Ventilatory behavior after hypoxia in C57BL/6J and A/J mice

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Han, Fang, Shyam Subramanian, Thomas E. Dick, Ismail A. Dreshaj, and Kingman P. Strohl. Ventilatory behavior after hypoxia in C57BL/6J and A/J mice. J Appl Physiol 91: 1962–1970, 2001.—Given the environmental forcing by extremes in hypoxia-reoxygenation, there might be no genetic effect on posthypoxic short-term potentiation of ventilation. Minute ventilation (V˙E), respiratory frequency (f), tidal volume (VT), and the airway resistance during chem-}

tentiation

ventilatory control; genetics; mouse strains; short-term po-

sponses to reoxygenation indicate that genetic mechanisms

pressure-volume curves were also performed. In 12 males for each strain, after 5 min of 8% O2 exposure, B6 mice had a predominant decrease in V˙E on reoxygenation with either air (−11%) or 100% O2 (−20%), due to the decline of f. In contrast, A/J animals had no ventilatory undershoot or f decline. After 5 min of 3% CO2-10% O2 exposure, B6 exhibited significant decrease in V˙E (−28.4 vs. −38.7%, air vs. 100% O2) and f (−13.8 vs. −22.3%, air vs. 100% O2) during reoxygenation with both air and 100% O2; however, A/J mice showed significant increase in V˙E (+116%) and f (+62.2%) during reoxygenation and significant increase in V˙E (+68.2%) during 100% O2 reoxygenation. There were no strain differences in dynamic airway resistance during gas challenges or in steady-state total respiratory compliance measured postmortem. Strain differences in ventilatory responses to reoxygenation indicate that genetic mechanisms strongly influence posthypoxic ventilatory behavior.

ventilatory control; genetics; mouse strains; short-term po-

tentiation

INTERMITTENT HYPOXIA AND REOXYGENATION occur in num-

erous pathological conditions. With abrupt termination of brief hypoxic episodes, minute ventilation (V˙E) and its components, such as respiratory frequency (f) and tidal volume (VT), exhibit complex time-dependent changes (22). For example, total ventilation can remain above baseline; terms for this, short-term potentiation of ventilation (STP) or ventilatory afterdischarge, refer to a gradual return of ventilation to prehypoxic baseline values after reoxygenation. STP occurs in rats (16), cats (32), goats (11), dogs (34), and awake or sleeping humans (3, 8, 12, 17); is activated by a central neural mechanism with slow dynamics; and is able to drive ventilation independent of both peripheral and central chemoreceptor inputs (21).

An inherited basis for ventilation and ventilatory responsiveness is present in human populations (5, 7, 20, 24, 33), and recent reviews discuss differences in ventilatory behavior during steady-state exposure to hypoxia or hypercapnia among unanesthetized rats and mice, indicating the presence of genetic influence (15, 28). Responses to reoxygenation, however, have not been a focus of studies of inheritance. Rapid reoxygenation might not engage genetic factors, because the environmental change could be too short or too strong (15). The hypothesis was that the time domain of rapid exposure to extremes in hypoxia and hyperoxia, as well as its novelty, would minimize any opportunity to identify genetic influences on the posthypoxic ventilatory behavior. The present study sought to determine whether posthypoxic ventilatory behavior differed between two strains of mice with different steady-state response to hypoxia. If there were no differences between different mice strains, the hypothesis of environmental predominance would be confirmed. Refutation of the hypothesis would indicate genetic influence on posthypoxic ventilatory responses.

METHODS

Animals

Experiments were performed on two strains of inbred C57BL/6J (B6) and A/J mice (Jackson Laboratory, Bar Harbor, ME). These strains differ in a variety of physiological traits; relevant to this study, they have been shown by others to exhibit differences in ventilatory behavior to steady-state exposure to hypoxia (28). All animals in this study were male and of similar age (15–16 wk). Animals were housed at Case Western Reserve University for at least 2 wk before testing and were provided food and water ad libitum. The study protocol was approved by the Case Western Reserve University School of Medicine Institutional Animal Care and Use Committee and was in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental Protocols

In the experiments involving unanesthetized mice, measurements were made between 6:00 and 8:00 PM, because...
the temperature profile of the two strains is similar at this time of day (J. Nadeau, personal communication). All experiments were carried out when the animals were quietly awake, as assessed by behavioral observation. Each animal was weighed, placed in the test apparatus, and allowed 60 min to acclimate to the chamber environment, with room air flowing through the plethysmograph.

