Elevated body temperature during sleep in orexin knockout mice

Takatoshi Mochizuki,1 Elizabeth B. Klerman,2 Takeshi Sakurai,3 and Thomas E. Scammell1

1Department of Neurology, Beth Israel Deaconess Medical Center; 2Division of Sleep Medicine, Brigham and Women’s Hospital, Boston, Massachusetts; and 3Department of Pharmacology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan

Submitted 16 December 2005; accepted in final form 2 March 2006

MATERIALS AND METHODS

Core body temperature (Tb) is influenced by many physiological factors, including behavioral state, locomotor activity, and biological rhythms. To determine the relative roles of these factors, we examined Tb in orexin knockout (KO) mice, which have a narcolepsy-like phenotype with severe sleep-wake fragmentation. Because orexin is released during wakefulness and is thought to promote heat production, we hypothesized that orexin KO mice would have lower Tb while awake. Surprisingly, Tb was the same in orexin KO mice and wild-type (WT) littermates during sustained wakefulness. Orexin KO mice had normal diurnal variations in Tb, but the ultradian rhythms of Tb, locomotor activity, and wakefulness were markedly reduced. During the first 15 min of spontaneous sleep, the Tb of WT mice decreased by 1.0°C, but Tb in orexin KO mice decreased only 0.4°C. Even during intense recovery sleep after 8 h of sleep deprivation, the Tb of orexin KO mice remained 0.7°C higher than in WT mice. This blunted fall in Tb during sleep may be due to inadequate activation of heat loss mechanisms or sustained activity in heat-generating systems. These observations reveal an unexpected role for orexin in thermoregulation. In addition, because heat loss is an essential aspect of sleep, the blunted fall in Tb of orexin KO mice may provide an explanation for the fragmented sleep of narcolepsy.

Orexin deficiency in mice strongly resembles human narcolepsy, with fragmented sleep-wake behavior and cataplexy (4, 21). Compared with wild-type (WT) mice, orexin knockout (KO) mice also have less LMA (13, 21), slower heart rate, and lower blood pressure (13). Orexin KO mice thus provide a unique opportunity to study how sleep-wake behavior, LMA, and autonomic dysfunction affect the regulation of Tb. Because the orexin neurons are mainly active during wakefulness (6, 15, 20), we hypothesized that orexin deficiency would reduce Tb, especially during wakefulness. We examined Tb in orexin KO mice with an emphasis on how Tb varies in relation to behavioral state and LMA.

MATERIALS AND METHODS

Animals. Founder orexin KO mice were on a C57BL/6J-129/SvEv background, and their offspring were backcrossed with C57BL/6J mice for 6–8 generations (21). These experiments used 7 male KO mice and 8 wild-type (WT) male littersmates, all 11 wk old and weighing 26–28 g. All experiments were approved by the Institutional Animal Care and Use Committees of Beth Israel Deaconess Medical Center and Harvard Medical School.

Surgery and EEG/EMG recordings. Mice were anesthetized with ketamine-xylazine (100 and 10 mg/kg ip) and implanted with EEG and electromyogram (EMG) electrodes, as described previously (21). EEG signals were recorded using two ipsilateral stainless steel screws (1.5 mm to the right of the sagittal suture; 1 mm anterior to bregma and 1 mm anterior to lambda). EMG signals were acquired by a pair of multistranded stainless steel wires inserted into the neck extensor muscles. A telemetric temperature in rats (31, 33, 37). Injection of orexin into the lateral hypothalamus that affect Tb, sleep-wake behavior, and motor activity (for reviews, see Refs. 30 and 32). Intracerebroventricular injection of orexin A increases Tb (39), perhaps, in part, by increasing wakefulness and locomotor activity (LMA) (5, 10). In addition, orexin may affect Tb through direct autonomic effects. The orexin neurons heavily innervate sympathetic preganglionic neurons of the spinal cord (16, 36) and activate sympathetic outflow; injection of orexin in the lateral ventricle or arcuate nucleus increases arterial blood pressure, heart rate, oxygen consumption, renal sympathetic nerve activity, plasma catecholamines, and colonic temperature in rats (31, 33, 37). Injection of orexin into the diagonal band of Broca also increases the temperature of interscapular brown adipose tissue in anesthetized rats (22). These findings suggest that orexin facilitates heat generation as a result of behavioral and/or sympathetic activation (12, 23). However, few of these studies have considered the effects of behavioral state on Tb control, even though orexin potently increases wakefulness.

