Effect of sleep/wake state on arterial blood pressure in genetically identical mice


Effect of sleep/wake state on arterial blood pressure in genetically identical mice. J. Appl. Physiol. 85(1): 366–371, 1998.—Genetic determinants may contribute to the large variability in arterial blood pressure responses to changes in sleep/wake state in humans. In this study, we developed techniques to examine the relationship between sleep/wake state and mean arterial pressure (MAP) in unrestrained, genetically identical mice (C57BL/6J; n = 9). The left common carotid artery was catheterized, and arterial blood gases were analyzed 24–48 h postsurgery to verify normal respiratory and metabolic function. The animals were then allowed to cycle naturally through sleep/wake states over a 3- to 4-h period while continuous polysomnography and arterial pressure measurements were made. The MAP decreased from quiet wakefulness to non-rapid-eye-movement sleep (9.8 ± 1.3 mmHg; P < 0.001) and further decreased from non-rapid-eye-movement to rapid-eye-movement sleep (9.7 ± 1.8 mmHg; P < 0.001). We conclude that the inbred strain of C57BL/6J mice exhibits a significant and consistent change in MAP related to sleep/wake state. Future studies can compare responses in this strain of mice with those in other inbred or transgenic mice to determine whether specific genes regulate arterial blood pressure responses to sleep/wake state.

arterial blood gases; heart rate; non-rapid-eye-movement sleep; rapid-eye-movement sleep

Studies in humans indicate that arterial blood pressure can change as a function of sleep/wake state (1, 3, 6, 9). Recent interest has focused on 24-h ambulatory monitoring to examine changes in arterial blood pressure between daytime and nighttime. In a large, multinational database of 24-h-monitoring ambulatory studies, mean arterial blood pressure (MAP) fell on average 14.3 mmHg between daytime and nighttime (11). However, this nocturnal fall in MAP was highly variable. In the population studied, the nocturnal MAP increased above daytime MAP in some subjects (0–5th percentile), but it decreased by 30 mmHg or more below daytime MAP in other subjects (95–100th percentile) (11). Staessen et al. (11) suggest that genetic factors may contribute to this variability in arterial blood pressure responses between daytime and nighttime. To date, no studies have examined the relationship between sleep/wake state and arterial blood pressure in the absence of genetic variability.

It is possible to eliminate genetic variability by performing studies in an inbred mouse strain. Unfortunately, the small size of mice makes chronic, invasive measurements of arterial blood pressure and sleep/wake state an arduous task. As a result, studies examining the relationship between arterial blood pressure and sleep/wake state have been conducted in larger, genetically heterogeneous animals (4, 7, 8, 17). In the present study, we developed techniques to simultaneously record arterial blood pressure and polysomnography in a common strain of inbred mice (C57BL/6J). We applied these techniques to determine the extent and variability of sleep/wake state changes in arterial blood pressure in genetically identical mice. Our findings indicate that sleep/wake state has a pronounced and uniform effect on arterial blood pressure in this inbred strain.

METHODS

Surgical procedures. Experiments were performed in nine male inbred mice (C57BL/6J; J axon Laboratory, Bar Harbor, ME). The animals were housed at the Johns Hopkins University in an antigen- and virus-free facility and subjected to a 12:12-h light-dark cycle. Animals were 104 ± 11 (SE) days old and weighed 30.1 ± 1.2 g. Two separate surgeries were performed in each animal. Anesthesia was induced and maintained by using halothane administered through a face mask.