During resting air breathing, ventilation, O2 consumption (\(V_\text{O}_2\)), and CO2 production (\(V_\text{CO}_2\)) were measured three times over a 15-min period to obtain baseline values. The animal was subsequently exposed to the following test gases: 100% O2 (hyperoxia), 3% CO2-10% O2-87% N2 (isocapnic hypoxia), 8% O2-92% N2 (poikilocapnic hypoxia), and 5% CO2-95% O2 (hypercapnia). Each gas challenge was given for 5 min. There was a 20-min interval between each test.

Hypoxia-reoxygenation effects on respiration were examined on the same group of mice during the second day. The test gases were room air (A), 100% O2 (O), 3% CO2-10% O2-87% N2 (isocapnic hypoxia; I), and 8% O2-92% N2 (poikilocapnic hypoxia; P). Mice were exposed to a given gas for 5 min, and test gases were flushed out of the chamber for 10 s. The following protocols outlined in Fig. 1 were presented in random order. Protocol A (AA) served as a sham test and included flushing the chamber with room air. Protocol B (AO) examined the difference in ventilation between room air baseline and a hyperoxic gas exposure. Protocol C (PA) examined the difference in ventilation between room air baseline and poikilocapnic hypoxia. Protocol D (PO) examined the difference in ventilation between room air baseline and reoxygenation with 100% O2 after 8% O2 exposure. Protocol E (IA) and Protocol F (IO) examined the difference in ventilation between room air baseline and reoxygenation with air (IA) or 100% O2 (IO) after 3% CO2-10% O2 exposure. There was a 20-min interval between each protocol.

Airway resistance. During measurements of airway resistance, mice were exposed to air, 3% CO2-10% O2-87% N2 (isocapnic hypoxia), 8% O2-92% N2 (poikilocapnic hypoxia), and 5% CO2-95% O2 (hypercapnia). Each gas challenge was given for 5 min with a 20-min interval between each challenge.

Specific Methods

Measurements of respiratory variables and chemoreponsiveness. Ventilation and metabolism were assessed using a whole body plethysmograph by the open-circuit method (26), modified for the unanesthetized and unrestrained mouse. Animals were placed in a round lucite chamber (600-ml volume) with an inlet port for the administration of test gases (see Experimental Protocols). An outlet port was connected to a vacuum source sufficient to create a bias flow of 300 ml/min (8–10 times \(V_\text{E}/100 \text{g}\)) through the chamber, as measured by a rotameter. Because respiration immediately after hypoxia was a focus of the experiment, test gases were flushed out of the chamber at a flow rate of 15 l/min for 10 s, and then the flow rate through the chamber was returned to baseline. The chamber was connected to one side of a differential pressure transducer (model DP45, Validyne Engineering), with a sensitivity of ±2 cmH2O, referenced to a chamber of equal volume. As the animal breathed, swings in chamber pressure were converted to a signal that represents \(V_\text{E}\) (4). The respiratory signals were recorded on a strip-chart recorder (Linercorder WR3320, Grahtpec) and also stored in a computer with respiratory acquisition software [LabView programming by Innovative Computer Engineering (I.C.E.), Cleveland, OH]. The fractional contents of CO2 and O2 (\(F_\text{CO}_2\) and \(F_\text{O}_2\)) were measured by sampling the gas exiting the chamber (Beckman OM-11 and LB-2 analyzers). \(V_\text{E}\) and \(V_\text{CO}_2\) were determined by the open-circuit method (26). A reference volume of 0.25 ml of air was repeatedly introduced into the chamber before and on completion of recording with the chamber empty, and the pressure swing was the calibration for a pressure swings related to \(V_\text{E}\), when the animal was tested in the chamber. A thermistor-hygrometer probe was placed inside the chamber to monitor chamber temperature, chamber humidity, and barometric pressure. Two setups were available, each using identical transducers and monitors. Animals were studied in tandem.