Orexin deficiency in mice strongly resembles human narcolepsy, with fragmented sleep-wake behavior and cataplexy (4, 21). Compared with wild-type (WT) mice, orexin knockout (KO) mice also have less LMA (13, 21), slower heart rate, and lower blood pressure (13). Orexin KO mice thus provide a unique opportunity to study how sleep-wake behavior, LMA, and autonomic dysfunction affect the regulation of Tb. Because the orexin neurons are mainly active during wakefulness (6, 15, 20), we hypothesized that orexin deficiency would reduce Tb, especially during wakefulness. We examined Tb in orexin KO mice with an emphasis on how Tb varies in relation to behavioral state and LMA.
with a 12:12-h light-dark (LD) cycle (30 lux; lights on at 7:00 AM and off at 7:00 PM) and a constant temperature of about 23°C.

The EEG/EMG signals were acquired using Grass Model 12 amplifiers (West Warwick, RI) and digitized at 128 Hz using a sleep scoring system (Sleep Sign, Kissei Comtec, Matsumoto, Japan). The signals were digitally filtered (EEG: 0.3–30 Hz, EMG: 2–100 Hz) and semi-automatically scored in 10-s epochs as wake, non-rapid eye movement (NREM), or rapid eye movement (REM) sleep. This preliminary scoring was visually inspected and corrected when appropriate. We operationally defined cataplexy to begin with an abrupt transition from an active wake period to atonia with EEG theta (4–9 Hz) activity and to end with an abrupt return from atonia and theta to an active wake period (21). On rare occasions, orexin KO mice had brief atonia during sustained wake periods that lacked clear theta or delta (0.5–4 Hz) activity, and these indeterminate episodes were scored as a fifth state. These atypical states accounted for only 0.2% of the total recording time and were omitted from further analysis. WT mice never had cataplexy or this fifth state. We recently presented a detailed description of the sleep-wake behavior of these mice (21).

**Analysis of body temperature and LMA at behavioral state transitions.** The signal from the telemetry transmitter was received by an antenna (RPC-1, Data Sciences International) below the recording cage and digitally acquired (Dataquest, Data Sciences International). Tb was sampled for 10 s at 5-min intervals. LMA was measured as movements around the cage and tallied in 5-min bins.

To examine the change in Tb and LMA at major behavioral state transitions, we analyzed sleep-wake behavior in 5-min bins. In an individual animal, when the amount of wakefulness in 5 min exceeded 60%, this bin was considered “wake”. During cataplexy, consciousness is preserved and some wake-promoting brain regions remain active (11). Therefore, cataplexy was included with wake when determining whether a 5-min bin met the above criterion. A “sleep” bin required more than 60% total sleep (NREM and REM) in 5 min. These 60% thresholds were chosen because the results parallel changes in Tb, and in preliminary analyses, we found that more stringent thresholds (70 or 80% wakefulness or sleep/5 min) markedly reduced the numbers of acceptable bins, especially in orexin KO mice. Two consecutive “wake” or “sleep” bins were considered the beginning of a wake or sleep episode, and an episode ended if interrupted by a bin of sleep or wake, respectively. We aligned the Tb and LMA data to the onset of the sleep or wake episodes. In this report, an epoch lasts 10 s, a bin lasts 5 min, and a sleep or wake episode lasts at least 10 min. A bout means one or more consecutive epochs of wake or sleep.