In the first surgery, the mice were instrumented with chronically implanted polysomnographic electrodes for determination of sleep/wake state. A midline incision was made to expose the skull and muscles immediately posterior to the skull. The underlying fascia was gently cleared from the skull surface, and two bilateral pairs of holes (0.015 cm) were drilled (drill bit E-HSD-97; Small Parts, Miami Lakes, FL) through the skull in the frontal and parietal regions. Four 40-cm electroencephalographic (EEG) electrodes were fashioned from Teflon-coated stainless steel wire (0.018-cm OD Teflon coated, 0.013-cm OD bare; A-M Systems, Everett, WA). The final 0.15 cm of each electrode was stripped of the Teflon coating, bent at 90°, and inserted into the skull through each of the predrilled holes. The four electrodes were bonded to the dorsal surface of the skull with dental acrylic (Land Dental, Wheeling, IL). Two 45-cm nuchal electromyographic (EMG) electrodes were made from Teflon-coated stainless steel wires...
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identical to those used for the EEG electrodes. The end of each of the EMG electrodes was fashioned into a small loop (−0.15 cm diameter) from which the Teflon coating was removed with a bunsen burner. The two EMG electrodes were stitched, with 6-0 silk, flat onto the surface of the muscle immediately posterior to the dorsal area of the mouse skull. The skin overlying the skull and posterior muscles was reapproxosed, and the six electrodes exited the skin dorsally ∼1.25 cm posterior to the point of EMG attachment. The total time from induction of anesthesia to recovery of consciousness was ∼30–40 min.

After surgery was performed, mice were housed individually in cages with open tops. The polysomnographic electrodes were loosely threaded through a circular plastic loop positioned 20 cm above the center of the cage. The loop could rotate through 360° with low friction. This setup enabled the weight of the wires to be offset while allowing the animal to move freely within the cage. All animals were allowed 3–5 days to recover from the first surgery before undergoing the second surgery.

In the second surgery, an arterial catheter was chronically implanted in the left carotid artery for measurement of arterial blood pressure and for sampling of arterial blood. The left common carotid artery was carefully exposed via a 0.5- to 1.0-cm midline incision in the ventral neck region. Care was taken to remain inferior to the carotid bifurcation and not to damage the vagus nerve. A 60-cm catheter fashioned from PE10 tubing (A-M Systems) was inserted ∼0.65 cm into the common carotid artery, ensuring that the catheter tip was in the thorax. The angle of the bevel of the catheter tip was the most important factor in determining the length of time the catheter remained patent. Essentially, the more blunt the catheter tip, the longer the catheter remained patent. In addition, we heated the catheter tip with a heat gun to smooth and round the edges. The catheter was tied in place and routed under the skin to exit on the dorsal surface of the neck immediately adjacent to the point of exit of the polysomnographic electrodes. The catheter was filled with a sterile 10% heparin-90% saline solution and tied off at its most distal point. The catheter was fixed to the polysomnographic electrodes with tape, which protected the catheter from interference. By the second day after the second surgery to ensure their long-term survival before they were killed (by using 1 ml pentobarbital sodium ip). The study was approved by the Johns Hopkins University Animal Use and Care Committee and complied with the American Physiological Society Guidelines.

Apparatus and methods of measurement. Arterial pressure measurements were made with pressure transducers (Cobe, Lakewood, CO) zeroed at midthoracic level. Calibrations were checked at the beginning and end of each experiment. A pen recorder (Grass Instruments; Quincy, MA) was used to record EEG activity, EMG activity, and arterial pressure. Signals from the pen recorder were digitized at 300 Hz (DI-200 data-acquisition board; Dataq Instruments, Akron, OH) and stored on optical disk with WinDaq/200 acquisition software (Dataq Instruments).

A 100-µl Hamilton glass syringe was used to withdraw arterial blood for analysis of blood-gas status. The tied distal end of the catheter was cut, and blood was allowed to flow back spontaneously. The Hamilton syringe was then attached to the catheter via either a 27- or 30-gauge needle inserted directly into the end of the Hamilton syringe. A sample of arterial blood (80–100 µl) was drawn into the syringe, placed on ice, and immediately analyzed on a blood-gas analyzer (IL BG3; Instrumentation Laboratory, Lexington, MA). The blood loss was replaced with an infusion of 200–300 µl of saline over 2–3 min.