There were no differences in chamber temperature (24.10 ± 0.03°C), chamber humidity (28.3 ± 0.2%), or barometric pressure (742.6 ± 0.2 Torr) that differed significantly with day of testing or by strain.

Measurement of Respiratory Mechanics

Airway resistance. To assess airway resistance for B6 and A/J mice during hypoxia or hypercapnia challenge (see Experimental Protocols), we used a different whole body plethysmograph (Buxco, Troy, NY) to measure a derived index named “enhanced pause (Penh),” reported by Hamelmann and coworkers (14) as a good index of airway resistance in unanesthetized and unrestrained mice. Briefly, measurements included pressure differences between the main chamber of the plethysmograph containing the animal and a reference chamber; a pneumotachograph with defined resistance in the wall of the main chamber acts as a low-pass filter and allows thermal compensation. Inspiration and expiration were recorded by establishing start inspiration and end-in-
spiration as the box pressure-time curve crosses the zero point. The maximum box pressure signal occurring during one breath in a negative or positive direction is defined as peak inspiratory pressure (PIP) or peak expiratory pressure (PEP), respectively. The relaxation time (Tr) is defined as the time of pressure decay to 36% of the total expiratory pressure signal. Penh, a dimensionless value used in this study to empirically monitor airway function, reflects changes in the waveform of the box pressure signal from both inspiration and expiration (PIP, PEP) and combines it with the timing comparison of early and late expiration (Pause). Penh is not a function of the absolute box pressure amplitude or f but rather a junction of the proportion of the pressure signal from inspiration and expiration and of the timing of expiration (Te).

\[
\text{Pause} = (\text{Te} - \text{Tr})/\text{Tr}
\]

\[
\text{Penh} = \text{Pause} \times \text{PEP}/\text{PIP}
\]

**Static pressure-volume curves.** Animals were anesthetized with intraperitoneal injections of a mixture of ketamine (25 mg/0.1 kg body wt) and xylazine (2.5 mg/0.1 kg body wt). After the trachea was cannulated, animals were killed with an overdose of ketamine and xylazine mixture given intraperitoneally. Static pressure-volume (P-V) curves were immediately performed while the animal was in a supine position, first with an intact chest wall and then when the thorax was opened widely by sternal incision. The inflation and deflation were performed using a 2-ml syringe. One minute before the stepwise inflation, 1 ml of air was given to ensure that all lung regions were fully opened. Airway pressure was measured by a pressure transducer (Argon). The inflation and deflation rates were 0.1 ml for every 10 s. The limits of the inflation and deflation airway pressures were 25 and 0 cmH2O, respectively. Sequential P-V loops, with and without chest wall effects, were generated for two complete cycles. Repeated inflation and deflation curves were reproducible; however, only the first one was used for the data analysis.

**Data Analysis**

Ventilatory parameters were measured continuously throughout the testing period and scored by computer using a respiratory-based software program (LabView programming by I.C.E). The following variables were calculated and analyzed: inspiratory VT (\(\mu l\)), f (breaths/min), VE (ml/min; \(\text{VT} \times f\)), VO2 (ml/min), VCO2 (ml/min), and respiratory quotient (VCO2/\(\text{VO2}\)). For each animal, f and VT were obtained from the mean of 20 consecutive breaths. VT and VE were normalized to the body weight of the animal. Sighs and sniffs were excluded from the analysis. Values for the steady-state VE and its components (VT, f) were determined during the fifth minute of exposure to each test gas. During non-steady state, VE, VT, and f were determined between 30 and 90 s after reoxygenation, when the FIO2 was 40% or higher (protocols B, D, and F) and after the switch to air (protocols A, C, and E). Penh was averaged for 1 min during exposure to each test gas and was scored (Buxco). Both compliance of the intact respiratory system (Crs; ml/cmH2O) and lung compliance (C; ml/cmH2O) were computed from the slopes of the P-V relationships between 2.5 and 7.5 cmH2O on deflation. Hysteresis in the P-V relationship was further quantified by integration of the area bounded by the inflation and deflation curves.

All results are expressed as means ± SD. Student's paired t-tests were used to evaluate whether each animal responded with significant increases in respiration during air compared with reoxygenation. Statistical significance between B6 and A/J mice was determined by one-way analysis of variance. P values < 0.05 were considered significant.

