Hypercapnic duty cycle is an intermediate physiological phenotype linked to mouse chromosome 5

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Schneider, H., S. P. Patil, S. Canisius, E. A. Gladmon, A. R. Schwartz, C. P. O’Donnell, P. L. Smith, and C. G. Tankersley. Hypercapnic duty cycle is an intermediate physiological phenotype linked to mouse chromosome 5. J Appl Physiol 95: 11–19, 2003; 10.1152/japplphysiol.01144.2002.—We hypothesized that upper airway obstruction (UAO) leads to a compensatory increase in the duty cycle [ratio of inspiratory time to respiratory cycle length (Ti/Ṫr)], which is determined by genetic factors. We examined the compensatory Ti/Ṫr response to 1) UAO and hypercapnia among normal individuals and 2) hypercapnia in different inbred strains, C3H/HeJ (C3) and C57BL/6J (B6), and their first- and second-generation (F2) offspring. 3) We then used the compensatory Ti/Ṫr response in the F2 to determine genetic linkage to the mouse genome. First, normal individuals exhibited a similar increase in the Ti/Ṫr during periods of hypercapnia (0.11 ± 0.07) and UAO (0.09 ± 0.06) compared with unobstructed breathing (P < 0.01). Second, the F2 offspring of C3 and B6 progenitors showed an average Ti/Ṫr response to 3% CO2 (0.42 ± 0.005%) that was significantly (P < 0.01) greater than that of the two progenitors. Third, with a peak log of the odds ratio score of 4.4, Ti/Ṫr responses of F2 offspring are genetically linked to an interval between 58 and 64 centimorgans (cM) on mouse chromosome 5. One gene in the interval, Dagk4 at 57 cM, is polymorphic for C3 and B6 mice. Two other genes, Adrb2k2 at 60 cM and Nos1 at 65 cM, have biological plausibility in mechanisms of upper airway patency and chemosensitivity, respectively. In summary, Ti/Ṫr may serve as an intermediate physiological phenotype for compensatory neuromuscular response mechanisms for maintaining ventilation in the face of UAO and hypoventilation and to help target specific candidate genes that may play a role in the expression of sleep-disordered breathing.

obstructive sleep apnea; respiratory phenotype; hypercapnia

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on careful phenotypic analysis. Moreover, the diversity of phenotypic expression of OSA requires clearly defined physiological characteristics to facilitate this analysis.

The overall purpose of this study is to establish that compensatory duty cycle responses to hypercapnia and UAO may serve as intermediate physiological phenotypes for studying the genetic basis of OSA. Specifically, we 1) compare the heterogeneity of compensatory duty cycle responses to hypercapnia and UAO in normal human individuals with the variability observed in different inbred strains and their offspring, and 2) establish genetic linkage between the duty cycle responses to hypercapnia as a specific physiological trait and the mouse genome, which has testable homology to the human genome. Our results demonstrate that the duty cycle response to hypercapnia and to UAO is a distinct compensatory neural response mechanism that serves as an intermediate physiological phenotype of sleep-disordered breathing and is linked to mouse chromosome 5.

METHODS

Experimental Approach

The following conceptual framework forms the basis for our approach to characterizing the respiratory phenotypes predisposing to hyperventilation during periods of UAO. According to the classic relationship (2, 18, 19) \( V_t = (V_T/T_I) \times (T_I/T_T) \), where \( V_t \) is the inspired minute ventilation, \( V_T \) is the tidal volume, \( T_I \) is the inspiratory time, \( T_T \) is the respiratory cycle length, and \( T_I/T_T \) is the inspiratory duty cycle, it can be seen that two distinct physiological components defend ventilation in response to UAO. The first component is the inspiratory airflow \( (V_T/T_I) \), whereas the second is the duty cycle. Although the mechanisms involved in stabilizing ventilation during sleep in the presence of UAO have not been well defined, UAO is known to increase ventilatory drive, which increases the mean inspiratory flow. If the upper airway collapses, however, such increases in drive cannot generate any increase in the mean \( V_T/T_I \), because inspiratory flow would be limited to a maximal level that cannot be exceeded as effort increases (27, 34). During periods of \( V_T/T_I \) limitation, therefore, increases in ventilatory drive can no longer prevent the individual from hyperventilating during sleep. Rather than increasing ventilatory drive and mean \( V_T/T_I \), patients can only preserve ventilation in the face of UAO by prolonging the \( T_I/T_T \).

