Altered circadian rhythm reentrainment to light phase shifts in rats with low levels of brain angiotensinogen

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Submitted 3 October 2005; accepted in final form 3 December 2005

Campos, Luciana A., Ralph Plegehm, José Cipolla-Neto, Michael Bader, and Ovidiu C. Baltatu. Altered circadian rhythm reentrainment to light phase shifts in rats with low levels of brain angiotensinogen. Am J Physiol Regul Integr Comp Physiol 290: R1122–R1127, 2006. First published December 8, 2005; doi:10.1152/ajpregu.00703.2005.—In this study, we aimed to investigate the adaptation of blood pressure (BP), heart rate (HR), and locomotor activity (LA) circadian rhythms to light cycle shift in transgenic rats with a deficit in brain angiotensinogen [TGR(ASrAOGEN)]. BP, HR, and LA were measured by telemetry. After baseline recordings (BLD), the light cycle was inverted by prolonging the light by 12 h and thereafter the dark period by 12 h, resulting in inverted dark-light (DL) or light-dark (LD) cycles. Toward that end, a 24-h dark was maintained for 14 days (free-running conditions). When light cycle was changed from BLD to DL, the acrophases (peak time of curve fitting) of BP, HR, and LA shifted to the new dark period in both SD and TGR(ASrAOGEN) rats. However, the readjustment of the BP and HR acrophases in TGR(ASrAOGEN) rats occurred significantly slower than SD rats. The LA acrophases changed similarly in both strains. When light cycle was changed from DL to LD by prolonging the dark period by 12 h, the reentrainment of BP and LA occurred faster than the previous shift in both strains. The re-adjustment of the BP and HR acrophases in TGR(ASrAOGEN) rats occurred significantly slower than SD rats. In free-running conditions, the circadian rhythms of the investigated parameters adapted in TGR(ASrAOGEN) and SD rats in a similar manner. These results demonstrate that the brain RAS plays an important role in mediating the effects of light cycle shifts on the circadian variation of BP and HR. The adaptive behavior of cardiovascular circadian rhythms depends on the initial direction of light-dark changes.

AS MOST OF THE BIOLOGICAL processes, the functionality of cardiovascular system exhibits a circadian pattern. The blood pressure (BP) and heart rate (HR) circadian rhythms are generally considered to follow the activity period, with high levels during the alert period of the day and low levels during sleep. The interest in studying the circadian variation of BP has arisen because of the finding that hypertensive patients with disrupted circadian rhythms are more prone to morbidity cardiovascular events and end-organ damage (24, 26). A role of the renin-angiotensin system (RAS) in BP variability 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 (17, 27), similar to secondary forms of hypertension in humans (“nondippers”). We demonstrated that ANG II can invert the circadian rhythm of BP (6). The ANG II-induced shift on BP circadian rhythm was not associated with alterations in 24-h rhythmicity of HR or locomotor activity, indicating that the circadian variability of BP and HR are differentially regulated. The circadian rhythms are controlled by an internal autonomous oscillator system in the brain that coordinates the rhythms of peripheral oscillators (9). Using a transgenic rat model [TGR(ASrAOGEN)] with low levels of brain angiotensinogen (AOGEN), we showed that the brain RAS is importantly involved in the ANG II-induced shift in BP circadian rhythm (6). These transgenic rats have up to 90% reduced AOGEN levels throughout the brain, hypotension, low plasma vasopressin levels (21), and altered regulation of body metabolism (16) and are less reactive to stress (4).

One of the most powerful mediators of the internal circadian biological rhythms is the 24-h light-dark cycle. Therefore, in the present study, we decided to investigate the possible involvement of brain RAS in the adaptation of the circadian rhythms of BP, HR, and locomotor activity (LA) to light cycle shifts. Transgenic rats with low levels of brain angiotensinogen [TGR(ASrAOGEN)] were compared with Sprague-Dawley (SD) rats. BP, HR, and LA were measured by telemetry.

METHODS

Adult (aged 5 mo) male transgenic rats [TGR(ASrAOGEN)] (n = 6) and age-matched Hanover SD rats (parent strain used as normal controls, n = 6) were used. The rats were housed individually, synchronized to a 24-h light-dark cycle (LD 12:12, light: 6 AM 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 water were supplied ad libitum.

Experimental protocols and data analysis. All experimental protocols were performed in accordance with the guidelines of the American Physiological Society and approved in advance by the local Animal Ethics Committee.

The rats underwent chronic implantation of a device that telemetrically monitors BP, HR, and motor activity (Data Sciences), as previously described (8). The catheter of the transducer was implanted into the abdominal aorta 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 about 14 days, when the telemetry tracing indicated reestablishment of the 24-h oscillations of BP and HR.

