats and SDR, then further breeding was done only with the SDR strain. All rats were synchronized to a 12-hour cycle of light (7 AM to 7 PM, 200 lux) alternating with darkness (7 PM to 7 AM, <0.1 lux). Standard chow (Hope Farms, the Netherlands) and tap water were allowed ad libitum; room temperature was maintained at 23°C.

Telemetry and Data Acquisition

The Dataquest III or IV system (Data Sciences Inc, St Paul, Minn) was used to measure telemetrically systolic blood pressure (SBP), diastolic blood pressure (DBP), and HR.6 The system measures the motility of the animals as well. The monitoring system consists of the transmitter (model TA11PA), the receiver panel...
Data Analyses

For analysis of rhythmicity, mean values were calculated for 30-minute intervals for each group of rats. The data were analyzed according to two strategies. First, an analysis of variance was performed. Second, a rhythm analysis was applied by means of the nonlinear least-squares fitting program PHARMFIT.²² Twenty-four-hour cosine and all partial Fourier curves including up to the fifth harmonic (4.8-hour period) were fitted as models to the data.²² The following equation was used: y = MESOR + Σ[amplitude(i) × cos[(x - acrophase(i)) × 2π/period(i)]], with i being the number of overlapping cosine functions. The program calculates estimates of the MESOR (midline estimating statistic of rhythm, ie, the rhythm-adjusted 24-hour mean), the amplitude (half of peak to trough of rhythmic change), and the acrophase (peak time of each component cosine function) of the harmonics together with the percentage of rhythm. The F test was used to test for zero amplitude.²²

Model comparison statistics over all fit models was done with the PHARMFIT subprogram SYNOPS²² according to Wald²⁵ as described in detail by McIntosh and McIntosh.²⁶ Briefly, likelihood was calculated for each fit model and transformed into a confidence value. Among the models with a confidence of at least 0.05, the mode with the smallest number of harmonics was regarded as “best fit.” To further characterize the rhythms, we calculated the absolute peak and trough values and the transition times through the MESOR and respective slope values from the best-fitting partial Fourier curves.²²

Results

Highly significant (P<.001 to .0001) time-dependent variations in SBP and DBP as well as HR were identified by analysis of variance in each of the four rat strains. Nonlinear rhythm analysis also revealed highly significant (P<.0001) daily variations of each variable in each strain. The 24-hour profiles in blood pressure showed a “fingerprint” type of strain-dependent pattern; the 24-hour profiles in HR, on the other hand, were fairly similar among the strains (Figs 1A to ID). Reproducibility of the strain-dependent rhythm patterns is documented in Fig 1, which shows telemetric data collected over a 4-day period. The results of the rhythm analyses are compiled in Table 1. For all strains and all parameters, the 24-hour period was the dominant one. A further significant improvement of fit (in general, 8% to 16%) in each parameter was obtained by including additional harmonics in the fitting procedure (Table 1). Most of the variability in HR (74% to 86%) was described by the 24-hour frequency. Of the additional harmonics, the 8-hour frequency was the most important for HR rhythms in all rat strains. Percentage of rhythm of the dominant 24-hour frequency was less for SBP and DBP (43% to 75%) in each strain. Whereas in WKY rats, SDR, and SHR the 8-hour frequency was the second in amplitude, in the transgenic strain the fourth harmonic (6-hour) took the second rank. Phase position of the acrophases and degree of amplitudes of all harmonics included determine the final pattern of best fits as shown in Fig 1.

The normotensive WKY strain was characterized by a rhythm-adjusted 24-hour mean (MESOR) in SBP, DBP of 115±0.3/79±0.2 mm Hg and in HR of 310±1.1 beats per minute (Table 1). The second normotensive SDR strain had slightly higher mean values, with a MESOR in SBP, DBP of 132±0.29/±0.2 mm Hg and in HR of 331±0.8 beats per minute. The mean HR in SHR (307±0.6 beats per minute) was similar to that in WKY rats, whereas the blood pressures of these animals were greater (176±0.3/123±0.2 mm Hg). In the TGR(mRen-2)27 animals, the mean 24-hour blood pressure values were 189±0.3/140±0.3 mm Hg, and their HR values (324±0.9 beats per minute) were similar to those in SDR (Table 1). Acrophases of the dominant 24-hour period in SBP and DBP occurred between 1:15 AM and 3:00 AM in WKY rats, SDR, and SHR, and acrophases in HR rhythms were shortly after midnight (Table 1). On the other hand, in TGR(mRen-2)27 rats, the acrophases for the dominant 24-hour period in SBP and DBP were identified in the resting phase (10:55 AM and 11:54 AM, respectively), whereas the respective acrophase in HR was still in the dark phase (11:34 PM) as found in the other rat strains (Table 1).