In humans, to test the ability of a normal individual to prolong the duty cycle in response to UAO, we controlled the level of UAO by lowering the nasal pressure (Pn) to negative levels, as previously described (26), and compared duty cycle responses to UAO between individuals. We further examined the effect of duty cycle responses in tracheostomized apneic patients. Accessing the trachea allowed us to administer a precise level of inspiratory air directly into the trachea, despite persistence of UAO, as previously described (25). Moreover, this technique also allowed us to administer a defined level of CO\(_2\) during sleep without changing the level of \( V_T/T_I \) and degree of UAO.

In mice, compensatory duty cycle responses were examined in two inbred mouse strains with genetic differences in the control of breathing. On the basis of previous findings in the B6 and the C3 strain (36), we predicted that a mild hypercapnic challenge would disclose differences in the duty cycle response between strains and among their offspring that would then allow a link to a specific genetic locus.

**Human Studies**

**Subjects.** Apneic patients. Tracheostomized patients, referred to the Johns Hopkins Sleep Disorders Center, were eligible for this study if they had OSA, as defined by a non-rapid eye movement (NREM) apnea-hypopnea index of greater than five episodes per hour and were free of clinical and laboratory evidence of the Pickwickian syndrome. Three such patients were recruited, with a mean age of 37.0 ± 13.1 yr and mean body mass index (BMI) of 44.5 ± 13.9 kg/m\(^2\).

Tracheostomy was employed because patients were either noncompliant \((n = 2)\) or refractory \((n = 1)\) to treatment with nasal continuous positive airway pressure. The protocol was approved by the Johns Hopkins Institutional Review Board, and informed consent was obtained from each patient.

**Normal individuals.** Individuals were recruited through both media interest and paid advertisements in local newspapers. Normal subjects were recruited and considered eligible if they were adults between 18 and 50 yr of age and had a BMI between 20.9 and 35.0 kg/m\(^2\), without habitual snoring. Individuals were excluded if they had any serious medical illness or an abnormality in a standard clinical examination or a lung function test (as defined by ratio of forced expiratory volume in 1 s to forced vital capacity <80% predicted, total lung capacity <80% predicted, lung CO diffusion capacity <80% predicted, resting daytime arterial P\(_{O_2}\) <55 Torr, and resting daytime arterial P\(_{CO_2}\) ≥45 Torr). Eleven normal subjects (mean age 36.4 ± 13.8 yr, mean BMI 29.8 ± 5.7 kg/m\(^2\)) participated in our protocol, which was approved by the Johns Hopkins Institutional Review Board, and informed consent was obtained from each individual.

**Experimental techniques.** Experimental apparatus. Pressure was controlled at the nose (Pn) or trachea over a range from −15 to 20 cmH\(_2\)O, as previously described (26). In brief, both a positive and a negative pressure were generated through a modified continuous positive airway pressure device (Moritz-CPAP, MAP Medizin-Technologie, Martinsried, Germany) that could be used to switch pressures from one level to the other. The outflow from this machine was connected in series to a pneumotachometer (model 3700A, Hans Rudolph, Kansas City, MO) and to either a nasal mask or to the patient's tracheostomy tube. Patients were each fitted with a no. 6 Shiley cuffless tracheostomy tube (6.4 mm ID, 10.8 mm OD, 76 mm length; Mallinckrodt, Hazelwood, MO).

**Polysomnography.** Standard polysomnographic monitoring was performed during all study protocols and included monitoring of electroencephalograms (C\(_3\)-A\(_2\), C\(_3\)-O\(_2\)), left and right electrooculograms, submental electromyogram, and electrocardiogram (modified V\(_2\) lead). Oxygen saturation was also monitored (Biox 3700, Ohmeda, Boulder, CO). Body position was monitored visually with infrared video cameras to maintain patients in a supine posture throughout the protocol.

**Respiratory monitoring.** Tidal airflow was monitored with a pneumotachometer affixed to a tight-fitting nasal mask. Pn was monitored through a side hole in either the nasal or face mask, and all pressures were monitored with Gould-Statham transducers (P23ID, Gould Electronics, Cleveland, OH).