During data collection periods, the animals remained in their single-occupancy cages. The stripped ends of the polysomnographic electrodes were attached to the Grass polygraph via an input cable (7P5B; Grass Astromed, West Warwick, RI), and the arterial catheter was connected to the pressure transducer. The weight of the electrode-catheter unit was supported by the fixation of the electrodes to the input cable ∼30 cm above the animal. The length of the electrode-catheter unit allowed the animal to move freely within the cage throughout the data collection period.

Experimental protocol. Experiments were conducted ∼24 h after the second surgery in seven animals and 48 h after the second surgery in two animals. An arterial blood-gas sample was taken between 0900 and 1100 on the day of the experiment, and polysomnograph and cardiovascular data were recorded between 1200 and 1700. The experiments were conducted in a quiet, partially darkened room. Each animal was permitted to acclimate for ∼30 min before data collection was begun. The protocol consisted of allowing the mice to cycle naturally through their normal sleep/wake states for 3–4 h while continuously recording polysomnography and arterial pressure.

Data analysis. Sleep/wake state was assessed over 30-s epochs from EEG and EMG recordings. Wakefulness was characterized by low-amplitude, high-frequency (0.5–2 Hz) EEG waves and high levels of EMG activity compared with the sleep states. Wakefulness was subdivided into quiet and active wakefulness, as defined by the absence or presence of movement-related, pen-blocking artifact in the EEG signal. Non-rapid-eye-movement (NREM) sleep was characterized by high-amplitude, low-frequency (0–1 Hz) EEG waves and an EMG activity considerably less than during wakefulness. Rapid-eye-movement (REM) sleep was characterized by low-amplitude, mixed-frequency (0.5–10 Hz) EEG waves, although the predominant pattern was a fixed-amplitude theta activity consistent with hippocampal theta rhythm previously reported in rodents (15). During REM sleep, the EMG activity was either equal to or less than that seen during NREM sleep, but the activity was always less than that seen during wakefulness. All sleep/wake assessments were made manually by one investigator. Reliability of sleep/wake staging was determined by two independent observers, who used a balanced, stratified sample (wakefulness, NREM sleep, and REM sleep) of sixty 30-s epochs from all mice used in the study. A kappa statistic of 0.95 (P < 0.01) was calculated, representing 96.6% agreement between the two independent observers.
MAP was calculated during quiet wakefulness, NREM sleep, and REM sleep as the average MAP from 30 s after the start of each sleep/wake state to the end of each particular state. HR was measured from the pulsatile arterial blood pressure over two 30-s periods taken at the points 30 s into and 30 s preceding the end of each sleep/wake state. For a REM cycle lasting 60 s, the HR was measured over the last 30 s of that particular REM period. The data were averaged to give a single value for MAP and HR during quiet wakefulness, NREM sleep, and REM sleep in each animal. These data were next pooled across animals.

Data were analyzed by using Crunch 4 (Crunch Software; Oakland, CA) and are reported as means ± SE. Within-subject one-way ANOVA with repeated measures was used to detect significant differences in arterial pressure and HR across sleep/wake states. If the ANOVA was significant, a Newman-Keuls test was used to identify which means were significantly different. Differences were considered significant if P < 0.05.

RESULTS

The arterial blood-gas status of the animals (n = 9) immediately before data collection was pH 7.384 ± 0.015 units; arterial PCO₂, 36.2 ± 1.1 Torr; arterial Po₂, 90.0 ± 4.5 Torr; arterial O₂ saturation, 96.6 ± 0.6%; and HCO₃⁻, 22.0 ± 0.6 mM. The time spent in each sleep/wake state, the number of cycles through each sleep/wake state, and the average time per cycle of each sleep/wake state are shown in Table 1. All nine animals exhibited NREM sleep, and seven of the nine animals cycled into REM sleep.