**RESULTS**

Respiratory variables and chemoresponsiveness were characterized for 12 mice of each strain; the average body weights were 29.6 ± 2.0 g for B6 mice and 25.0 ± 1.8 g for A/J mice (P < 0.05). Respiratory mechanics were determined on 10 other male mice of each strain; in these protocols, the average weights were 29.7 ± 2.3 and 28.6 ± 2.0 g (P > 0.05), respectively.

**Ventilatory Behavior During Resting Air Breathing**

Table 1 summarizes the variations in ventilatory behavior and metabolism between the two strains during resting breathing. Group data are shown for each trait variable. Metabolic measures and ventilatory values are provided both as unadjusted and as adjusted values for body weight. The results show that significant differences existed between strains in regard to f and VT at rest, but not in VE, a finding that persisted after adjustment for either VCO2 or VO2. Once corrected for body weight, differences in VE reached statistical significance. At rest, A/J mice exhibited a slower, deeper breathing pattern compared with B6 animals.

**Respiratory Responses to Steady-State Hyperoxia, Isocapnic Hypoxia, Poikilocapnic Hypoxia, and Hypercapnia**

An example illustrating the effect of 100% O2, 3% CO2-10% O2, 8% O2, and 5% CO2 on respiration in one animal from each strain is shown in Fig. 2A. Average results for 12 animals are summarized in Table 2. During 5 min of 100% O2 exposure, the f and VT at rest, but not in VCO2 and VCO2, were unaffected in both B6 and A/J mice. VCO2 increased slightly in A/J mice (P = 0.013) but not in B6 mice. Thus A/J animals maintained ventilation at higher values than B6 animals. Differences in regard to f and VT while animals breathed 100% O2 were similar to those observed at 100% air.
those observed while animals breathed room air. With a 5-min 5% CO2 challenge, as would be expected, both VT and f were significantly increased in all animals. Strain differences in VT and f observed during resting and 100% O2 breathing were in general more pronounced: A/J mice still breathed more slowly and deeply than the B6 animals. However, significant differences in VE as well as VE/body wt were not observed. Further lowering the inspired oxygen to 8% O2 also resulted in a significant increase in VE in both mice. This increase in respiration was due to increases in f as well as VT. VE differences between strains remained as that during resting breathing. However, the breathing pattern to achieve VE was notably different from that of the resting breathing: f was significantly lower and VT was higher in B6 mice. Ventilatory parameters, including f, VT, and VE, increased significantly in both strains on 3% CO2-10% O2 inhalation for 5 min. Comparison between B6 and A/J mice revealed that there were significant differences in VT and VE between the two strains but no difference in f. During the course of the 5-min hypoxia exposure, both B6 and A/J mice showed hypoxia ventilatory decline or “roll-off” phenomenon. From the first to the fifth minute, the magnitude of decrease in regard to VE (20 ± 9.1 vs. 18 ± 9.1%; B6 vs. A/J), VT (12 ± 10 vs. 11 ± 6.2%), and f (10 ± 7.5 vs. 9 ± 6.9%) had no difference between the two strains (P > 0.05).

Measurements of ventilatory responsiveness to chemoreceptor inputs are approximated by the differences in ventilation and its components between appropriate baseline values. Figure 2B is a histogram showing the
Table 2. Ventilatory behavior during 100% O₂, 5% CO₂, 8% O₂, and 3% CO₂-10% O₂

