Sleep apnea in mice: a useful animal model for study of SIDS?

Akira Nakamura a, Tomoyuki Kuwaki a, b, *

a Department of Autonomic Physiology, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan
b Department of Molecular and Integrative Physiology, Graduate School of Medicine, Chiba University, Inohana 1-8-1, Chuo-ku, Chiba 260-8670, Japan

Abstract

Although the incidence of sudden infant death syndrome (SIDS) has been decreased by education programs to avoid sleeping in prone position, the pathological mechanisms of SIDS have not fully been understood. Basic research on sleep apnea using experimental animals may help further understanding and prevention of SIDS because the syndrome is thought as inability to wake up from respiratory arrest (apnea) during sleep. Although several animal models of sleep apnea have been described previously, mice would be useful experimental animals in that these animals are frequently used in genetic engineering. Those considerations prompted us to establish a method for measuring ventilation of mice concomitantly with electroencephalography and electromyography for assessing sleep–wake states. Normal wild-type mice developed two types of central sleep apneas (CSA), that is, post-sigh and spontaneous apneas, as normal humans do. Moreover, post-sigh apneas in mice were observed exclusively during slow-wave sleep (SWS) while spontaneous apneas were seen in both SWS and rapid eye movement (REM) sleep. These characteristics are very similar to those of sleep apneas in healthy human infants and children. Therefore, mice seem to be a promising experimental animal model for studying the genetic and molecular basis of respiratory regulation and dysregulation during sleep in humans, especially infants and children. However, we should keep in mind limitations in studying mice as an animal model of SIDS, since they are nocturnal rodents and they sleep in the prone position.

Keywords: Sudden infant death syndrome; Sleep apnea; Mice

1. Introduction

Sudden infant death syndrome (SIDS) is thought as inability to wake up from respiratory arrest (apnea) during sleep [1,2]. Paradoxically, however, retrospective study showed low incidence of sleep apnea in SIDS victims before they died [3]. We do not know at present whether there is fundamental difference between physiologically returnable sleep apnea and irreversible one. We do not know even precise mechanisms how sleep apnea occurs. Therefore, basic research on sleep apnea using experimental animals may help further understanding and prevention of SIDS.

On the other hand, there is ample evidence suggesting a genetic link in etiology of SIDS [4,5], although the gene(s) that is responsible for pathogenesis of SIDS has not been identified so far. Therefore, use of mice, especially genetically engineered mice, seems promising not only to understand the still unknown mechanisms of sleep apnea, but also to elucidate molecular basis of SIDS.

In this short review, we will briefly summarize classification and clinical aspects of sleep apneas and then address experimental methods and new findings in our recent study using mice on sleep-related regulation of breathing [6]. Future directions and limitations using genetically engineered mice will also be discussed.

2. Classification of sleep apneas

There are two major forms of sleep apneas: obstructive sleep apneas (OSA) and central sleep apneas (CSA). Sleep apneas are classified as obstructive in type if the presence of chest or abdominal wall motion associated with the absence of airflow at the mouth or nose. They are classified as central in type if the absence of chest or abdominal wall motion associated with the absence of airflow at the mouth or nose. Sleep apneas are observed not only in sleep apnea syndrome patients but also in healthy humans [7,8].
Prevalence of sleep apneas in children is quite different from that in adults. In adult human, apneas and hypopneas were found to occur in 24% of males and 9% of females [9]. CSA accounts for less than 11% of total prevalence of CSA and OSA [10]. On the other hand, in normal infants and children, CSA is more frequent than OSA. Marcus et al. [11] reported that normal children (mean age 9.7 ± 4.6, range 1.1–17.4 years, n = 50) had 0.1 ± 0.5 sleep apneah of total sleep time. Eighteen percent of the subjects had OSA, while 30% of them showed CSA. Recently, Tang et al. [12] reported that 18% had OSA and 92% had CSA in 433 healthy children of 8–11 years old. Although percentage varies depending on either the number of subjects or the differences in methodologies, we can safely say that CSA is more frequent than OSA in normal infants and children [13].

3. Central sleep apneas in children and adolescents

3.1. Detailed classification and frequency of central sleep apneas in human children and adolescents

Although detailed classification and terminology are not commonly approved to date, CSA may be further classified into either of three types in healthy children [3,12,14]. First type is associated with the preceding sigh and second type with preceding movement. They are termed as “post-sigh” and “post-movement” apneas, respectively. The third type of CSA, arising without the preceding sighs or movements, is termed as “isolated” apneas [12]. Among 433 children of 8–11 years old, 398 (92%) had isolated CSA, 350 (81%) had post-sigh CSA, and 363 (84%) had post-movement CSA [12]. Isolated, post-sigh, and post-movement types were observed at the rate of 0.9 ± 1.6, 0.9 ± 1.6, and 0 ± 0.5 events/h, respectively.