The fragmented sleep of orexin KO mice (21) could alter Tb. Thus, to produce consolidated sleep, we used gentle handling to deprive WT and KO mice of sleep for 8 h beginning at lights on (7:00 AM). In this experiment, analysis of Tb focused on the subsequent recovery sleep at 3:00–4:00 PM.

**Analysis of ultradian rhythmicity in body temperature, locomotor activity, and wakefulness.** We used a cosine spectrum analysis (CirCADia, Behavioral Cybernetics, Cambridge, MA) to measure the periods and amplitudes of the ultradian rhythms of Tb, LMA, and wakefulness. During the dark period, mice often have long, consolidated episodes of wakefulness, sometimes lasting several hours. In our preliminary analysis, these very long episodes of wakefulness interfered with the analysis of ultradian rhythmicity, so the present analysis focused on the 12-h light period. Tb, LMA, and behavioral state data in 5-min bins were fit using a linear, least squares cosine analysis focused on the 12-h light period. Tb, LMA, and behavioral state data in 5-min bins were fit using a linear, least squares cosine analysis focused on the 12-h light period.

For controls, we randomized the same data series chronologically and analyzed them the same way.

**Statistical analysis.** All results are expressed as means ± SE except Fig. 2, which shows data from individual mice. Time-course changes in Tb and LMA were averaged across animals and compared between WT and KO mice using two-way, repeated-measures ANOVAs with post hoc, two-tailed Student’s t-tests.

**RESULTS**

**Hourly variations in Tb, LMA, and wakefulness.** Across 24 h, the Tb of orexin KO mice was slightly higher than that of WT mice (Fig. 1) (genotype × time, F = 1.844, P < 0.01). The mean Tb of orexin KO mice was higher during the 12-h dark period (37.6 vs. 37.3°C in WT and KO, respectively, P < 0.05) but not different during the 12-h light period (36.0 and 36.1°C).

The hourly amounts of wakefulness did not differ between WT and orexin KO mice, but orexin KO mice had less LMA than WT mice (genotype × time, F = 1.589, P < 0.05). This reduction in movement was most apparent during the dark period, though orexin KO mice still had a normal daily pattern of LMA. Specifically, the amount of LMA during the dark period was 3 times higher than during the light period in both groups. These normal amounts of wakefulness and reduced LMA cannot account for the unexpectedly higher Tb of orexin KO mice during the dark period.

![Fig. 1. Orexin KO mice have slightly higher body temperature (Tb) despite lower locomotor activity (LMA).](http://ajpregu.physiology.org/ by 10.22033.6 on June 15, 2017)
Short-term variations in Tb, LMA, and wakefulness. To better understand the control of Tb in orexin KO mice, we examined Tb, behavioral state, and LMA in 5-min bins. Viewed on this time scale, individual WT mice had long episodes of wakefulness at the beginning of the dark period that often lasted several hours (Fig. 2). These were followed by recurring episodes of wakefulness lasting 20–40 min in an ultradian pattern. The Tb of WT mice increased soon after each wake episode began and decreased about 2°C during sleep. Changes in LMA closely paralleled the rhythms of wakefulness and Tb, with large bursts of LMA during the dark period.

In contrast, orexin KO mice had fragmented wakefulness and much less variation in Tb. Ultradian rhythms were much less apparent, but Tb still dropped during the light period. LMA was less than in WT littermates, especially during the dark period. All orexin KO mice exhibited these dampened rhythms in wakefulness, Tb, and LMA.

We examined these ultradian rhythms using a cosine fitting analysis (Fig. 3). During the 12-h light period, WT mice had clear rhythms of Tb, LMA, and wakefulness with periods of about 100 min (compared to the randomized data, condition·time, Tb: F = 3.586, P < 0.0001, LMA: F = 2.277, P < 0.0001, wake: F = 2.772, P < 0.0001). These rhythms were much less apparent in orexin KO mice; the amplitudes of their rhythms at the 90- to 110-min periods were reduced by 64% (Tb), 55% (LMA), and 40% (wake) compared with WT mice. The amplitudes of these rhythms in orexin KO mice did not differ statistically from the randomized data.

