Alterations in Blood Pressure and Heart Rate Variability in Transgenic Rats With Low Brain Angiotensinogen

Ovidiu Baltatu, Ben J. Janssen, Giampiero Bricca, Ralph Plehm, Jan Monti, Detlev Ganten, Michael Bader

Abstract—To study whether the brain renin-angiotensin system plays a role in the long-term and short-term control of blood pressure and heart rate variability, we examined in transgenic rats [TGR(ASrAOGEN)] with low brain angiotensinogen levels the 24-hour variation of blood pressure and heart rate. Telemetry recordings were made during basal and hypertensive conditions induced by a low-dose subcutaneous infusion of angiotensin II for 7 days. Short-term blood pressure and heart rate variability were evaluated by spectral analysis, and as a measure of baroreflex sensitivity, the average transfer gain between the pressure and heart rate variations was calculated. During the angiotensin II infusion in control but not TGR(ASrAOGEN) rats, the 24-hour rhythm of blood pressure was inverted (5.8±2 versus −0.4±1.8 mm Hg/group of day-night differences of blood pressure, P<0.05, respectively). In both the control and TGR(ASrAOGEN) rats, the 24-hour heart rate rhythms remained unaltered and paralleled those of locomotor activity. The transfer gain between 0.3 to 0.6 Hz was significantly higher in TGR(ASrAOGEN) than in control rats during control (0.71±0.1 versus 0.35±0.06, P<0.05) but not during angiotensin II infusion (0.6±0.07 versus 0.4±0.1, P>0.05). These results demonstrate that the brain renin-angiotensin system plays an important role in mediating the effects of angiotensin II on the circadian variation of blood pressure. Furthermore, these data indicate that a permanent deficiency in the brain renin-angiotensin system alters the reflex control of heart rate in rats. (Hypertension. 2001;37[part 2]:408-413.)

Key Words: renin-angiotensin system ■ blood pressure ■ baroreflex ■ circadian rhythm ■ brain

Multiple clinical studies have implicated blood pressure (BP) and heart rate (HR) variability in the diagnosis and prognosis of arterial hypertension and cardiovascular diseases. It has been shown, for instance, that patients with essential or secondary forms of hypertension can be divided into 2 groups: “dippers” and “nondippers.” In “dippers,” circadian rhythm of BP is preserved, whereas “nondippers” lack the characteristic nocturnal fall in BP. Cross-sectional studies have indicated that target-organ damage is more pronounced in “nondippers” than in “dipper” patients with comparable clinical blood pressure. Furthermore, a circadian pattern becomes obvious in the occurrence of acute cardiovascular diseases such as ischemia, infarction, stroke, and sudden death, and investigators are using new chronotherapeutic approaches in antihypertensive therapy to exploit the knowledge of circadian rhythms to reduce these events. Furthermore, it has been demonstrated that short-term (beat-to-beat) variations of BP and HR contain information about the activity of the autonomic nervous system, and power spectral analysis of these parameters shows promise for studying the mechanisms involved in cardiovascular disease.

There is evidence that in humans, the HR and HR variability (HRV) can be genetically determined. Recently developed approaches based on genetically modified animal models offer the opportunity to advance our understanding of the role that single genes play in the regulation of cardiovascular rhythms. The role of the renin-angiotensin system (RAS) in blood pressure variability (BPV) has been indicated by studies on transgenic hypertensive TGR(mREN2)27 rats with an overactive RAS. TGR(mREN2)27 rats manifest an inverted circadian rhythm of BP similar to secondary forms of hypertension in humans (“nondippers”).

Using a transgenic rat model with reduced brain angiotensinogen [TGR(ASrAOGEN)], we have recently shown that the brain RAS modulates the slow pressor response to low doses of angiotensin (Ang) II. These transgenic rats exhibit up to 90% reduced angiotensinogen levels throughout the brain, reduced drinking response to intracerebroventricular renin, hypotension, and low plasma vasopressin levels. In the current study, we evaluated in the same model to which extent the long-term and short-term BPV is affected by the brain RAS. Because the pressor response to Ang II may be in part related to an increased sympathetic tone, which is critical for the regulation of BPV and HRV, experiments were conducted in normotensive as well as hypertensive conditions induced by an infusion of low-dose Ang II.

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Methods

Rat Strains
Adult (aged 5 months) male transgenic rats [TGR(ASrAOGEN)] (n=12) and age-matched Hanover Sprague-Dawley rats (SD, parent strain used as normal controls) (n=11) were obtained from the animal facilities of the Max Delbrück Center for Molecular Medicine, Berlin, Germany. The rats were housed individually, synchronized to a 12-hour light-dark cycle (light: 6AM to 6 PM, 200 lux; dark: 6 PM to 6 AM, <0.1 lux), at ambient temperature 23±2°C. A standard rat diet (ssniff R-ZUCHT) and tap water were supplied ad libitum. Before starting the experimental protocols, rats underwent an acclimatization period for at least 10 days.