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>f, breaths/min</th>
<th>Vₜ, μl</th>
<th>Vₜ/body wt, μl/g</th>
<th>Vₑ, ml/min</th>
<th>Vₑ/body wt, ml·min⁻¹·g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>12</td>
<td>160 ± 16.3†</td>
<td>82 ± 16.6ª</td>
<td>2.8 ± 0.59ª</td>
<td>13 ± 2.5†</td>
<td>0.44 ± 0.09ª</td>
</tr>
<tr>
<td>A/J</td>
<td>12</td>
<td>135 ± 27.5</td>
<td>121 ± 25.2</td>
<td>4.8 ± 1.0</td>
<td>16 ± 4</td>
<td>0.64 ± 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100% O₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>12</td>
<td>271 ± 25.2ª</td>
<td>166 ± 28.3ª</td>
<td>5.6 ± 0.9ª</td>
<td>45 ± 8.2</td>
<td>1.5 ± 0.28</td>
</tr>
<tr>
<td>A/J</td>
<td>12</td>
<td>186 ± 27.4</td>
<td>237 ± 53.6</td>
<td>9.6 ± 2.5</td>
<td>45 ± 14.7</td>
<td>1.8 ± 0.62</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>5% CO₂</td>
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<td></td>
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</tr>
<tr>
<td>C57BL/6J</td>
<td>12</td>
<td>204 ± 21.5ª</td>
<td>169 ± 29.8ª</td>
<td>5.7 ± 0.94</td>
<td>34.5 ± 5.8</td>
<td>1.16 ± 0.19ª</td>
</tr>
<tr>
<td>A/J</td>
<td>12</td>
<td>271 ± 32</td>
<td>144 ± 22.6</td>
<td>5.8 ± 1.0</td>
<td>38.8 ± 5.4</td>
<td>1.56 ± 0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8% O₂</td>
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<td></td>
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</tr>
<tr>
<td>C57BL/6J</td>
<td>12</td>
<td>231 ± 38.2</td>
<td>142 ± 23.5ª</td>
<td>4.8 ± 0.89ª</td>
<td>33.2 ± 9.0ª</td>
<td>1.1 ± 0.34ª</td>
</tr>
<tr>
<td>A/J</td>
<td>12</td>
<td>238 ± 37.2</td>
<td>189 ± 43.6</td>
<td>7.7 ± 1.9</td>
<td>45.8 ± 14.9</td>
<td>1.8 ± 0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3% CO₂-10% O₂</td>
<td></td>
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</tr>
</tbody>
</table>

Values are means ± SD; n, no. of animals. *Significantly different compared with A/J mice, P < 0.01. †Significantly different compared with A/J mice, P < 0.05.

relative differences between strains in regard to chemoresponsiveness. As shown in Fig. 2B, the two strains differ substantially in regard to f response to 3% CO₂-10% O₂, 8% O₂, and 5% CO₂ challenges. The percent increases in Vₜ during 100% O₂, 3% CO₂-10% O₂, and 5% CO₂ relative to room air were not different between the two strains but were significantly different during 8% O₂ exposure. The relative increase in Vₑ with inhalation of 100% O₂, 3% CO₂-10% O₂, 8% O₂ and 5% CO₂, compared with resting breathing, is similar in the two strains. In summary, the magnitudes of Vₑ changes to hypoxia and/or hypercapnia between B6 and A/J animals were the same; however, the breathing pattern to achieve the same Vₑ change was notably different between the two strains.

Ventilatory Behavior With Hypoxia-Reoxygenation

Vₑ and its components (f, Vₜ) during dynamic testing were determined ~40 s after 100% O₂ or air was given. The results are shown in Table 3 and Fig. 3. In protocol A, Vₜ, f, and Vₑ remained within the range observed during baseline conditions in both strains. With transition from air to 100% O₂ (protocol B), f decreased and Vₜ increased significantly in both B6 and A/J mice. Vₑ decreased significantly in B6 mice but remained unchanged compared with baseline in A/J mice. In protocol C, during air breathing after 5 min of 8% O₂ exposure, there was a nonsignificant trend toward increase in f in A/J mice; Vₜ and Vₑ were not significantly different from that during air baseline. In contrast, B6 mice showed a significant decrease in f and Vₑ and a significant increase in Vₜ. In protocol D, on posthypoxic reoxygenation, hyperoxia resulted in a prompt reduction in f and Vₑ and an increase in Vₜ in B6 mice; in A/J mice, all of these remained within the range observed during baseline. In protocols E and F, after 5 min of isocapnic hypoxia exposure, B6 mice still exhibited significant decrease in Vₑ and f and significant increase in Vₜ during reoxygenation with both air and 100% O₂; however, A/J mice showed significant increases in Vₑ, Vₜ, and f during air reoxygenation and significant increases in Vₑ and Vₜ during 100% O₂ reoxygenation.

Airway Resistance

Table 4 summarizes the Penh values during resting breathing, 5% CO₂, 8% O₂, and 3% CO₂-10% O₂ exposure.