The experimental protocols were performed in conscious and unrestrained rats. To study the 24-h cardiovascular variability, the

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system was set to monitor BP, HR, and LA at 5-min intervals. Data of three successive days were extracted under basal conditions, and Dataquest LabPRO software was used to store and process the data. Data were extracted as systolic BP, diastolic BP, HR, and LA.

After baseline recordings in basal LD (bLD) conditions, the light cycle was inverted by prolonging one light period by 12 h, resulting in an inversion of the light-dark cycle (DL 12:12). After 14 days, the light-dark cycle was returned to the initial condition, by prolonging one dark period by 12 h. In addition, we studied the adaptation of the circadian rhythms of SD and TGR(ASrAOGEN) rats to free-running conditions (24 h of darkness for 14 days).

The circadian rhythm analysis was performed by fitting of partial Fourier series to the data, as previously described (6, 28). Shortly afterward, the following function was used: f(t) = mesor + ∑ [amplitude, * cos(t) - acrophase, i, 2π/period length, i], with the period length fixed at 24 h. The following parameters of the cosinor analysis were analyzed: acrophase (peak time of curve fitting), MESOR (rhythm-adjusted mean), and amplitude (half rhythm parameters). Considering that the curve fitting was fixed for 24 h, the parameter “acrophase” represents the time of the day when the peak of curve fitted into the investigated variable (BP, HR, or LA) occurred.

Statistical analysis. The comparisons for multigroup and multifactorial analyses were done with a two-way ANOVA and by Kruskal-Wallis one-way ANOVA on ranks for multiple-group comparisons. Data are expressed as means ± SE. P < 0.05 was regarded as significantly different.

RESULTS

Baseline levels of systolic and diastolic BP were lower in the TGR(ASrAOGEN) compared with SD rats (systolic BP: 119.8 ± 1.5 vs. 126.6 ± 1.6 mmHg, diastolic BP: 83.9 ± 1.4 vs. 90.6 ± 2.3 mmHg, respectively), as previously observed (8). Baseline levels of heart rate and locomotor activity were not different between the rat strains.

When the light cycle was changed from bLD to DL, the acrophases of BP, HR, and LA shifted to the new dark period in both SD and TGR(ASrAOGEN) rats. However, the readjustment of the BP acrophases in TGR(ASrAOGEN) rats occurred significantly slower than SD rats (Fig. 1). At day 7, the BP acrophases were adjusted to the new light cycle in both strains. The HR acrophase readjustment was also significantly delayed in TGR(ASrAOGEN) rats, and a small, but significant, difference from those of SD rats still remained after 14 days. The LA acrophases changed equally in both strains.

When the light cycle was changed from DL to LD by prolonging the dark period by 12 h, the reentrainment of BP and LA occurred faster than the previous shift (Fig. 2). The readjustment of the BP and HR acrophases, but not of the LA, occurred significantly slower in TGR(ASrAOGEN) rats than SD rats.

We initiated free-running conditions by switching off the light, and a reentrainment period occurred that was close to the circadian rhythm for all parameters studied (Fig. 3). No differences between the SD and TGR(ASrAOGEN) rats in the circadian parameters of BP, HR, or LA were observed.

DISCUSSION

The main findings from the present study are 1) the brain RAS may play an important role in mediating the effects of light cycle shifts on the circadian variation of BP and HR, and 2) the brain RAS does not play a role in the maintenance of the internal circadian rhythms, at least for BP, HR, or LA.

The BP and HR circadian rhythm reentrainment induced by shifts in the ambient light-dark schedule is significantly slower in TGR(ASrAOGEN) rats with low levels of brain angiotensinogen than in their control group. These alterations appeared irrespective of the direction of the light-dark shift. Light is considered as the major environmental stimulus to entrain the internal circadian biological rhythms. The only known mammalian oscillators that can be entrained by light are located in the hypothalamic suprachiasmatic nucleus (9). This indicates the existence of a hierarchical model of internal oscillators, in which the SCN represents the master clock (Zeitgeber) linking the environmental stimuli (e.g., light) and the internal circadian clocks (9). Neural outputs from the suprachiasmatic nucleus (SCN) may affect the cardiovascular system through hormonal, sympathetic, or parasympathetic signals (12, 13). Brain areas that are connected with the SCN and that regulate circadian rhythms include the pineal gland and paraventricular nucleus. All of these brain areas contain RAS components (2, 7). The TGR(ASrAOGEN) rats used in this study have inhibited production of AOGEN throughout the brain. Thus, from the present study, we cannot identify at which level of the central circadian machinery the RAS could have been acting. For instance, the TGR(ASrAOGEN) rats have low levels of the pineal melatonin (3). One possible mechanism on the modulatory effect of melatonin on the circadian rhythms of BP and HR is through its influence on the autonomic nervous system (19). Also, the TGR(ASrAOGEN) rats may have altered the output of the autonomic nervous system, because they have altered the functionality of brain stem nuclei, such as the rostral ventrolateral medulla (5) or nucleus tractus solitarii (14, 15). The last possibility is that the intimal mechanism responsible for the actual findings resides just within the SCN. The current concept indicates that the 20,000 cells forming the SCN are functionally heterogeneous and may be grouped in subregions that serve distinctly separate functions (1). Furthermore, ANG II has heterogeneous effects on different SCN neurons (22, 23, 25). All of these hypotheses have yet to be elucidated.