Figure 1 is a compressed sample trace in one animal, showing EEG, EMG, and arterial blood pressure recordings during different sleep/wake states. This trace demonstrates the distinct differences in EEG and EMG activity that characterize the sleep/wake states. Figure 2 shows decompressed recordings from the same animal shown in Fig. 1 and highlights the differences in EEG and EMG activity between quiet wakefulness, NREM sleep, and REM sleep. Figures 1 and 2 also reveal the effect of sleep/wake state on arterial pressure. The MAP fell from quiet wakefulness to NREM sleep and again from NREM sleep to REM sleep. There was also a slowing of the heart from quiet wakefulness to NREM sleep, but there was no obvious difference in HR from NREM to REM sleep (Fig. 2).

### Table 1. Total time, no. of cycles, and time per cycle during periods of active and quiet wakefulness, NREM sleep, and REM sleep in chronically instrumented, unrestrained mice

<table>
<thead>
<tr>
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<th>Active Awake</th>
<th>Quiet Awake</th>
<th>NREM</th>
<th>REM</th>
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<tbody>
<tr>
<td>Total time, %</td>
<td>6 ± 3</td>
<td>42 ± 6</td>
<td>49 ± 6</td>
<td>3 ± 1</td>
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<tr>
<td>No. of cycles</td>
<td>4.4 ± 0.6</td>
<td>5.9 ± 0.5</td>
<td>4.4 ± 0.6</td>
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<tr>
<td>Time per cycle, min</td>
<td>26.3 ± 5.1</td>
<td>17.0 ± 2.3</td>
<td>1.3 ± 0.2</td>
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Values are means ± SE; n = no. of mice. NREM sleep, non-rapid-eye-movement sleep; n = 9. REM sleep, rapid-eye-movement sleep; n = 7. Periods of active and quiet wakefulness, n = 9. Length of experiments averaged 204 ± 25 min.

Fig. 1. Representative recording showing electroencephalogram (EEG), electromyogram (EMG), and arterial blood pressure (Part) vs. time in non-rapid-eye-movement (NREM) sleep, rapid-eye-movement (REM) sleep, and quiet wakefulness in a chronically instrumented, unrestrained mouse. Note: compressed time scale makes it difficult to distinguish changes in EMG activity between NREM and REM sleep that are evident for uncompressed data in same animal in Fig. 2.
The effect of sleep/wake state on MAP is shown in Fig. 3 for individual and pooled data. During quiet wakefulness, the animals' MAP was 117.8 ± 2.5 mmHg. The MAP fell in all nine animals from quiet wakefulness to NREM sleep (9.8 ± 1.3 mmHg; \( P < 0.001 \)). The 5th and 95th percentiles for the fall in MAP from quiet wakefulness to NREM were 7.8 and 11.8 mmHg, respectively. The MAP also fell from NREM to REM sleep (9.7 ± 1.8 mmHg; \( P < 0.001 \)) in all seven animals that cycled into REM sleep.

During quiet wakefulness, the animals' HR was 610 ± 20 beats/min. HR fell in eight of the nine animals from quiet wakefulness to NREM sleep (44 ± 13 beats/min; \( P < 0.05 \)). However, there was no consistent change in HR between NREM (566 ± 25 beats/min) and REM sleep (564 ± 20 beats/min), with HR increasing in

Fig. 2. Representative traces taken from Fig. 1 showing EEG and EMG activity, Part, and heart rate (HR) in a chronically instrumented, unrestrained mouse during quiet wakefulness, NREM sleep, and REM sleep.

Fig. 3. Mean arterial pressure (means ± SE) measured during periods of quiet wakefulness (\( n = 9 \)), NREM sleep (\( n = 9 \)), and REM sleep (\( n = 7 \)) in chronically instrumented, unrestrained mice.
three animals, decreasing in three animals, and not changing in one animal.