**Data acquisition.** All pressures, flows, and polysomnographic parameters were amplified and recorded continuously on a polygraph recorder (Grass recorder, Astromed, Warwick, RI). Signals from analog amplifiers were also digitized at 100 Hz and stored on optical disk for off-line analysis.
DUTY CYCLE RESPONSES ARE LINKED TO MOUSE CHROMOSOME 5

(AUO were taken from the last just before the sustained drop in Pn. Duty cycle responses to timing indexes were calculated in the unobstructed condition. In this experiment, normal individuals initiated low- to assess for differences in duty cycle between baseline and insufflation, as previously described (3, 26). When subjects had stable NREM sleep, the Pn was then lowered abruptly for five breaths repeatedly over a range encompassing zero airflow. The passive maximal Vt vs. Pn relationship was described from breaths at various Pn levels (3, 26) and were solved to determine the passive Pcrit as the Pn at zero flow. A short, sustained period of UAO was then induced experimentally by lowering the Pn 1 cm above the passive Pcrit level and maintaining it at this level for 3 min. At this Pn level, UAO ensued, depending on the magnitude of compensatory neural responses to UAO. Duty cycle responses during periods of UAO were then compared with the baseline (unobstructed breathing periods at holding pressure).

EXPERIMENT 2: EXAMINING DUTY CYCLE RESPONSES TO MILD HYPERCAPNIA AND AIRFLOW LIMITATION DURING SLEEP. In this experiment, we determined ventilatory responses to J 3% hyperoxic CO₂ and 2% marked reductions in the inspiratory flow during stable NREM sleep in the tracheostomized apneic patients. The level of flow was controlled by utilizing the transtracheal insufflation technique, as previously described (25). In brief, patients’ tracheostomies were capped, and 15 l/m of 40% O₂/air were insufflated directly into the trachea after UAO had occurred during stage 2 NREM sleep. This flow rate was shown to stabilize the sleep breathing pattern while the upper airway remained obstructed during each inspired cycle (25), making the patients solely dependent on the insufflated air. The transtracheal insufflation of air was maintained for ~5 min of sleep, and either the level of airflow was then reduced to 5 l/m or a blend of 3% CO₂-40% O₂ was added. The timing parameters during these conditions were compared with the baseline (high-flow condition).

Analytic methods. INDEXES OF SLEEP-DISORDERED BREATHING. Standard polysomnographic techniques were utilized to determine the indexes of sleep-disordered breathing, as previously described (33). In brief, an apnea was defined by the complete cessation of airflow for >10 s. Hypopnea was defined as a >50% reduction in airflow associated with either an arousal from sleep or >4% oxyhemoglobin desaturation. The mean baseline and average low oxyhemoglobin saturations for the apneas and hypopneas were calculated. Assessing TT/Tt responses to UAO. The baseline TT/Tt was measured during periods without UAO when Pn was at +5 cmH₂O (holding pressure). Respiratory pattern indexes (Tt, Tr, and Tt/Tt) were calculated at two time points. Baseline timing indexes were calculated in the unobstructed condition just before the sustained drop in Pn. Duty cycle responses to UAO were taken from the last five breaths of the sustained period of UAO or, if an arousal occurred during the period, the last five breaths before that arousal. The difference in the duty cycle between baseline and UAO was taken to determine the strength of compensatory responses of the respiratory pattern control to UAO.

Statistical analysis. Paired two-tailed t-tests were utilized to assess for differences in duty cycle between baseline and UAO in the normal subjects and between hypercapnic and low-flow conditions in the apneic patients (Minitab, State College, PA). A value of P < 0.05 was considered to be statistically significant.

Animal Studies

Animals. Male C3H/HeJ and C57BL/6J progenitor strains and their B6C3F1/J (F1) offspring were purchased from Jackson Laboratory (Bar Harbor, ME). Male and female F1 mice were also purchased to establish breeding colonies at the Johns Hopkins Bloomberg School of Public Health. Intercross progeny (F2) were generated from F1 progenitors and weaned at 4–5 wk of age. From the breeding colonies, randomly selected male F2 offspring (n = 69) were housed in cages of four to six animals for an additional 6–12 wk. Water and mouse chow (Agway Pro-Lab RMH 1000) were provided ad libitum. All animal protocols were reviewed and approved by the Animal Care and Use Committee of the Johns Hopkins Bloomberg School of Public Health.

