Sleep-Related Changes in Cardiovascular Neural Regulation in Spontaneously Hypertensive Rats

Terry B.J. Kuo, MD, PhD; Cheryl C.H. Yang, PhD

Background—Sleep has significant effects on cardiovascular neural regulation. The aim of this study is to explore the possible change in sympathetic vasomotor activity and baroreflex sensitivity associated with spontaneous hypertension during each stage of the sleep-wake cycle.

Methods and Results—Polysomnographic analysis was performed in freely moving spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) during their normal daytime sleep. Continuous spectral analyses of electroencephalogram and electromyogram were performed to define active waking, quiet sleep, and paradoxical sleep. Low-frequency power of the arterial pressure variability (BLF) was quantified to provide an index of sympathetic vasomotor activity. Spontaneous baroreflex sensitivity was assessed (1) by the slopes of the regression lines of the mean arterial pressure and R-R intervals pairs that ascended (BrrA) or descended (BrrD) successively and (2) by the magnitudes of the arterial pressure and R-R intervals transfer functions in the high-frequency (BrrHF) or low-frequency (BrrLF) ranges. SHR had significantly higher mean arterial pressure during each of the sleep-wake states. Although the values of BLF, BrrA, BrrD, BrrHF, and BrrLF in SHR did not differ from those of WKY during active waking, SHR had a significantly higher BLF and lower BrrA, BrrD, BrrHF, and BrrLF compared with WKY during quiet sleep and paradoxical sleep.

Conclusions—SHR had enhanced sympathetic vasomotor activity but attenuated baroreflex sensitivity during sleep although each phenomenon was not evident when awake. (Circulation. 2005;112:849-854.)

Key Words: baroreceptors ■ blood pressure ■ nervous system, autonomic ■ hypertension ■ sleep

The cause of essential hypertension is multifactorial and complex. The neural mechanism is especially noteworthy because it provides a rationale for current clinical treatments with adrenoceptor antagonism using α-, β-, or combined blockers. In support of this hypothesis, animal studies have revealed information about enhanced basal sympathetic nerve activity, augmented pressor, and sympathoexcitatory response to stimuli in spontaneously hypertensive rats (SHR) compared with normotensive Wistar-Kyoto rats (WKY). Several investigators showed that medullary vasomotor centers and related neural pathways may play important roles in the pathogenesis of hypertension in SHR. The data support the hypothesis that sympathetic hyperfunction may play an important role in the cause of essential hypertension.

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The neural mechanism of essential hypertension, however, is not without controversy. For example, hypertensive humans and animals have been shown to have higher, similar, or even lower sympathetic functions compared with their normotensive counterparts. It should be noted that most of the previous comparisons were made without a detailed classification of the state of consciousness. Because sleep is an important part of humans' and other mammals' lives and leads to significant changes in the autonomic nervous system (ANS) activity, a systematic study of ANS functions in essential hypertension during each stage of the sleep-wake cycle warrants detailed exploration.

In previous studies, researchers from our laboratory developed a simple and quantitative analysis to explore the interaction between cerebral cortical and autonomic functions during sleep. The methodology, which simultaneously analyzes electroencephalogram, electromyogram, and heart rate variability, may be applied in both human and animal experiments on sleep-related ANS functions. Using this technique, we demonstrated that SHR have a significant cardiac sympathovagal imbalance, with increased sympathetic modulation during sleep, although it was less evident when awake.

In the previous study of the sleep-related sympathovagal imbalance of SHR, the heart rate variability indexes were used to indicate sympathetic modulations. Representation of vagal activity with the high-frequency power of heart rate...
variability (HF) has been widely accepted, but the quantitative estimate of cardiac sympathetic modulation using heart rate variability is still under debate, especially in rat studies. With the application of telemetry, the present study analyzed the changes of arterial pressure variability between SHR and WKY during the states of active waking (AW), quiet sleep (QS), and paradoxical sleep (PS) to explore neurogenic vasomotor activities. Transfer function analysis of arterial pressure variability and heart rate variability was also applied to estimate changes in cardiac baroreflex sensitivity. All of these noninvasive ANS indexes were applied to test whether a significant change in sympathetic vasomotor activity and/or baroreflex sensitivity occurred during sleep in SHR.