Experimental Protocols
All experimental protocols were performed in accordance with the guidelines for the human use of laboratory animals by the Max Delbrück Center for Molecular Medicine and approved by local authorities.

The rats underwent chronic implantation of a device that telemetrically monitors BP, HR, and motor activity (Data Sciences). The telemetry system consists of a radiofrequency transmitter (TA11PA-C40), a receiver panel, and an acquisition system (IBM compatible). For the implantation of the transmitter, rats were anesthetized with 10 mg/100 g body wt ketamine (Ketavet; Parke-Davis) plus 0.02 mg/100 g body wt xylazine (Rompun; Bayer). The catheter of the transducer was implanted into the abdominal aorta just below the bifurcation of the renal arteries, and the sensor itself was fixed to the peritoneum. After implantation, the rats were allowed to recover from the operation for 13 to 15 days, when the telemetry tracing indicated reestablishment of the 24-hour oscillations of BP and HR. To induce hypertension, Ang II was infused subcutaneously by osmotic minipump Alzet (model 2001, Alza Corp) at a rate of 100 nanograms per kilogram per minute for 7 days, as previously described.18

Figure 1. Telemetry tracing of systolic BP and HR in SD and TGR(ASrAOGEN) during basal conditions and Ang II infusion (averaged values per group of study). Filled bars on x axis represent dark period (6 PM to 6 AM); open bars represent light period (6 AM to 6 PM).

Figure 2. Acrophases of systolic BP, HR, and locomotor activity in SD (n=5) and TGR(ASrAOGEN) (n=6) in basal conditions and after 7 days of Ang II infusion. asTGR indicates TGR(ASrAOGEN) rats. Values are mean±SEM. *Significantly different (P<0.05) compared with values in basal conditions.
The experimental protocols were performed in conscious and unrestrained rats. To study the 24-hour cardiovascular variability, the system was set to monitor arterial pressure, HR, and locomotor activity at 5-minute intervals. Three-day periods were extracted in basal conditions and at the end of Ang II infusion. To study the short-term cardiovascular variability and spontaneous baroreflex function, beat-to-beat values of BP and HR were extracted from the waveform recordings obtained between 2 and 4 pm, when locomotor activity is lowest in the rat. Data were extracted during basal conditions and in the seventh day of Ang II infusion. Dataquest LabPro software was used to store and process the data.

Variability Analysis

The circadian variability was analyzed as described previously. Data processed by Dataquest LabPro software were extracted as systolic arterial pressure, HR, and locomotor activity. Three-day interval data were further analyzed by fast Fourier curve fitting, by transferring it into the analysis software developed by Witte et al. The following function was used: \( f(t) = \text{mesor} + \left(\text{amplitude}_{i}\times \cos(t - \text{acrophase}_{i})\right) / (\text{period length}_{i}) \), with the period length fixed at 24 hours.

Short-term cardiovascular variability was analyzed as previously described. For this analysis, the interbeat interval identified by the pulse interval (PI) rather than HR was used. The 2-hour beat-to-beat tracings were divided into segments of 200 seconds each. Because an equidistant sample rate is required for spectral analysis, relative stationary segments were resampled at 20 Hz by cubic interpolation. After the resulting time series was linearly detrended and a Hanning window was applied, spectral power of BP and PI were calculated by fast Fourier transform algorithm. For each animal, spectral power was then averaged over sequential data segments in 3 different frequency ranges: (1) a high-frequency band (HF, 0.6 to 3 Hz), (2) a mid-frequency band (MF, 0.3 to 0.6 Hz), and (3) a low-frequency band (LF, 0.07 to 0.3 Hz). These bands contain rhythmic oscillations related to (1) the respiratory cycle, (2) the autonomic nervous system, and (3) peripheral vascular control mechanisms, respectively. As an index of baroreflex sensitivity, the transfer (TF) gain between BP and PI variations in these frequency bands was calculated, together with the coherence and phase relation between the two signals. In rats, the TF gain of the mid-frequency band is generally taken as the most reliable index of baroreceptor activity because the linear coupling between BP and PI oscillations is generally highest in this region, and these oscillations probably result from resonance phenomena in the baroreceptor reflex. Furthermore, by direct nerve recordings, sympathetic oscillations at this frequency have been found. However, baroreceptor modulation of HR occurs also at frequencies <0.3 Hz as was found in sinoaortic denervation studies.

Statistical Analysis

The comparisons for multigroup and multifactorial analyses were done with a 2-way ANOVA and by Kruskal-Wallis 1-way ANOVA on ranks for multiple group comparisons. Changes versus control values (before Ang II infusion) were studied also by statistical analysis with Student’s paired t test. The criterion for significant differences between groups of study was \( P<0.05 \). Data are presented as mean±SEM.