Whole body plethysmography. Ventilatory parameters in mice were determined by whole body plethysmography, and a comprehensive description of this method has been described elsewhere (7, 33, 36). In review, the breathing pattern was monitored by the barometric technique, after the chamber was completely sealed for a period of <1 min. At a constant chamber volume, changes in pressure due to inspiratory and expiratory temperature fluctuations were measured by using a differential pressure transducer (model 8510 B-2, Endevco), and the oscillations of chamber pressure were recorded on a strip-chart recorder (model 7 D polygraph, Grass). The pressure signal was also transmitted to a dedicated computer, from which breathing frequency, Vt, and Tt were determined. Pressure transducer calibrations were performed daily by using a 50-μl gas-tight syringe, while the chamber temperature approximated experimental ambient conditions. Ventilatory function was evaluated at baseline and during mild hypcapnic challenge (inspired CO₂ fraction = 0.03 and inspired O₂ fraction = 0.21 in N₂) for 3–5 min.

Mouse genotyping. After the breathing measurements, DNA samples were isolated from 52 randomly selected F2 mice. Primers surrounding DNA microsatellite markers (n = 176) polymorphic between C3 and B6 mice were purchased from Research Genetics (Huntsville, AL). The primers were used to amplify sample DNA, and genotypes of the F2 offspring were determined by comparing the allelic size of a simple sequence repeat to the known allele size of the parental strains. Markers were selected to establish 10- to 15-centimorgan (cM) intervals between two loci and to provide coverage of the entire mouse genome with 95% confidence. Linkage analysis was performed by using the computer software programs Mapmaker-EXP and Mapmaker-QTL (14, 15). Linkage was inferred when the log of the odds ratio (i.e., called a LOD score) in favor of linkage over nonlinkage was 1,000:1 (i.e., LOD > 3.3). The candidate region surrounding the putative quantitative trait loci (QTL) was examined for genes with biological relevance to ventilatory control and polymorphisms between C3 and B6 strains. Finally, comparative mapping was performed to determine homologies between the mouse and human genomes.

Statistical analysis. One-Way ANOVA was utilized to assess for differences in duty cycle between strains, and a paired two-tailed t-test was utilized to assess for differences in duty cycle between baseline and hypercapnia (Minitab). Bonferroni’s post hoc comparisons were performed to determine the source of significance when significant differences were found by the ANOVA. A value of P < 0.05 was considered statistically significant.
RESULTS

Human Studies

Experiment 1: Effect of UAO on duty cycle responses in normal subjects. The effects of UAO on timing indexes are illustrated in one subject in Fig. 1. UAO was induced by lowering the nasal pressure (Pn) from 5 cmH2O (A) to -5 cmH2O (B) to induce UAO. Respiratory pattern indexes (T_I, T_T, and T_I/T_T) are shown for the baseline, unobstructed condition (A) and for the first 5 breaths immediately after the induction of UAO. As shown in the tables in Fig. 1, A and B, the duty cycle was markedly elevated during UAO compared with the period with unobstructed breathing.

In Fig. 2, individual and mean data for the duty cycle responses to UAO are shown. Duty cycle responses increased from 0.39 ± 0.6 (SD) to 0.49 ± 0.1 (SD) at the baseline and UAO time points, respectively (P < 0.01). The findings indicate that UAO leads to a substantial increase in T_I/T_T in normal individuals. The change in duty cycle from baseline increased 0.09 ± 0.02 on average (P < 0.05), but individual data points (Fig. 2B, right) illustrate a substantial variability (0.03–0.18) of the change from baseline to obstructed periods.

Experiment 2: Duty cycle responses to mild hypercapnia and severe airflow limitation during sleep in apneic patients. In Fig. 3, the effect of CO2 and airflow limitation on the duty cycle is illustrated for one patient (RS). Three conditions were obtained in each patient. In condition 1 (high flow, no CO2), air was insufflated at a rate (15 l/m) of 40% O2-60% N2 (Fig. 3A). In condition 2, 3% CO2 was added, while the airflow was maintained at the high-flow rate (Fig. 3B). In condition 3, the insufflated airflow was reduced to 5 l/m in 40% O2-60% N2 to produce severe flow limitation (low flow, no CO2). The duty cycle increased substantially during both the hypercapnia and the low-flow condition. Similar findings were observed in all patients. Figure 4 shows the pooled data for duty cycle responses to hypercapnia and severe flow limitation and indicate that both stimuli are potent compensatory mechanisms during periods of UAO.