3.2. Central sleep apneas as sleep-stage-dependent phenomenon

Concerning post-sigh and post-movements apneas, sleep stage dependency has been reported. Namara et al. [14] reported that in 10 normal infants, post-sigh apneas were more frequent during NREM sleep than during REM sleep. Although the examined children vary as to age and gender in those previous reports, there seems a tendency that post-sigh apneas are observed more frequently in NREM than in REM sleep.

As to isolated apneas, on the other hand, we cannot say anything about sleep stage dependency from available literature.

3.3. Mechanism of central sleep apneas in human children and adolescents

Mechanisms of CSA in both children and adults are yet to be understood. Very few studies considered the mechanisms of isolated apneas in human children [12]. However, concerning the post-sigh apneas, at least two possibilities have been proposed. One possibility, “CO2 threshold hypothesis”, is that the prior sigh-induced arterial hypocapnia would diminish CO2-driving force for the next breathing. This is thought to be a major cause of CSA at least in heart failure patients [16]. Second possibility, “mechanoreceptor hypothesis”, is that the prior sigh-induced stretch receptor activation in the airway would activate vagal afferent and neurally inhibit initiation of the next breathing [9]. Both hypotheses may account for the mechanisms of post-sigh apneas. However, it is too difficult to test those hypotheses in human children.

As to sighs, they are thought to serve to prevent lung atelectasis during sleep [17]. However, the mechanisms of sighs also remain to be elucidated. According to McNamara et al. [14], cortical arousals were not observed with any of the sighs that were followed by an apnea in infants and children. On the other hand, the sighs in adult humans can be associated with cortical arousals [18]. Thus, sighs in children seem quite different from those in adults. Sighs might have to be subclassified according to whether they are associated with cortical arousal or not. It seems intriguing to point out that age-linked differences (or in other words, developmental changes) in sighs may be related to the possible maturational difference between cortical arousal and the so-called “brainstem arousal” [19,20].

Interestingly, Kahn et al. [3] reported that post-sigh apneas were significantly less frequent in the future SIDS group. This observation may provide us an important key to elucidate the pathophysiological basis of SIDS, as well as for the physiological basis of post-sigh apneas in healthy children.

4. Sleep apnea in mice

Although several animal models of sleep apnea have been described during the last two decades [21–23], no report had been available on mice until recently. Mice are particularly intriguing in that these animals are frequently used in genetic engineering. Actually, use of transgenic mice has already allowed us to investigate the possible effects of specific genes on certain respiratory functions (see Section 5).

We introduce here our recently established method [6] for measuring ventilation of mice concomitantly with electroencephalogram (EEG) and electromyogram (EMG) for assessing sleepwake state. We also introduce some results from our study especially those that are relevant to SIDS research.
4.1. Measurements of sleep apneas in normal mice

We have recently established a method to record ventilation concomitantly with EEG and EMG in freely moving mice using body plethysmography (Fig. 1) [6]. In brief, at least 7 days after surgical implantation of electrodes for EEG and EMG into the parietal bone and nuchal muscle, respectively, breathing was measured by whole body plethysmography of a flow-through system. Tidal volume, respiratory frequency, minute volume, as well as frequencies of sighs and apneas were calculated during quiet waking, slow-wave sleep (SWS, corresponds human NREM sleep), and REM sleep. Apnea was defined as cessation of plethysmographic signals for at least two respiratory cycles, similar to the criterion used for sleep apnea in humans (cessation of breathing for more than 10 s, i.e., one or two missed breaths). All the recordings were 6 h in length and were performed from 1000 to 1600 h, which is the resting period for nocturnal mice. During the entire recording period, the mouse was allowed to move freely in the chamber. Each chamber was continuously flushed with a gas mixture at a rate of 500 ml/min, either room air (RA) or hypoxic (15% O2) or hyperoxic (100% O2) or normoxic hypercapnic (5% CO2–21% O2–N2) gas mixtures. In one additional animal, EMG of the external intercostal muscle in the eight intercostal space was recorded instead of EEG and neck EMG to check whether the absence of the plethysmographic signal (i.e., apnea) was associated with disappearance of intercostal EMG.

4.2. Sleep apneas in normal adult mice

4.2.1. Two types of central sleep apneas

As well as in human and rats [22,23], normal (wild-type) adult mice suffered from considerable numbers of sleep apneas (~30 times/h). Since basal respiratory frequency in mice was 100–150 min⁻¹, one apnea would be observed in every 200–300 breaths. This ratio seems within the normal range since criteria for sleep apnea syndrome in humans (>5 times/h) correspond one or more apnea in every 120 breaths when basal respiratory frequency is 10 min⁻¹.

Two types of sleep apneas were observed in normal mice. One type was defined as post-sigh apnea, following the prior augmented breath (sigh), while another type was spontaneous one arising without the prior augmented breath. Both apneas are central in type because cessation of respiratory signal in whole body plethysmography was accompanied with disappearance of intercostal EMG.