Results

Circadian Variability of BP, HR, and Locomotor Activity

Figure 1 shows a telemetry tracing for SD and TGR(ASrAOGEN) rats (average values per group of study) and demonstrates the 24-hour rhythms of BP and HR during control conditions and during the Ang II infusion. The acrophases and mean day-night differences of the BP and HR diurnal variations in the TGR(ASrAOGEN) rats in basal conditions were not significantly different from those of SD rats (Figures 2 and 3). In SD rats, the circadian rhythm of BP was significantly deviated by the subcutaneous Ang II infusion, with the acrophase occurring during the daylight period (Figure 2) and hence inverted mean day-night differences (Figure 3). Opposite to the SD, the circadian variation of BP of the TGR(ASrAOGEN) rats was not significantly affected by the subcutaneous Ang II infusion (Figures 2 and 3).

In contrast to the BP, in both the SD and TGR(ASrAOGEN) rats, the 24-hour HR rhythms remained unaltered and paralleled those of locomotor activity during the subcutaneous Ang II infusion, with acrophases occurring during the night period (Figures 1 and 2). The mean day-night differences of HR and locomotor activity remained negative, and the acrophases occurred in the night period (Figures 1 and 2).

Short-Term Variability of BP and PI

The Table shows the averaged values of systolic BP and HR from data extracted both for circadian (3-day segment) and short-term (2-hour segment during the lowest locomotor activity period of the rat) periods. The analysis of the 3-day period of systolic BP is in agreement with the previously published observations, showing an attenuation in the development of hypertension in the TGR(ASrAOGEN) rats. The analysis of the 2-hour segment of systolic BP shows a lower (but not statistically significant) systolic BP in TGR(ASrAOGEN) than in SD rats. The HR was not different

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Systolic BP Average</th>
<th>HR Average</th>
<th>Systolic BP Average</th>
<th>HR Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD Basal</td>
<td>128±1.4</td>
<td>324.4±5.6</td>
<td>120.4±1.8</td>
<td>320.9±5.9</td>
</tr>
<tr>
<td>+ Ang II</td>
<td>168.2±0.9†</td>
<td>310.1±6.1</td>
<td>156.3±8.8†</td>
<td>311.7±13.6</td>
</tr>
<tr>
<td>TGR(ASrAOGEN) Basal</td>
<td>122.0±1.6</td>
<td>325.7±8.0</td>
<td>115.3±0.8</td>
<td>297.1±4.3</td>
</tr>
<tr>
<td>+ Ang II</td>
<td>152.0±3.5†</td>
<td>325.9±8.5</td>
<td>142.5±10.5†</td>
<td>294.9±8.2</td>
</tr>
</tbody>
</table>

*Significantly different (\( P<0.05 \)) in comparison to values in SD rats; †Significantly different (\( P<0.05 \)) in comparison to values in basal conditions.
between SD and TGR(ASrAOGEN) and was not affected by Ang II treatment. The short-term variation of BP and PI analysis and results are summarized in Figure 4. During baseline conditions as well as during Ang II infusion, power spectra of BP were not different between SD and TGR(ASrAOGEN). In contrast, PI power spectra were different between TGR(ASrAOGEN) and SD rats. Mid- and low-frequency power of PI was significantly higher in TGR(ASrAOGEN) than in SD rats in both baseline (3.05±0.69 ms² versus 1.03±0.25 ms²) and hypertensive conditions (2.36±0.44 ms² versus 1.1±0.24 ms²). In control conditions, the coupling between the oscillations of BP and PI, as indicated by the average coherence, was highest in the mid-frequency band (0.4 Hz) both in SD and TGR(ASrAOGEN) (Figure 4). During the Ang II infusion, the average coherence between BP and PI in this band was still significant but less pronounced. In control conditions, the average TF gain between BP and PI in TGR(ASrAOGEN) than in SD rats (Figure 5) in MF bands. During Ang II infusion, such differences in TF gain remained statistically significant in the very low-frequency band (0.02 to 0.07 Hz) and LF band but not in the MF band.