Animal Studies

As shown in Fig. 5, the T_I/T_T responses at baseline and during 3% CO2 vary among C3 and B6 progenitors and their F1 offspring. At baseline, the B6 strain dem-
onstrated a significantly \((P < 0.05)\) lower Ti/Tt response compared with C3 mice (Fig. 5A). In addition, Ti/Tt responses were significantly \((P < 0.05)\) greater in F1 mice compared with both progenitors. Each strain demonstrated a significant \((P < 0.01)\) increase in Ti/Tt during 3% CO2. The variation between progenitors at baseline was obscured during 3% CO2 because of a greater increase in Ti/Tt in B6 compared with C3 mice (Fig. 5B). However, the Ti/Tt response during 3% CO2 remained significantly \((P < 0.05)\) elevated in F1 mice relative to both progenitor strains.

In Fig. 6, segregation plots show Ti/Tt responses to 3% CO2 for C3 and B6 progenitors and their first- and second-generation offspring. In Fig. 6A, the F2 mice showed an average Ti/Tt response to 3% CO2 \((0.42 ± 0.005)\) that was significantly \((P < 0.01)\) greater than that of the two progenitors, and the range was not distinguishable from F1 responses. In Fig. 6B, the inheritance pattern is shown for individual changes in Ti/Tt from baseline (room air) to 3% CO2. The significantly augmented Ti/Tt response in B6 mice is consistent with a greater CO2 ventilatory sensitivity com-

\[\begin{align*}
\text{A} & \quad (\text{high flow, 40% O}_2/\text{RA}) \\
\text{B} & \quad (\text{high flow, 3% CO}_2/40\% \text{ O}_2/\text{RA}) \\
\text{C} & \quad (\text{low flow, 40% O}_2/\text{RA})
\end{align*}\]

\[\begin{align*}
\frac{V}{(\text{ml/s})} & \quad 250 \\
\frac{T_i}{(\text{cmH}_2\text{O})} & \quad 10 \\
\text{SaO}_2 & \quad 100 \\
\text{CO}_2 & \quad 5 \\
\text{EMG}_{\text{Dia}} & \quad 50
\end{align*}\]

Fig. 3. Effects of hypercapnia (CO2) and severe flow limitation on timing mechanisms are illustrated in 1 subject. Compared with the high-flow condition without CO2 (A), Ti/Tt increased substantially during both the high-flow hypercapnic (B) and the severe flow-limited condition (C). Prach, tracheal pressure; CO2, level of administered CO2; SaO2, arterial oxygen saturation; EMG_{Dia}, electromyogram of the diaphragmatic muscle; RA, room air.

Fig. 5. Average duty cycle (means ± SE) responses at baseline and during 3% CO2 are depicted for C3 and B6 progenitors \((n = 20 \text{ mice/strain})\) and their F1 offspring \((n = 14 \text{ mice})\). A: the average duty cycle is significantly greater in F1 mice compared with both progenitors. B: the change in duty cycle from baseline to 3% CO2 is significantly greater in B6 mice relative to C3 and F1 mice. *\(P < 0.05\), B6 differs from the other 2 strains. †\(P < 0.05\), F1 differs from the other 2 strains.

Fig. 4. Effect of hypercapnia and severe inspiratory flow limitation is depicted for the apneic patients \((n = 3)\). A: average duty cycle increased from the high-flow \((15 \text{ l/m})\) O2/RA (O2) condition to the high-flow hypercapnia (CO2) condition and the low-flow \((5 \text{ l/m})\) O2 condition. B: the change from baseline high-flow O2 did not significantly differ between both respiratory challenges. Values are means ± SD. NS, not significant. See text for details.
pared with that in both the C3 progenitor and the F1 offspring. Seventy percent of the variation of the duty cycle in the F2 mice is explained by the inheritance pattern, whereas 30% of the variation among responses in the F2 generation is explained by the locus on chromosome 5. All in all, the distribution of F2 offspring. *P < 0.05 vs. the other 2 strains.

DISCUSSION

In the present study, we conducted parallel experiments in inbred mice and humans to explore the genetic basis for variation in duty cycle responses to UAO. We hypothesized that UAO leads to a compensatory physiological phenotype for compensatory neuro-muscular response mechanisms that are important in maintaining ventilation in the face of an increased load, such as seen with airflow limitation, OSA, and hypoventilation. Moreover, homology with the human genome will help target specific candidate genes that may play a role in the pathogenesis and expression of sleep-disordered breathing.