Changes in Tb at the onset of spontaneous sleep. Because the Tb of orexin KO mice appeared to vary less with changes in behavioral state, we analyzed Tb around the onset of spontaneous sleep episodes (Fig. 4). During the wake episodes in the dark period, both groups had similar average Tb (around 37.7°C), even though orexin KO mice had less LMA and slightly less wakefulness. Once WT mice started sleeping, their Tb dropped about 1.0°C in the initial 15 min and remained low through the rest of the sleep episode. In contrast, the Tb of orexin KO mice dropped much less (genotype·time, F = 6.636, P < 0.0001), falling only 0.4°C in the first 15 min of sleep (P < 0.01). This blunted fall in Tb is probably not caused by persistent muscle activity because both KO and WT mice had almost no LMA after the onset of sleep. Although the durations of sleep-wake bouts were not considered in this analysis, gross differences in behavioral state did not contrib-

Fig. 2. Orexin KO mice have less variable Tb and very fragmented sleep-wake behavior compared with WT mice. A: when viewed in 5-min bins, a typical WT mouse has a long episode of wakefulness at the beginning of the dark period followed by many episodes of wakefulness in an ultradian pattern. Large variations in Tb and LMA parallel these wake episodes. B: a typical orexin KO mouse has very short wake episodes at all times, and Tb is less variable than in WT mice. LMA is low even during the dark period.
ute either because both groups had similar amounts of sleep and similar types of sleep (REM sleep accounted for 12% and 13% of the total sleep in WT and KO mice, respectively). The pattern was similar during the light period: the Tb of WT mice dropped 0.8°C after 15 min of sleep, but the Tb of orexin KO mice decreased only 0.4°C (genotype \times time, F = 5.182, P < 0.0001). Both groups had similar amounts of sleep and similar ratio of REM sleep to the total sleep in the light period (WT 16%; KO 13%). These results demonstrate that orexin KO mice have higher Tb during sleep, and this cannot be easily explained by differences in the amount or type of sleep.

Changes in Tb at the onset of spontaneous wakefulness. We also examined the Tb of these mice as they transitioned from sleep to wakefulness (Fig. 5). During the dark period, the Tb of WT mice increased 1.2°C over the first 15 min of wakefulness. The average Tb of orexin KO mice during sleep was 0.9°C higher than that of WT mice (from −15 to 0 min, genotype \times time, F = 11.061, P < 0.0001), and it rose 0.6°C during the first 15 min of wakefulness (P < 0.05 compared with the gain in WT mice). After 20 min of wakefulness, both groups of mice plateaued at the same temperature, even though orexin KO mice had less LMA (genotype \times time, F = 2.347, P < 0.01) and slightly less wakefulness (genotype \times time, F = 1.947, P < 0.05). During the light period, the Tb of WT mice rose 1.1°C after 15 min of wakefulness. Orexin KO mice had a slightly higher initial Tb, and their temperature rose 0.5°C after 15 min of wakefulness. Later, in the wake episode, the Tb of orexin KO mice did not achieve the same steady level as in WT mice (genotype \times time, F = 7.787, P < 0.0001), possibly because the wake bouts of orexin KO mice during the light period are so short (21). Orexin KO mice also had less LMA after wake onset (genotype \times time, F = 2.868, P < 0.01). These results illustrate that when they produce sustained wakefulness, orexin KO mice maintain a normal temperature.