Methods

Animal Preparation

Experiments were carried out on adult male SHR (n = 10) and WKY (n = 10). The rats were obtained from the Animal Center of Tzu Chi University of Taiwan with guidelines established by the Position of the American Heart Association on Research Animal Use. These experimental procedures have been approved by the Institutional Animal Care and Use Committee of Tzu Chi University.

The detailed surgical procedure for the implantation of the electric and pressure sensors has been described in detail previously. In brief, electrodes for the parietal electroencephalogram, nuchal electromyogram, and ECG were implanted at appropriate positions when the rats were 8 to 10 weeks old. A telemetry transmitter (TA11PA-C40, Data Sciences) was also implanted to record arterial pressure signals. The tip of the arterial catheter was inserted into the abdominal aorta.

Protocol

After surgery, the rats were given antibiotics (chlortetracycline) and housed individually in cages for 1 week of recovery. To allow the rats to become habituated to the experimental apparatus, each animal was placed in the recording environment at least 2 times (1 h/d) before testing. On the day of the recording, a 30-minute period was allowed for the rat to become familiar with the chamber. Then, the biological signals and behaviors were synchronously recorded for 6 hours (10:30 AM to 4:30 PM) in a sound-attenuated room.

Measurements

Electroencephalogram, electromyogram, and ECG signals were amplified 10 000-fold but with different selections for filter bandwidths. The electroencephalogram was filtered at 0.3 to 70 Hz; the electromyogram was filtered at 0.3 to 70 Hz, respectively. The behaviors were recorded with a digital video recorder connected to another computer. The acquired data were analyzed online but were simultaneously stored on optic disks for subsequent offline verification.

Sleep Analysis

Sleep analysis was performed according to a recently developed and semi-automated computer procedure, which has previously been described in detail. The procedure discriminate the consciousness states into AW, QS, and PS, and the scoring was confirmed by an experienced rater with the assistance of the video recordings. Briefly, continuous power spectral analysis was applied to the electroencephalogram and electromyogram signals, from which the mean power frequency of the electroencephalogram (MPF) and the power magnitude of the electromyogram were quantified. For each time segment, the sleep-wake stage was defined as AW if the corresponding MPF was greater than a predefined MPF threshold (T_tmpf), and the electromyogram power was greater than a predefined electromyogram power threshold (T_tmpg), as QS if the corresponding MPF was less than the T_tmpf and the electromyogram power was less than the T_tmpg, and as PS if the corresponding MPF was greater than the T_tmpf and the electromyogram power was less than the T_tmpg. If the MPF was less than T_tmpf and the electromyogram power was greater than T_tmpg, the stage would not be determined and corresponding cardiovascular signals would not be analyzed. T_tmpf and T_tmpg of each animal were defined manually by the rater and were constant for the whole recording period. The time series of MPF first underwent a histogram analysis from which 2 separate populations related to AW/PS complex and QS could be identified. Thus, T_tmpf could be set to discriminate these 2 populations. The histogram of the electromyogram time series also had 2 populations but were related to AW and QS/PS complex. Therefore, T_tmpf could be set to discriminate these 2 populations. QS is also known as slow-wave sleep, whereas PS is equivalent to rapid-eye-movement sleep.

Cardiovascular Variability Analysis

The detailed analytical procedures of arterial pressure variability and heart rate variability have also been described in detail. Briefly, the mean arterial pressure (MAP) was obtained by the integration of the arterial pulse contour. The R-R interval (RR) was estimated continuously from the digitized ECG signals. The stationary MAP and RR were resampled and interpolated at 64 Hz to provide continuity in the time domain; then, they were truncated into 16-second time segments with 50% (8-second) overlap. These sequences were analyzed with the fast Fourier transform after application of the Hamming window. We generated the average periodograms and transfer functions continuously from every 7 successive signals. Because the sleep-wake stage might change frequently, only the average periodograms and transfer functions generated from the time segments that had an identical sleep-wake stage were statistically analyzed. The high-frequency power (BHF; 0.6 to 2.4 Hz) and low-frequency power (BLF; 0.06 to 0.6 Hz) of the MAP spectrogram and the high-frequency power (HF; 0.6 to 2.4 Hz) and normalized low-frequency power (LF%; 0.06 to 0.6 Hz) of the RR spectrogram were quantified. BLF, LF%, and HF provided markers of sympathetic vasomotor activity, cardiac sympathetic modulation, and cardiac vagal activity, respectively.