Like hypertension and diabetes, OSA is certainly a complex trait. As such, a multigenic process modifies the genetic basis for disease etiology. Hence, to study the genetic etiology of sleep-disordered breathing, complex disease phenotypes must first be dissected to discern intermediate phenotypes that precede other clinical manifestations. Another major difficulty in human genetic studies is the complexity and the heterogeneity among individual human genomes. This obstacle is somewhat overcome by using extremely large numbers of subjects needed to perform association or linkage studies. Alternatively, our approach exploits the comparative linkage maps between the mouse and human genomes. A principal step in this alternative strategy is establishing an intermediate phenotype that is comparable between mice and humans. In the present study, we determined the duty cycle response associated with breathing mild hypercapnia as one possible, but distinct, respiratory phenotype, essential to the pathogenesis of sleep-disordered breathing. Our data indicate that the duty cycle response tests compensatory mechanisms responsible for defending ventilation in the face of UAO.
We identified the duty cycle as a distinct physiological component that may play a pivotal role in defending ventilation in response to UAO. However, the duty cycle contains a physiological ceiling of ~0.6, at which no further increases of the duty cycle are possible (2, 18, 19). For example, if the duty cycle during unobstructed breathing is already >0.5, an individual would be able to increase the duty cycle and minute ventilation by only 20%, whereas another with a duty cycle of 0.3 at baseline may increase the duty cycle and ventilation by 200%. Therefore, the duty cycle during unobstructed breathing may determine an individual’s ability to respond to UAO. Nevertheless, the strength in the present study is derived from our controlling the level of UAO and examining the duty cycle responses to UAO relative to baseline, unobstructed breathing. We found that comparable levels of UAO in normal individuals exhibited substantial variation in the magnitude of duty cycle response, even when the baseline duty cycle was similar between subjects. The upper airway response varied between 0.02 and 0.18, indicating that the duty cycle in response to UAO may serve as an intermediate physiological trait that may provide a link to specific genetic factors relevant to the expression of OSA.

To further explore the genetic basis for duty cycle variations, we conducted genetic linkage studies in inbred mouse models. In these models, we used a mild hypercapnic (3%) challenge on the basis of the observation in our human protocol, in which mild hypercapnia produced similar duty cycle responses as UAO. The mechanisms for similar increases in the duty cycle during both conditions are not entirely clear. Whereas hypercapnia increased the duty cycle incrementally, UAO led to an immediate increase in duty cycle, indicating that other neural reflex mechanisms might be involved (19). Nevertheless, irrespective of the precise mechanism involved, the similarity of duty cycle responses between both stimuli in each patient indicates that a mild hypercapnic stimulus in a murine model may also disclose intermediate physiological phenotypes. Indeed, when we determined duty cycle responses to mild hypercapnia in inbred strains of mice and their offspring generations, we found that the F2 offspring showed an increase in the hypercapnic duty cycle response and a variation of this response that overlapped those of the progenitors, indicating that the duty cycle variation may be genetically regulated. Taken together, the mild hypercapnic stimulus disclosed specific intermediate phenotypes that can be used to examine the genetic basis for duty cycle responses.

Our results in the animal studies showed that a putative QTL on mouse chromosome 5 modulates duty cycle responses. The interval on mouse chromosome 5 containing the QTL is centered at ~58 cM from the centromere (i.e., D5Mit239). The putative QTL implies that a gene or suite of closely positioned genes differs in nucleotide sequence to modulate variation in hypercapnic duty cycle. Within a broader interval surrounding the QTL, only two genes, Dagk4 and Gus, are characterized as differing in nucleotide sequence between C3 and B6 strains. The product of the Dagk4 gene, in particular, is one of a family of diacylglycerol kinases, which play a pivotal role in signal transduction conversion of diacylglycerol, a lipid second messenger, to phosphatidic acid (20). The role of diacylglycerol kinases in upper airway function and adaptation is unclear.