Figure 4. Comparison of average spectral powers of systolic BP and PI between SD (n=6) and TGR(ASrAOGEN) (n=6) rats during basal conditions and Ang II infusion. Average frequency-dependent changes in coherence are given in lower plots. Values are means per group of study. LF (0.07 to 0.3 Hz), MF (0.3 to 0.6 Hz), and HF (0.6 to 3 Hz) band values are shown. *Significantly different (P<0.05) between TGR(ASrAOGEN) and SD rats. asTGR indicates TGR(ASrAOGEN) rats.
Discussion
The main new findings of this study are (1) Ang II inverts day-night rhythm of BP but not of HR or locomotion in SD rats. (2) The fact that this is not observed in TGR(ASrAOGEN) rats points to a central role of the brain RAS in this process. (3) Short-term HRV but not BPV is elevated in TGR(ASrAOGEN) rats and hence the TF gain between these two, as an index of baroreflex sensitivity, is also higher. This indicates that a permanent deficiency in brain RAS can alter the reflex control of HR. Peripheral Ang II infusion did not generally alter this pattern. (4) Neither long-term nor short-term BPV is altered under basal conditions in TGR(ASrAOGEN).

The SD rats infused with a slow pressor dose of Ang II (this study) and the transgenic hypertensive rats TGR(mREN2)27 with overactive RAS30 develop an inverted 24-hour rhythm of BP and are therefore reliable study models for human secondary variability in BP and HR are differentially regulated.12,20 We and rhythmicity of HR, contributing to the concept that the circadian rhythm in SD rats was not associated with alterations in 24-hour variability of BP in SD but not TGR(ASrAOGEN) rats indicates that brain RAS is also a crucial factor that determines 24-hour BP rhythm. Also noticeably, the Ang II–induced shift on BP circadian rhythm in SD rats was not associated with alterations in 24-hour rhythmicity of HR, contributing to the concept that the circadian variability in BP and HR are differentially regulated.12,20 We and others have previously shown that the central nervous system is importantly involved in the circadian cardiovascular rhythmicity because the ablation of the suprachiasmatic nucleus (SCN) abolished the cardiovascular circadian rhythmicity.28,29 It is well known that the SCN contains a high concentration of Ang II receptors30 that may influence its neuronal activity.31 In the present study, the alterations in BP rhythm were not associated with disturbances in HR and locomotor 24-hour variability. This might indicate that the effect of Ang II does not occur in the SCN, because lesions of the SCN also alter HR and locomotor activity.28 Although it has been shown that Ang II can induce differential effects in different neuronal populations within the SCN, there is no evidence for specific sites in this nucleus to regulate BP and HR. Where in the brain Ang II causes its effects is not known.

HRV but not BPV is enhanced in TGR(ASrAOGEN). Hence, it is not surprising that the TF gain is also enhanced. Ang II does not considerably affect these parameters in TGR(ASrAOGEN) or SD rats. The reasons for that are not clear. If Ang II would increase sympathetic tone, then probably steady-state HR would have been higher and the 0.4-Hz BPV would have been enhanced because the 0.4-Hz BP rhythm in rats is directly coupled to sympathetic tone.25 The 0.4-Hz rhythm in HR is coupled both to sympathetic tone and vagal tone and sensitive to both atropine and β-blockers.32 Thus, this suggests that in TGR(ASrAOGEN), the vagal tone is higher because the HR during the time period tested was lower.

Pharmacological approaches have indicated a role of the brain RAS in fast adaptive changes of BP. For instance, the components of the RAS are present in cardiovascular-regulatory brain areas,33 and pharmacological manipulations of the system in these regions affect BP and the sensitivity of the baroreceptor reflex.34 Importantly, as indicated by the TF gain, the TGR(ASrAOGEN) rats have an exaggerated spontaneous baroreflex sensitivity in comparison to the SD rats, indicating that a lifetime deficiency in brain angiotensinogen production can affect baroreflex. These results confirm the inhibitory role of the brain RAS in the modulation of the baroreflex,34–37 and it can be concluded that a properly operating RAS inside the blood-brain barrier is necessary to maintain the baroreflex function. Moreover, the difference in TF gain between the two strains is maintained during the induction of hypertension, indicating that peripheral administration of Ang II does not reverse the alterations observed in the TGR(ASrAOGEN) rats during basal conditions. The fact that the induction of hypertension in both rat strains did not significantly alter TF gain correlates with previous observations regarding the ability of the brain angiotensin peptides to change the sensitivity of the baroreceptor reflex in a pressure-independent manner.34,38

It has been proposed that cardiac hypertrophy can be one of the causes of reduced baroreflex sensitivity in hypertension.39 Interestingly, however, despite the cardiac hypertrophy induced by the slow pressor dose of Ang II,18 the baroreflex sensitivity was only slightly attenuated without reaching statistical significance, as it has been observed in a similar study in rabbits.40

Summary
The results obtained from this study indicate that normal activity of the brain RAS importantly contributes to the short-term cardiovascular variability and spontaneous baroreflex activity and to the modulation of the circadian rhythm of BP. These results have potentially interesting implications for developing diagnosis and treatment strategies. For instance, the penetrability through the blood-brain barrier of a drug affecting the RAS may be an important parameter to consider in therapeutic regimens.
Acknowledgments

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References


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