Spontaneous baroreflex sensitivity was estimated by MAP-RR transfer function and MAP-RR linear regression as described previously. In brief, for the transfer function analysis, the transfer magnitude at frequency of optimal coherence was estimated in the high-frequency (BrHF) and low-frequency (BrLF) ranges. For the sequence analysis, the slope of the linear regression between the MAP and RR pairs that were ascending simultaneously was estimated as the BrRA. The slope of the linear regression between the MAP and RR pairs that were descending simultaneously was estimated as the BrR. At least 3 beats were used to calculate the slope, and a slope was considered valid if MAP was well correlated (r² > 0.85) with RR. The data length for sequence analysis was 56 seconds, which was synchronous with the spectral analysis. Although each 6-hour sleep experiment repeatedly produced a large amount of cardiovascular data, we grouped these data into the 3 sleep-wake states and calculated their respective means. Thus, each experiment produced only 3 kinds of data, namely AW, QS, and PS, for each cardiovascular parameter.

Statistical Analysis

BHF, BLF, and HF were logarithmically transformed to correct the skewness of the distribution. Different effects of the 2 animal groups (WKY and SHR) and the 3 sleep-wake states (AW, QS, and PS) were assessed with 2-way ANOVA. When indicated by a significant F statistic, differences between states were isolated through the use of post hoc comparisons with the Student-Newman-Keuls test. Comparisons between 2 sets of data were performed with
the Student t test. Statistical significance was assumed for $P < 0.05$.

Data are presented as mean ± SEM.

Results

The polysomnographic recordings, coupled with the telemetric arterial pressure recordings, allowed complete and simultaneous measurements of electroencephalogram, electromyogram, ECG, and arterial pressure signals from which sleep staging, heart rate variability, and arterial pressure variability could be analyzed. Figure 1 demonstrates a representative example of WKY and SHR during daytime recording. Both rat strains had frequent transitions of the sleep-wake states.

The WKY had significant changes in arterial pressure and heart rate spectrograms, along with the sleep-wake transitions (Figure 1A). The sleep-related changes in the cardiovascular variabilities, however, were not as evident in the SHR (Figure 1B). In general, the WKY had weaker BLF but stronger HF during sleep. In addition, we noted that the upper limit of BLF and the lower limit of HF of the WKY were not far from those of SHR. However, SHR appeared to lose the ability, at least partially, to decrease BLF and to increase HF as WKY did during sleep.

To deal with the frequent changes in the sleep-wake states, the average periodogram and transfer function analysis during stable AW, QS, and PS conditions were performed. Figure 2 supports the idea that the differences in cardiovascular variabilities between WKY and SHR were more evident during sleep, either QS or PS. The group data are summarized in the Table and Figure 3. ANOVA detected significant effects of animal group and sleep-wake state on all MAP, RR, BHF, BLF, HF, and LF% ($P < 0.05$) but detected a significant animal group by sleep-wake state interaction on only BHF, BLF, and LF% ($P < 0.05$). Although SHR and WKY had similar RR, BHF, BLF, and LF% during AW, SHR had significantly higher BHF, BLF, and LF% during QS and PS. SHR also had a significantly higher MAP but lower HF during all stages. For the baroreflex examination (Figure 4), ANOVA detected very significant effects ($P < 0.01$) of animal group, sleep-wake state, and animal group by sleep-wake state interaction on all BrrLF, BrrHF, BrrD, and BrrA. The 4 indexes of spontaneous baroreflex sensitivity led to consistent results: Although SHR had baroreflex sensitivity similar to WKY during AW, they had significantly lower baroreflex sensitivity during QS and PS. It was also true that WKY had a significant trend to higher baroreflex sensitivity during QS and PS, whereas the trend for SHR was more ambiguous.