4.2.2. Post-sigh apnea in mice was sleep-stage dependent

Interestingly, post-sigh apneas were exclusively observed during SWS, although sighs without following apnea were seen in both SWS and REM sleep. As to spontaneous (isolated) apnea, on the other hand, there was no such clear relationship to sleep stages. Sigh-apnea coupling mechanisms in mice is supposed to be sleep-stage dependent.

4.2.3. Similarities of sleep apnea in mice and that in human children

As mentioned above, sleep apnea in adult normal mice is similar to that in normal human children in two points. First, both are predominantly CSA. Second, post-sigh apnea in both humans and mice is predominantly observed in SWS. This is one of the reasons why we think that mouse may be a useful animal model for study of SIDS.

4.3. Mechanisms of sleep apneas in normal mice

Although the mechanisms of sleep apneas in mice are not fully elucidated in our study, we examined "CO2 threshold hypothesis" by changing composition of the inspiratory gas. In our study, post-sigh apneas arose even in hypercapnic condition. This result suggested that post-sigh apnea in normal mice could not result solely from the possible
hypocapnia after the augmented breath. However, we cannot exclude the possibility that the CO₂ threshold may be reset to a higher level under continuous hypocapnic condition. It would be necessary to evaluate breath-by-breath PaCO₂ before making any conclusions on this issue.

Moreover, in order to elucidate the precise mechanisms of post-sigh apneas, we obviously need to know the physiological basis of sighs. In general, they are thought to be related to pulmonary stretch reflexes preventing collapse of alveoli. They may be important particularly in sleeping period when ventilation is usually attenuated. However, this explanation does not account for the sleep-stage-dependent mechanisms of sigh- apnea coupling in mice because post-sigh apneas arise exclusively during SWS but not during REM sleep when minute volume was much more attenuated than in SWS and waking.

As to spontaneous apneas, we can only point out that those were observed mainly in REM sleep. In REM sleep, ventilation was unstable in normal mice as in normal humans. Irregular rhythm of respiration may result in spontaneous apnea.

In order to investigate pediatric sleep apneas, it would be interesting to study infant mice. EEG recording may be difficult because of their small size and because dam may not care operation-damaged children. However, we have shown that whole body plethysmography is applicable even to newborn mice [24]. We do not know whether infant mice suffer from SIDS and it would be worth testing.

5. Future research for understanding of the genetic and molecular basis of sleep apneas using mice

Mouse may be a useful animal model for study of SIDS from the following two reasons. First, as mentioned above, there is resemblance between mice and human in that type of sleep apnea is associated with sleep stage. Namely, normal mice developed post-sigh apneas exclusively in SWS, as healthy human children and adolescents have post-sigh apneas predominantly in NREM sleep. Elucidating the mechanism of the post-sigh apneas in mice may shed light on the understanding pathophysiological basis of SIDS, taken together with a reduced number of post-sigh apneas in SIDS [3].

Second, genetic engineering can be easily applied to mice, compared to other mammal animal models. At present, the genetic and molecular bases of central sleep apnea and related disorders have been gradually unveiled by molecular genetic methods. For example, some mutations of genes have been reported in congenital central hypoventilation syndrome (CCHS) patients [25–27]. The transgenic mouse deficient in some of those genes actually showed the ventilatory failure and confirmed causative relationship in the gene in CCHS [28]. Taking advantages of such characteristics of mice, we will be able to study the genetic and molecular basis of post-sigh and spontaneous apneas.

One intriguing example of transgenic mice for studying sleep apneas may be prepro-orexin knockout mice, which has already contributed to unveiling the neurobiology of sleep–wake regulation, particularly the pathophysiological basis of human narcolepsy [29]. Orexin, discovered in 1998, is a neuuropeptide serving as neurotransmitter in the central nervous systems. The cell bodies containing orexin are located exclusively in the lateral and dorsal hypothalamic areas and their axons diffusely innervate almost the entire central nervous system including those regulating respiration [30]. In addition to sleep–wake regulation, orexin system also contributes to regulation of feeding behavior and hemodynamic adjustment to emotional stress [31]. Moreover, in our preliminary experiment, we found that prepro-orexin knockout mice showed spontaneous apneas much more frequently than normal controls in both SWS and REM sleep (unpublished observation). Interestingly, also in narcolepsy patients, the numbers of central sleep apneas were increased [32]. These findings strongly suggest that orexin system may serve to preserve appropriate ventilation during sleep.

We should keep in mind that, however, there are limitations in studying mice as animal models of SIDS. Mice are quite different from human, in that they are nocturnal rodents and that they sleep in the prone position. Moreover, no one knows at present whether revealing mechanisms of sleep apnea in normal animals directly lead to elucidation of mechanisms in irreversible cessation of ventilation in SIDS. We admit that genetically engineered mice will be better but not the best or the sole animal model for the study of SIDS. Obviously, basic research on respiratory regulation is further needed.

References