Table 2 gives the transition times through the MESOR and the respective slope values of the best-fitting partial Fourier curves²² for SBP, DBP, and HR. These data clearly demonstrate that in WKY rats, SDR, and SHR, transition from nightly peak to daily trough, ie, negative slope values of best fit in SBP, DBP, and in HR, is observed around
FIG 1. Plots show 24-hour rhythms in blood pressure (BP) and heart rate (HR) in male normotensive Wistar-Kyoto (WKY) rats (panel A), normotensive Sprague-Dawley rats (SDR) (panel B), spontaneously hypertensive rats (SHR) (panel C), and hypertensive transgenic TGR(mRen-2)27 rats (TGR) (panel D) as monitored by telemetry. Telemetric data on systolic and diastolic BP and HR were registered 4 days in sequence. Shown are mean values±SEM of 30-minute intervals of 8 WKY rats, 10 SDR, 11 SHR, and 9 TGR rats of each strain; best nonlinear fit by a partial Fourier analysis (Table 1) is given by the solid line.
TABLE 1. Blood Pressure and Heart Rate Rhythms in Two Normotensive Rat Strains and Spontaneously Hypertensive and Hypertensive Transgenic Rats

<table>
<thead>
<tr>
<th>Strain/Condition</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>HR (bpm)</th>
<th>MESOR (SBP)</th>
<th>MESOR (DBP)</th>
<th>MESOR (HR)</th>
<th>24-Hour component</th>
<th>Amplitude (SBP)</th>
<th>Amplitude (DBP)</th>
<th>Acrophase</th>
<th>Rhythm (%)</th>
<th>Rhythm (best fit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar-Kyoto normotensive</td>
<td>115.6±0.3</td>
<td>79.4±0.2</td>
<td>309.9±1.1</td>
<td>2.08±0.3</td>
<td>1.35±0.2</td>
<td>0.45±0.9</td>
<td>57.5</td>
<td>55.7</td>
<td>74.9</td>
<td>68.0*</td>
<td>83.2‡</td>
<td></td>
</tr>
<tr>
<td>Sprague-Dawley normotensive</td>
<td>131.7±0.2</td>
<td>90.7±0.2</td>
<td>331.0±0.8</td>
<td>6.8±0.3</td>
<td>5.5±0.2</td>
<td>43.8±1.6</td>
<td>71.2</td>
<td>75.1</td>
<td>82.3</td>
<td>80.6*</td>
<td>84.4*</td>
<td></td>
</tr>
<tr>
<td>Spontaneously hypertensive</td>
<td>175.6±0.3</td>
<td>122.5±0.2</td>
<td>306.7±0.6</td>
<td>6.1±0.4</td>
<td>4.0±0.3</td>
<td>45.9±1.1</td>
<td>49.5</td>
<td>42.7</td>
<td>85.7</td>
<td>66.1§</td>
<td>63.9§</td>
<td></td>
</tr>
<tr>
<td>TGR(mRen-2)27 transgenic</td>
<td>188.5±0.3</td>
<td>8.0±0.4</td>
<td>324.3±0.9</td>
<td>4.29</td>
<td>3.67</td>
<td>31.77</td>
<td>65.8</td>
<td>71.5</td>
<td>74.2</td>
<td>80.6§</td>
<td>88.8§</td>
<td></td>
</tr>
</tbody>
</table>

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were monitored by telemetry for 4 consecutive days. A partial Fourier curve, consisting of 24+8+6+4.8-hour components (*), 24+8+4.8-hour components (†), 24+12+8+4.8-hour components (‡), and 24+12+8+6+4.8-hour components (§), was fitted to the data. Significance of improvement of fit by adding additional harmonics to the dominant 24-hour period (see text) was tested by multiple model comparison. Shown are rhythm-adjusted mean (MESOR), amplitude, acrophase (peak time of rhythm), and percentage of rhythm of the dominant 24-hour period together with improvement of fit by adding the harmonics. Values are mean±SD.

TABLE 2. Transition Times Through MESOR and Respective Slope Values as Calculated From Best-Fitting Partial Fourier Curves of Rhythms in Systolic and Diastolic Blood Pressures and Heart Rate in Four Rat Strains