Discussion

It is well known that blood pressure measurements during sleep differ from measurements when awake. However, sleep can further be divided into QS and PS. Evidence has indicated that cerebral and sympathetic activities during PS are higher than those during QS and are similar to those during AW. AW, QS, and PS, representing 3 states of consciousness, have their own specific characteristics. Thus, cardiovascular data of the different sleep-wake states should be analyzed and compared separately. Previously, sleep staging was considered a complex and somewhat mysterious study. However, it is possible to divide consciousness into AW, QS, and PS through the use of simple criteria. A recent study from our laboratory demonstrated that SHR had less sleep time, poorer sleep quality, and a greater tendency to wake up from QS compared with WKY. With our staging system, the cardiovascular variability and baroreflex parameters of specific AW, QS, or PS state could be compared in the present study.

The physiological significance of arterial pressure variability has been broadly studied with frequency domain analysis. Through high-quality collection of blood pressure signals and the Fourier transform, investigators found 2 major components in arterial pressure variability, the respiratory-related

Figure 1. Continuous and simultaneous analysis of sleep-wake state, arterial pressure variability, and heart rate variability during 60 minutes of daytime sleep in 1 WKY (A) and 1 SHR (B). Sleep stage, including AW, PS, and QS, was automatically assigned by computer program according to states of mean power frequency of electroencephalogram and nuchal electromyogram. MAP and RR and their corresponding power spectrograms (BPSD and HPSD) are displayed. Also shown are temporal alterations in BHF and BLF powers of arterial pressure variability and HF and LF% of heart rate variability. Ranges of frequency for BHF, BLF, HF, and LF are denoted on right of spectrograms. nu indicates normalized units.
high-frequency component (BHF) and the vasomotor-related low-frequency component (BLF). Our previous study in rats has demonstrated that BLF may reflect excitation in the brainstem vasomotor center and that this reaction depends on intact sympathetic functions. A follow-up study showed greater BLF in SHR than in WKY during the anesthetized state. In freely moving rats, Stauss et al found that SHR had BLF similar to WKY, whereas Friberg et al demonstrated elevated BLF in SHR. Their studies, however, did not classify the state of consciousness of the rats. It is interesting that with our classifications, SHR and WKY showed no differences in BLF and BHF during AW. Once the rats entered sleep, either QS or PS, SHR developed less depression of arterial pressure variability, resulting in higher BLF and BHF. The data

### MAP and RR During AW, QS, and PS in WKY and SHR

<table>
<thead>
<tr>
<th></th>
<th>AW</th>
<th>QS</th>
<th>PS</th>
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<tbody>
<tr>
<td>MAP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>105.3±2.3</td>
<td>98.1±2.0†</td>
<td>100.4±1.6</td>
</tr>
<tr>
<td>SHR</td>
<td>135.5±2.7*</td>
<td>128.2±2.3†</td>
<td>128.9±2.2*</td>
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<tr>
<td>RR, ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>162.1±1.7</td>
<td>185.0±3.3†</td>
<td>189.2±3.0†</td>
</tr>
<tr>
<td>SHR</td>
<td>162.4±3.2</td>
<td>175.1±4.2†</td>
<td>176.5±4.5†</td>
</tr>
</tbody>
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Data are expressed as mean±SEM.

*P<0.05 vs WKY by Student t test; †P<0.05 vs AW by Student-Newman-Keuls test.

Figure 2. Illustrative example of time- and frequency-domain analyses of cardiovascular variabilities in WKY and SHR during AW, QS, and PS. Sixty-four seconds of MAP and RR are displayed, from which their corresponding average periodograms (BPSD and HPSD) are analyzed. Also shown are cross spectrograms showing coherence and transfer magnitude between BPSD and HPSD. Significant responses are denoted by coherence ≥0.5 or by heavy line in magnitude of transfer function.


indicated that SHR had higher sympathetic vasomotor activity during sleep. These observations were also compatible with the previous study\textsuperscript{16} based on heart rate variability analysis.

It should be noted that arterial pressure variability, even in the low-frequency range, is not due exclusively to the neurogenic vasomotor activity in some conditions. Even in well-controlled and well-defined experimental conditions, it provides only indirect information on the sympathetic vasomotor activity. For example, it has been shown that the vagal-mediated respiratory sinus arrhythmia may contribute to BLF, especially when the respiration is slow or irregular.\textsuperscript{35} However, if the respiratory rate is well separated from the vasomotor frequency, BLF is rather independent of vagal influence.\textsuperscript{21} Thus, it is especially noteworthy to observe the data during QS when the respiratory rate is regular and the BLF appeared not to be contaminated by respiratory sinus arrhythmia (Figure 2).