Changes in Tb during recovery sleep. Orexin KO mice have fragmented sleep (4, 21) that may disrupt the normal fall in Tb during sleep episodes. To produce more consolidated sleep, we deprived WT and orexin KO mice of sleep for 8 h and then analyzed the subsequent recovery sleep (Fig. 6). Both WT and orexin KO mice started sleeping about 5 min after the end of sleep deprivation. The Tb of WT mice dropped 1.6°C in the first 15 min of recovery sleep, then further decreased to a steady state of around 34.7°C in the next 30 min of recovery sleep (2.6°C lower than at sleep onset). Orexin KO mice showed a similar initial drop of Tb (1.3°C over the first 15 min of the recovery sleep; statistically no different from the drop in WT mice) but then plateaued 0.7°C higher than WT mice (genotype \times time, F = 4.26, P < 0.0001). Neither LMA nor the amounts of wake in the recovery period differed between groups.
This difference in Tb during recovery sleep is probably not influenced by brief awakenings. Orexin KO mice had more awakenings than WT mice in the first 20 min of the recovery period (Fig. 6, bottom), but both groups had similar falls in Tb during this interval. In the next 25- to 60-min period, orexin KO mice had persistently higher Tb, even though the number and duration of awakenings were the same as seen in WT mice. Thus simple fragmentation of sleep is unlikely to explain the smaller drop in Tb of orexin KO mice.

DISCUSSION

We found that the Tb of orexin KO mice falls much less than normal during sleep. Although prior studies implied that orexin KO mice should have lower Tb during wakefulness, we found that Tb is normal during wakefulness despite substantially less LMA. These surprising observations highlight an essential role for orexin in the control of Tb.

Technical considerations. A few technical aspects of our experiments warrant comment. First, several minutes are required for Tb to change in mice, so we analyzed the relation between Tb, LMA, and sleep-wake behavior by collapsing data into 5-min bins. Although WT mice often have brief bouts of sleep and wakefulness that are not fully represented by this time scale (21), the 5-min bins of sleep-wake behavior closely paralleled the changes in Tb over time. Second, we defined a bin as wake or sleep when an animal spent more than 60% of the 5 min awake or asleep because analysis with more stringent criteria (70% or 80% of the 5 min) substantially reduced the number of acceptable bins. Nevertheless, this criterion nicely permits demonstration of ultradian and state-dependent changes in Tb.

Orexin KO mice have elevated Tb during sleep. During sleep, Tb is regulated at a lower level (2, 19). In part, this results from decreased heat production from reduced muscle activity, less nonshivering thermogenesis, and lower basal metabolism. Tb also falls because of an increase in heat loss from tail, ear, and skin vasodilation. In humans, vasodilation of hands and feet correlates well with the onset of sleep (14). The mechanisms that produce these responses are not fully understood, but sleep-promoting neurons in the preoptic area also activate heat loss mechanisms (1, 9, 12, 19, 38). Thus a fall in Tb is an essential aspect of sleep.

Prior studies suggested that orexin deficiency should result in lower sympathetic activity, and so it is surprising that orexin KO mice have higher Tb during sleep. However, people with narcolepsy also have slightly higher than normal Tb during sleep (24, 28), and this abnormal response can be explained by several mechanisms.

First, with the onset of spontaneous sleep, Tb falls more slowly in orexin KO mice, suggesting that heat loss mechanisms might not be fully engaged. Perhaps, orexin KO mice readily fall asleep (21), even without much activity in sleep-

![Fig. 4. During spontaneous sleep, the Tb of orexin KO mice decreases less than normal.](http://ajpregu.physiology.org/)

During the dark (left) and light (right) periods, Tb drops substantially during sleep in WT mice, but the decrease is much smaller in orexin KO mice. Before they enter a sleep episode, orexin KO mice are less consistently awake and have less LMA. The data series for orexin KO mice is shorter during the dark period because they rarely sleep for longer than 20 min during this interval. *P < 0.05; **P < 0.01.
promoting preoptic neurons. Because these preoptic neurons also activate heat loss mechanisms, Tb would fall less during sleep.

