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, WKYCrI BR) and SHR (strain, SHRNCrI BR) 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.7 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.8 The system measures the motility of the animals as well. The monitoring system consists of the transmitter (model TA11PA), the receiver panel...
(model BPR86), a consolidation matrix (BCM-100), a
computer, and accompanying software (DATAQUEST IV
program). The receiver is a flat panel, which is placed
underneath the animal's cage. In that way, the animal with
its implanted transmitter is allowed to move freely and
undisturbed while blood pressure, HR, and activity are
monitored.

Rats weighing 160 to 200 g were anesthetized with
enflurane (Abbott, FRG). The catheter of the sensor
was inserted into the abdominal aorta below the bifur-
cation of the renal arteries, and the transmitter itself
was attached with a suture to the peritoneum. The
animals were housed singly in plastic cages, each of
which was situated above a receiver panel. Eight WKY
rats, 10 SDR, 9 heterozygote TGR(mRen-2)27 rats, and
11 SHR were studied. Three weeks after insertion of the
transmitters, the experiments were begun, and data
were collected for 4 consecutive days.

The following data-sampling procedure was applied:
Blood pressure of each rat was monitored every 5 minutes
as a waveform curve for 10 seconds. Peaks and troughs in
the blood pressure curve were detected, and SBP, DBP, and
HR sample values were calculated by the DATAQUEST
software. Motility was monitored as changes in transmitter
signal strength due to transmitter locomotion and was
collected for each 5-minute interval. For further evalua-
tion, mean values were calculated for intervals of 30
minutes, ie, from six samples each. Individual data from
each animal were averaged over 30-minute intervals and
exported from the DATAQUEST program in an ASCII
format. Data were then transferred to a spreadsheet
program (LOTUS 1-2-3) and the mean values±SEM were
calculated for the rats of each strain.

The experiments were approved by the German fed-
eral regulations regarding experiments in animals and
were duly licensed (17a-19c 20/15-F95/02). These regu-
lations meet fully the requirements of the American
Physiological Society.

Data Analyses

For analysis of rhythmicity, mean values were calcu-
lated 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. Twenty-four-hour
co sine and all partial Fourier curves including up to the
fifth harmonic (4.8-hour period) were fitted as models
to the data. 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 func-
tion) of the harmonics together with the percentage of
rhythm. The F test was used to test for zero amplitude.

Model comparison statistics over all fit models was done
with the PHARMFIT subprogram SYNOPS according to
Wald as described in detail by McIntosh and McIntosh.
Briefly, likelihood-ratio tests were employed 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.

Results

Highly significant (P<.001 to .00001) time-dependent
variations in SBP and DBP as well as HR were identi-
fied by analysis of variance in each of the four rat
strains. Nonlinear rhythm analysis also revealed highly
significant (P<.00001) daily variations of each variable
in each strain. The 24-hour profiles in blood pressure
showed a "fingerprint" type of strain-dependent pat-
tern; the 24-hour profiles in HR, on the other hand,
were fairly similar among the strains (Figs 1A to 1D).
Reproducibility of the strain-dependent rhythm pat-
tern 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 domi-
nant 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 addi-
tional 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±0.2/91±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). The
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 curves 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></th>
<th>MESOR (mm Hg, bpm)</th>
<th>Amplitude (mm Hg, bpm)</th>
<th>Acrophase</th>
<th>Rhythm (%)</th>
<th>Rhythm (best fit)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>24-Hour component</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wistar-Kyoto normotensive rat (n=8)</td>
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<tr>
<td>SBP</td>
<td>115.6±0.3</td>
<td>5.9±0.4</td>
<td>2.08 AM±13</td>
<td>57.5</td>
<td>68.0*</td>
</tr>
<tr>
<td>DBP</td>
<td>79.4±0.2</td>
<td>3.7±0.3</td>
<td>1.35 AM±17</td>
<td>44.4</td>
<td>55.7†</td>
</tr>
<tr>
<td>HR</td>
<td>309.9±1.1</td>
<td>43.8±1.6</td>
<td>0.45 AM±09</td>
<td>74.9</td>
<td>83.2‡</td>
</tr>
<tr>
<td>Sprague-Dawley normotensive rat (n=10)</td>
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</tr>
<tr>
<td>SBP</td>
<td>131.7±0.2</td>
<td>6.8±0.3</td>
<td>2.12 AM±09</td>
<td>71.2</td>
<td>80.6*</td>
</tr>
<tr>
<td>DBP</td>
<td>90.7±0.2</td>
<td>5.5±0.2</td>
<td>1.16 AM±08</td>
<td>75.1</td>
<td>84.4*</td>
</tr>
<tr>
<td>HR</td>
<td>331.0±0.8</td>
<td>45.9±1.1</td>
<td>0.22 AM±06</td>
<td>82.3</td>
<td>92.4§</td>
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<tr>
<td>Spontaneously hypertensive rat (n=11)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>175.6±0.3</td>
<td>6.1±0.4</td>
<td>3.07 AM±15</td>
<td>49.5</td>
<td>66.1§</td>
</tr>
<tr>
<td>DBP</td>
<td>122.5±0.2</td>
<td>4.0±0.3</td>
<td>1.25 AM±16</td>
<td>42.7</td>
<td>63.9§</td>
</tr>
<tr>
<td>HR</td>
<td>306.7±0.6</td>
<td>40.3±0.8</td>
<td>0.13 AM±05</td>
<td>85.7</td>
<td>94.9§</td>
</tr>
<tr>
<td>TGR(mRen-2)27 transgenic hypertensive rat (n=9)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SBP</td>
<td>188.5±0.3</td>
<td>8.5±0.4</td>
<td>10.55 AM±10</td>
<td>65.8</td>
<td>80.6§</td>
</tr>
<tr>
<td>DBP</td>
<td>139.7±0.3</td>
<td>8.0±0.4</td>
<td>11.54 AM±09</td>
<td>71.5</td>
<td>80.7*</td>
</tr>
<tr>
<td>HR</td>
<td>324.3±0.9</td>
<td>43.5±1.3</td>
<td>11.34 PM±07</td>
<td>74.2</td>
<td>88.8§</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.

the onset of the rats’ resting phase; and transition from daily trough to nightly peak, ie, positive slope values, is observed at the onset of darkness (Table 2). In transgenic rats, however, transition of best fit from peak to trough (negative slope factors) in SBP and DBP occurred at the late light phase (4:39 PM and 6:01 PM, respectively), leaving the HR transition from peak to trough undisturbed (6:23 AM).

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></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><strong>Curve rise</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>SBP</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>SBP</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>SBP</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>SBP</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.

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 hypertensive and essentially hypertensive humans. The SHR, on the other hand, the maximum values for both SBP and DBP were observed during the animals’ resting period. The HR pattern remained unperturbed and was not different from that observed in the other three rat strains.

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

![Motor Activity](image)

**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.

**References**

Circadian blood pressure variation in transgenic hypertensive rats.
B Lemmer, A Mattes, M Böhm and D Ganten

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