<table>
<thead>
<tr>
<th>Curve</th>
<th>Time of transition through MESOR</th>
<th>Slope at transition time (mm Hg/h, bpm/h)</th>
<th>Time of transition through MESOR</th>
<th>Slope at transition time (mm Hg/h, bpm/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar-Kyoto normotensive rat</td>
<td>7:36 PM</td>
<td>4.29</td>
<td>7:24 AM</td>
<td>-5.57</td>
</tr>
<tr>
<td>DBP</td>
<td>7:12 PM</td>
<td>3.67</td>
<td>7:12 AM</td>
<td>-3.67</td>
</tr>
<tr>
<td>HR</td>
<td>7:15 PM</td>
<td>31.77</td>
<td>6:54 AM</td>
<td>-26.41</td>
</tr>
<tr>
<td>Sprague-Dawley normotensive rat</td>
<td>7:22 PM</td>
<td>4.09</td>
<td>7:29 AM</td>
<td>-5.46</td>
</tr>
<tr>
<td>DBP</td>
<td>7:05 PM</td>
<td>3.08</td>
<td>7:13 AM</td>
<td>-4.59</td>
</tr>
<tr>
<td>HR</td>
<td>6:37 PM</td>
<td>26.19</td>
<td>7:04 AM</td>
<td>-32.25</td>
</tr>
<tr>
<td>Spontaneously hypertensive rat</td>
<td>8:01 PM</td>
<td>1.66</td>
<td>7:26 AM</td>
<td>-6.46</td>
</tr>
<tr>
<td>DBP</td>
<td>7:06 PM</td>
<td>2.97</td>
<td>7:02 AM</td>
<td>-5.74</td>
</tr>
<tr>
<td>HR</td>
<td>6:39 PM</td>
<td>25.52</td>
<td>6:45 AM</td>
<td>-29.21</td>
</tr>
<tr>
<td>TGR(mRen-2)27 transgenic hypertensive rat</td>
<td>7:56 AM</td>
<td>6.19</td>
<td>4:39 PM</td>
<td>-3.24</td>
</tr>
<tr>
<td>DBP</td>
<td>8:00 AM</td>
<td>4.64</td>
<td>6:01 PM</td>
<td>-0.66</td>
</tr>
<tr>
<td>HR</td>
<td>5:52 PM</td>
<td>28.38</td>
<td>6:23 AM</td>
<td>-34.40</td>
</tr>
</tbody>
</table>

MESOR, midline estimating statistic of rhythm, ie, rhythm-adjusted 24-hour mean; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. Transition times were calculated from peak to trough and vice versa as indicated by negative or positive slope factors. Best fits are as given in Table 1.
Because the parental rats of the transgenic animals, WKY rats and SDR, did not show such a dissociation in blood pressure and HR, the phase shift in the blood pressure rhythms in TGR(mRen-2)27 rats must be attributed to the introduction of the mouse Ren-2 salivary gland renin gene into the genome.

Thus, the TGR(mRen-2)27 rats show a dissociation of circadian blood pressure and HR patterns, which is similar to the situation described in secondary forms of hypertension in humans. The midday blood pressure values we observed in TGR(mRen-2)27 rats are in agreement with casual tail-cuff measurements, which are generally obtained in midmorning. A detailed study of hormonal diurnal patterns has not been performed in these rats. Furthermore, whether or not an altered circadian rhythm in sympathetic discharge occurs in the transgenic rats is not known. Additional attention to these variables may shed light on the mechanisms responsible for the altered circadian profile in blood pressure of TGR(mRen-2)27 rats. The fact that the circadian rhythm of blood pressure in TGR(mRen-2)27 rats was out of phase with the animals’ activity indicates that motor function has little effect on the diurnal variation in blood pressure.

In conclusion, our data show that the introduction of the mouse Ren-2 gene into the rat not only resulted in severe hypertension but also greatly changed the usual circadian pattern of blood pressure profile, with peak values during the dark phase. The SHR, on the other hand, exhibited a pattern not different from normotensive rats. We believe that our observation provides a model of secondary hypertension with respect to circadian blood pressure variation. The cause for this rhythm dissociation in blood pressure to HR is not known at present either for human secondary hypertension or for these transgenic rats. However, the fact that the introduction of a second renin gene resulted in that pattern may provide a clue with respect to mechanisms responsible for the phenomenon.

FIG 2. Plots show 24-hour patterns in motor activity in male normotensive (Wistar-Kyoto [WKY]) rat and Sprague-Dawley rat [SDR]), essentially hypertensive (spontaneously hypertensive rat [SHR]), and transgenic hypertensive rat (TGR) strains as monitored by telemetry. Movements were collected for 5-minute intervals and averaged over 30 minutes. Shown are mean values of 8 to 11 rats monitored for 4 days continuously to demonstrate nocturnal activity in any rat strain.

Interestingly, the motility patterns displayed the expected circadian rhythms for all strains; i.e., highest activity was observed during the dark phase (Fig 2).

Discussion
The important findings in this study were that the circadian rhythms in blood pressure and HR of WKY rats, SDR, and SHR but not of TGR(mRen-2)27 rats mirrored the rats’ activity patterns, in that peak values were observed in the rats’ activity phase. The circadian patterns in blood pressure and HR of WKY rats, SDR, and SHR are similar to patterns observed in normotensive and essentially hypertensive humans. These observations support the notion that elevated blood pressure in SHR closely resembles essential hypertension in humans. The circadian rhythm of blood pressure profile, with peak values during the dark phase, the SHR, on the other hand, exhibited a pattern not different from normotensive rats. We believe that our observation provides a model of secondary hypertension with respect to circadian blood pressure variation. The cause for this rhythm dissociation in blood pressure to HR is not known at present either for human secondary hypertension or for these transgenic rats. However, the fact that the introduction of a second renin gene resulted in that pattern may provide a clue with respect to mechanisms responsible for the phenomenon.

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Hypertension. 1993;22:97-101
doi: 10.1161/01.HYP.22.1.97

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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