The statistical differences on the BP and HR rhythm reentrainment observed between the TGR(ASrAOGEN) rats and their control group are not associated with alterations on the LA. This further supports the notion that the circadian rhythms of BP, HR, and LA are controlled by different mechanisms (6, 17, 20). The circadian rhythm of LA may be induced or regulated through the SCN projections to the thalamic areas involved in the locomotor control (10). The circadian rhythm of HR is not caused by activity and may be induced or regulated by the SCN through multisynaptic autonomic pathways, including the PVN (20). Also, the present and other studies indicate that the light-induced rhythm reentrainment of BP and HR could be mediated through similar mechanisms (19). Furthermore, continuous exposure to light suppresses the circadian rhythm of BP and HR (11). Differently from environmental light stimuli, nonephotic stimuli, such as ANG II, may induce a dichotomy in the circadian rhythms of BP and HR. ANG II acts as a hormonal modulator and induces a light-independent inversion of the circadian rhythm of BP but not of the...
HR or LA (6). Thus photic (environmental) and nonphotic (hormonal) stimuli induce different mechanisms of the central clock circuitry to alter BP or HR circadian rhythms. The brain RAS appears to be involved in the effects of both types of stimuli.

The exposure to constant dark conditions induced similar alterations in the circadian rhythm of BP, HR, and LA rhythms, in both TGR(ASrAOGEN) and SD rats. Removing the light-dark rhythm, one of the most potent modulators of the circa-

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**Fig. 1.** Reentrainment of circadian rhythm from basal 12:12-h light-dark conditions (b1 to b3) to inverted light rhythm (12:12-h dark-light, DL1 to DL14). Inversion was done by prolonging one light phase for 24 h. Plotted are group means ± SE of acrophases for systolic and diastolic blood pressure (BP), heart rate (HR), and locomotor activity. White area represents the light period; grey area represents the dark period. \( P < 0.01 \), significantly different between SD and transgenic rats with low levels of brain angiotensin [TGR(ASrAOGEN)].
dian rhythms, permits the expression of the endogenous circadian clocks, causing a situation called a “free-running” condition. In such circumstances, the SCN neurons exhibit rhythms with periods slightly different from 24 h (1, 18). Because no differences were observed between the transgenic rats with low levels of brain angiotensinogen and their controls, the brain RAS does not appear to have a role in the basal maintenance of the circadian rhythms of BP and HR.

Fig. 2. Reentrainment of circadian rhythm from 12:12-h dark-light conditions (DL1 to DL2) to light-dark light rhythm (12:12-h light-dark, LD1 to LD14). Inversion was done by prolonging one dark phase for 24 h. Plotted are group means ± SE of acrophases for systolic and diastolic BP, HR, and locomotor activity. White area represents the light period; grey area represents the dark period. $P < 0.01$, significantly different between SD and TGR(ASrAOGEN) rats.
Fig. 3. Adaptation of circadian rhythm from 12:12-h light-dark conditions (LD1 to LD2) to 24-h dark (12 h dark-dark, DD1 to DD14). Plotted are group means ± SE of acrophases for systolic and diastolic BP, HR, and locomotor activity. White area represents the light period; grey area represents the dark period.
This study provides evidence for the involvement of brain RAS in the resynchronization of the endogenous clock for BP and HR to a new shifted light-dark cycle. The intimate mechanisms by which the brain RAS acts on the central clock circuit need to be further elucidated.

ACKNOWLEDGMENTS

The authors would like to thank Reika Langanki for excellent technical assistance.

GRANTS

Luciana A. Campos was a recipient of a State of São Paulo Research Foundation doctoral and a Max-Delbrück-Center for Molecular Medicine postdoctoral fellowship. The work presented was funded in part by a grant from Deutsche Forschungsgemeinschaft (Grant BA1374/11–1).

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