The QTL interval also contains other genes with biological relevance to the control of breathing and upper airway control. For example, the Nos1 gene, located at 65 cm on mouse chromosome 5, has been shown to be instrumental in regulating peripheral chemosensitivity (16). Mice deficient in neuronal nitric oxide synthase (Nos1 knockout mice) have shown augmented hypoxic ventilation compared with wild-type mice (13a). One group of investigators hypothesized that nitric oxide derived from Nos1 inhibits peripheral chemosensory input in response to hypoxia (16). Variation in peripheral chemosensory input may likely manifest differences in duty cycle among the F2 off-

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spring of C3 and B6 progenitors. Another potential candidate gene within the QTL interval that has biological relevance to upper airway control is the Adrbk2 gene at 60 cM. The patency of the lower airways is likely dependent on β2-adrenergic-receptor activation (17). A family of G-protein-coupled receptor kinases, such as β2-adrenergic-receptor kinase, is chiefly involved in desensitizing the receptor by phosphorylation. Variation in β2-adrenergic-receptor kinases has been postulated to account for desensitization differences among different airway cell types (17). In the present study, sequence variation in the Adrbk2 gene between strains and among their F2 progeny may modulate the lower airway patency, as mediated by differences in β2-adrenergic-receptor sensitization mechanisms. Comparative linkage maps for the mouse and human genomes suggest homology for Nos1 and Adrbk2 genes on human chromosomes 12 and 22, respectively (13a, 37). In addition, there is suggestive evidence for the Dagk4 gene to be located on human chromosome 4 (5).

A limitation of the present study is that the duty cycle is known to depend on several nongenetic factors that may have confounded our results. Although we reduced potential confounders by confining our analysis of duty cycle responses during periods of stable NREM sleep in our human subjects, the duty cycle may not merely be a reflection of the inherent respiratory pattern control but also of dissociations between neural and mechanical timing and differences in upper airway resistances that may influence the expiratory and Vt/Ti dynamics (19) among individuals and across different mice strains. We did not determine indexes of airflow dynamics during unobstructed breathing at baseline, in either our human or murine experiments. Thus we cannot entirely rule out differences in the airflow dynamics as a determinant for duty cycle differences in humans and murine strains. In addition, the variable increases in duty cycle may also be a result of variations in the neural reflex pathway, including afferent inputs, central responses, and variation in the efferent signal, such as variability in vagal tone (12, 38). Although we did not precisely control all aspects of the neural reflex pathway between experimental conditions, the tracheostomy allowed us to control the level of Vt/Ti and the degree of UAO best (24), while independently changing the inspiratory CO2 content, as outlined in Experimental Approach in METHODS. We found that, in humans, the duty cycle increased similarly during periods of UAO and mild hypercapnia. The similarity of the duty cycle response to both stimuli led us conclude that the duty cycle may serve as an intermediate physiological phenotype for neuromuscular compensatory mechanisms to a respiratory load. Moreover, irrespective of whether baseline duty cycle is primarily controlled by inherent differences of the respiratory pattern control or differences in airflow dynamics and reflex pathways, our results show that the duty cycle during unobstructed breathing and the strength of the duty cycle response may determine an individual’s ability to defend ventilation in the face of UAO.

Implications

There are several physiological, genetic, and clinical implications of our approach. First, our conceptual approach allows for the dissection of ventilation into two major physiological components, of which the duty cycle determines an individual’s ability to defend ventilation in the face of UAO. Individuals failing to augment VT/Ti, therefore, would be predisposed to the development of hypoventilation. Second, studies in twins suggest that the duty cycle has a genetic trait (31, 32). Our experiments in the murine models now clearly establish the duty cycle response to mild hypercapnia as a physiological intermediate and genetically determined trait. Comparative linkage maps for the mouse and human genomes suggest homology for Nos1 and Adrbk2 genes on human chromosomes 12 and 22, respectively (37). The homology between the murine and human genome strongly supports testing the modulatory role of these genes in the regulating ventilatory pattern control in humans. Moreover, studies can now be designed to test whether variations in the compensatory duty cycle responses are primarily due to polymorphisms in specific regions identified by QTL or to environmental components. Third, although specific risk factors are known to predispose patients with UAO to the development of hypoventilation, it is unclear why individuals vary markedly in their susceptibility to this disorder. A major advantage of our approach is that a physiological trait can be assessed in normal individuals before the clinical manifestation of OSA. Moreover, by assessing both the baseline and response characteristics of the respiratory pattern control, it may be possible to quantify an individual’s susceptibility or resistance to the development of nocturnal hypoventilation in the presence of OSA.

In summary, our findings begin dissecting and characterizing distinct physiological phenotypes of ventilation when UAO is induced experimentally during sleep. Our analytic approach allows us to determine the physiological basis for susceptibility or resistance to hypoventilation in the face of UAO and to risk stratify these individuals on the basis of defined physiological parameters. Finally, examination of the physiological phenotype in segregant offspring of inbred mouse strains with a known genetic variation in chromosome 5, which may have correlates in the human genome (37).

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