Evidence shows that hypertensive subjects have lower baroreflex sensitivity. However, most human and animal experiments were performed while the subject was anesthetized or the state of consciousness was not identified.\textsuperscript{36–38} Information on subjects during sleep has not been researched. Traditionally, baroreflex sensitivities have often been evaluated by injecting pressor or depressor agents such as phenylephrine or nitroprusside. However, the procedure is potentially dangerous and may cause some stress in the subjects. During sleep, elicited blood pressure changes may alter the sleep-wake state or even wake the subjects.\textsuperscript{39} In contrast, the noninvasive technique of baroreflex analysis has its merits. Through a computer algorithm, baroreflex sensitivity can be estimated from the spontaneous fluctuations in blood pressure and heart rate without manipulating blood pressure artificially.\textsuperscript{20,40} In the present study, when the technique was applied in the sleep experiment, the 4 baroreflex sensitivity indexes did not show any differences between SHR and WKY during AW. However, as soon as the rats achieved the state of sleep, WKY had 2-times-more baroreflex sensitivity than SHR. That the sleep triggers an augmentation of baroreflex in normotensive rats confirmed the findings of the previous human studies.\textsuperscript{41,42} It has been proposed that the phenomena may be due to the rapid changes in cardiac vagal circuits.\textsuperscript{42} The mechanism underlying the inability to augment baroreflex sensitivity during sleep in SHR is not clear and warrants further exploration.

The causes of sympathetic hyperactivity during sleep may be linked to structural pathological changes. Animal experiments suggested that an elevation of vasomotor center-blood pressure control\textsuperscript{2} or depression of nitric oxide activity in the brainstem vasomotor center\textsuperscript{13} may play a role in spontaneous hypertension. However, the central nervous system deals with external information and thinking while awake. External changes may cause various psychological stimulations that influence autonomic functions; therefore, ANS activities are often variable, making it difficult to determine a stable index. On the contrary, the brain mainly manages reflective information from the body during sleep and is less interrupted by the external information\textsuperscript{44}; the experimental results during sleep may be more related to body conditions. Because sleep-related pathological changes may be subtle and prone to influence by the conscious state, using a technique that does not interfere with sleep is the basic requirement to study these changes.

Despite the common behavior of AW, QS, and PS, the sleep habit in rats is different from that in humans. For example, rats sleep in daytime with a sleep cycle of \textsuperscript{90} minutes; humans sleep in nighttime with a sleep cycle of \textsuperscript{90} minutes. Thus, it is still questioned whether hypertensive humans have similar sleep-related changes. Nevertheless, both this study exploring arterial pressure variability and baroreflex and a previous study\textsuperscript{16} exploring heart rate variability lead to a consistent message: The changes in the cardiovascular neural regulation in SHR were particularly evident during sleep. In other words, the switching of the sympathovagal balance toward the vagal limb during sleep was not apparent in the SHR. This loss of normal physiological function is likely to cause problems, including sleep disorders and even hypertension. Further studies of the underlying mechanisms in rats and related changes in humans are worth investigating. The data during awake states do not represent the whole story of circulation. Therefore, a sleep study is necessary to completely research the pathophysiology of the cardiovascular system.

\textbf{Conclusions}

Compared with WKY, SHR had enhanced vasomotor activity but attenuated cardiac baroreflex sensitivity during sleep, although each phenomenon was not evident when awake.

\textbf{Acknowledgments}

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\begin{center}
Figure 4. Comparisons of spontaneous baroreflex sensitivity between WKY and SHR during AW, QS, and PS. Four indexes were used for this analysis: magnitudes of transfer function between MAP and RR signals at frequency ranges of 0.06 to 0.6 Hz (BrLF) and 0.6 to 2.4 Hz (BrHF) and slopes of linear regression between MAP and RR pairs that under successively descending (BrD) and ascending (BrA) changes. Values are presented as mean±SEM; n=10 rats per group. *P<0.05 vs WKY by Student t test; †P<0.05 vs AW; ‡P<0.05 vs QS by Student-Newman-Keuls test.
\end{center}
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