In support of this idea, the Tb of orexin KO mice falls at a normal, rapid rate during the initial sleep episode after sleep deprivation. The activity of ventrolateral preoptic area neurons is particularly high during recovery sleep after sleep deprivation (35), and the initial, rapid fall in Tb in orexin KO mice may reflect strong activation of these and other sleep-generating preoptic neurons that reduce Tb. However, this hypothesis is not fully satisfying because, despite presumably similar sleep drive, the Tb of orexin KO mice then remains at a higher level. This persistently higher Tb in orexin KO mice is not a consequence of fragmented sleep-wake behavior, because both groups of mice have the same number of awakenings during this period.

A second explanation is that orexin deficiency may directly impair mechanisms that reduce Tb. Stimulation of the caudal lateral hypothalamus triggers tail vasodilation in rats (41), and the lateral hypothalamic orexin neurons may contribute to this response. Specifically, intracerebroventricular injection of orexin A can reduce Tb in fasted or cold-adapted rats housed in a cool environment (34). Furthermore, the orexin neurons directly innervate many brain regions implicated in thermoregulation, including the preoptic area, dorsomedial hypothalamus, ventromedial hypothalamus, posterior hypothalamus, periaqueductal gray matter, medullary raphe, and intermedio-lateral cell column of the spinal cord (3, 16, 18, 26). These neurons work together to regulate Tb depending on physiological needs, and orexin may be a key signal to control or synchronize the activity of these neurons. Although orexin neurons are mainly active during wakefulness (6, 15, 20), it is possible that orexin has lingering effects during sleep that reduce heat production and facilitate heat loss. A future study of tail or other distal skin temperature in orexin KO mice could demonstrate less vasodilation during sleep.

A third possibility is that the reduced fall in Tb could be caused by sustained thermogenic activity during sleep. Orexin deficiency may reduce sympathetic tone because orexin KO mice have lower blood pressure (13). If orexin deficiency reduces sympathetic activity and LMA during wakefulness, then other heat-generating mechanisms may compensate to produce normal Tb during wake. Thermogenic endocrine signals might be upregulated to maintain heat production, normalizing Tb during wake, but producing an inappropriately high Tb during sleep. Similarly, thermogenic neural mechanisms might be persistently active to compensate for the loss of orexin. Further studies are needed to determine whether neural or endocrine signals inappropriately drive thermogenesis in orexin KO mice.
Orexin and ultradian rhythms. The circadian rhythms of sleep-wake behavior, Tb, and LMA do not require orexin (7, 21), but the ultradian rhythms of Tb and wakefulness are clearly attenuated in orexin KO mice. Few researchers have examined ultradian rhythms in mice, but the Tb of mice varies rapidly with changes in behavioral state, probably because of their small thermal mass. WT mice exhibit large-amplitude and parallel rhythms of Tb and wakefulness with a period of about 100 min. In contrast, orexin KO mice appear to have much smaller ultradian rhythms. Most likely, this reduction in rhythmicity is due to three masking effects: orexin KO mice have smaller falls in Tb during sleep; LMA is reduced, so Tb may not rise as rapidly during active wakefulness; and the duration of wake and sleep bouts is much shorter, so there is less opportunity for sustained increases or decreases in Tb (21).

Implications. A moderate fall in Tb may be necessary for good-quality sleep (8). Tb falls less than normal during sleep in individuals with poor-quality sleep from aging, insomnia, or disorders of autonomic dysfunction, such as multisystem atrophy (17, 27, 40). No model of orexin neurobiology has adequately explained why people and animals with narcolepsy have fragmented sleep. If reduced heat production and activation of heat loss mechanisms are essential for sleep, then a smaller fall in Tb may contribute to the fragmented sleep of narcolepsy.

ACKNOWLEDGMENTS

We thank C. B. Saper, K. Yoshida, and M.P. Anderson for their thoughtful comments on this manuscript. We thank A. Crocker and S. McCormack for their technical assistance.

GRANTS

This study was supported by National Institutes of Health Grants MH-62589, HL-60292, and HD-045459.

REFERENCES