**DISCUSSION**

This study demonstrates that the C57BL/6J inbred strain of mice exhibits significant and consistent changes in arterial blood pressure related to sleep/wake state. In the present study, the average fall in MAP of 9.8 mmHg from quiet wakefulness to NREM sleep (Fig. 3) is quantitatively similar to the average fall of 14.3 mmHg reported from daytime to nighttime in a large database of human studies that used 24-h monitoring of ambulatory subjects (11). However, in contrast to the human data, the sleep-related fall in MAP was particularly uniform among animals in our study (5th and 95th percentiles = 7.8 and 11.8, respectively). Thus, in the C57BL/6J inbred strain of mice, there was a significant and consistent decrease in MAP from wakefulness to NREM sleep.

Genetic homogeneity may not be the only factor responsible for the consistent fall in MAP from wakefulness to NREM sleep in the present study. Indeed, Radulovacki et al. (8) report consistent and significant decreases in MAP related to sleep/wake state in outbred Sprague-Dawley rats. Thus limited variability in age and environmental factors also may have contributed to the tightness of the data reported in our study. Human studies show that the degree of lowering of blood pressure from daytime to nighttime decreases as a function of age (11). Furthermore, environmental factors have been proposed to account for the observation that nighttime blood pressures are higher in black American adolescents than in white American adolescents (5). Therefore, the consistent decrease in MAP between wakefulness and NREM sleep in inbred mice in the present study may be caused by not only the absence of genetic variability but also, in part, by reduced variability associated with age and environment. As such, however, the mouse provides a unique model with which to systematically explore the relative roles of genes, age, and environment on sleep/wake state differences in MAP.

The present study demonstrated, in addition to differences between wakefulness and NREM sleep, a systematic fall in arterial blood pressure from NREM to REM sleep (Figs. 1–3). Studies examining 24-h monitoring in ambulatory humans do not distinguish between NREM and REM sleep. However, periods of REM sleep are much shorter in mice (~1–2 min; see Table 1) than in humans, apparently varying as an inverse function of metabolic rate (14). Furthermore, our analyses in mice did not distinguish differences in MAP between phasic and tonic REM. Such differences between phasic and tonic REM may be important, because the occurrence of muscle twitches during REM sleep in humans produces surges in arterial blood pressure (10). Thus it is presently unclear what factors account for differences in arterial blood pressure patterns between mice and humans during REM sleep.

The techniques developed for this study enabled simultaneous measurement of polysomnography and arterial blood pressure in mice without compromising sleep architecture or cardiorespiratory function. The distribution of time spent in wakefulness (48 ± 6%), NREM sleep (49 ± 6%), and REM sleep (3 ± 1%) during our 3- to 4-h experiment was almost identical to that reported by Walsh et al. (16) in the same strain of mice over 10 consecutive days of data recording (wakefulness, 54.3%; NREM sleep, 40.1%; REM sleep, 5.6%). Arterial blood-gas measurements verified that normal respiratory and metabolic function existed in the mice at the time of data collection. Furthermore, all animals regained any postoperative weight loss and survived long term. Thus, despite the invasiveness of the methods used in this study, the animals maintained normal sleep architecture and cardiorespiratory function.

Sleep/wake state control of arterial blood pressure may be of pathophysiological significance. It has been proposed that the ability to lower blood pressure from daytime to nighttime may protect against cardiovascular morbidity and mortality (2, 12, 13). The data in the present study demonstrate that inbred mice exhibit such a lowering of MAP from wakefulness to sleep, and the magnitude of the decrease is highly reproducible among animals. We have, therefore, provided a framework with which to begin examining the effects of genes, environment, and age on the variability in sleep/wake state control of arterial blood pressure in humans. In particular, identifying inbred or transgenic mice that do not exhibit sleep-related decreases in MAP may allow us to determine whether specific genes are responsible for regulating blood pressure during sleep. Thus, studies of polysomnography and MAP in mice may lead us to the underlying causes of abnormal blood pressure responses to sleep/wake state in humans.

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