Table 3. Ventilatory behavior during air and 100% O₂ reoxygenation

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>f, breaths/min</th>
<th>Vₜ, μl</th>
<th>Vₑ, ml/min</th>
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<tr>
<td>Air baseline</td>
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<tr>
<td>C57BL/6J</td>
<td>12</td>
<td>167 ± 11ª</td>
<td>76 ± 12†</td>
<td>12.8 ± 2.7</td>
</tr>
<tr>
<td>A/J</td>
<td>12</td>
<td>144 ± 18</td>
<td>86.0 ± 15</td>
<td>14.1 ± 2.5</td>
</tr>
<tr>
<td>Air-100% O₂</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>12</td>
<td>107 ± 25ª</td>
<td>95 ± 16.7ª</td>
<td>10.1 ± 3.0†</td>
</tr>
<tr>
<td>A/J</td>
<td>12</td>
<td>122 ± 26</td>
<td>104.8 ± 17.8</td>
<td>12.9 ± 3.8</td>
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<td>Air-air</td>
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<tr>
<td>C57BL/6J</td>
<td>12</td>
<td>163 ± 20ª</td>
<td>75 ± 16ª</td>
<td>12.3 ± 3.4</td>
</tr>
<tr>
<td>A/J</td>
<td>12</td>
<td>147 ± 21</td>
<td>99.8 ± 19</td>
<td>14.6 ± 3.0</td>
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<tr>
<td>C57BL/6J</td>
<td>12</td>
<td>109 ± 19ª</td>
<td>98 ± 24.3ª</td>
<td>10.2 ± 4.3</td>
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<tr>
<td>A/J</td>
<td>12</td>
<td>174 ± 49</td>
<td>93.7 ± 20.6</td>
<td>16.9 ± 7.7</td>
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<td>8% O₂-100% O₂</td>
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</tr>
<tr>
<td>C57BL/6J</td>
<td>12</td>
<td>82.1 ± 20ª</td>
<td>124 ± 32ª</td>
<td>10.0 ± 3.4†</td>
</tr>
<tr>
<td>A/J</td>
<td>12</td>
<td>139 ± 24</td>
<td>101.5 ± 28</td>
<td>14.8 ± 7.5</td>
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<td>3% CO₂/10% O₂-air</td>
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<tr>
<td>C57BL/6J</td>
<td>12</td>
<td>121 ± 13.7ª</td>
<td>94.5 ± 15.3ª</td>
<td>11.4 ± 2.1ª</td>
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<td>A/J</td>
<td>12</td>
<td>214 ± 31</td>
<td>135 ± 19</td>
<td>30.3 ± 8.5</td>
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<td>3% CO₂/10% O₂-100% O₂</td>
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<tr>
<td>C57BL/6J</td>
<td>12</td>
<td>103.5 ± 22ª</td>
<td>98.7 ± 19.6ª</td>
<td>10.2 ± 3.0ª</td>
</tr>
<tr>
<td>A/J</td>
<td>12</td>
<td>155.1 ± 27.1</td>
<td>125.1 ± 13</td>
<td>19.3 ± 3.7</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of animals. *Significantly different compared with A/J mice, P < 0.01. †Significantly different compared with A/J mice, P < 0.05.
The A/J mice demonstrated a slightly but significantly (P < 0.05) greater Penh compared with B6 mice during resting breathing. As would be expected, both 5% CO₂ and 8% O₂ caused a significant increase of f in both strains. Although f was different between the two strains, the Penh values were the same. When exposed to 3% CO₂-10% O₂, both f and Penh had no significant differences. This suggests that the strains related changes in f or Vt after challenge with testing gases cannot be accounted for by a strain difference in airway resistance, because Penh values during test gases exposure in these two strains of spontaneously breathing mice were the same. Therefore, at the end of the hypoxic exposure, dynamic mechanics were similar between the two strains.

Static Mechanical Properties of the Lungs and Chest Wall

With the chest wall intact, there were no detectable strain differences between deflation curves, and both the Crs (0.049 ± 0.006 vs. 0.055 ± 0.009 ml/cmH₂O, B6 vs. A/J; P > 0.05) and the hysteresis (50.2 ± 9.3 vs. 55 ± 5.4 cm², P > 0.05) were similar. After excision of the chest wall, between 2.5 and 25 cmH₂O on deflation, A/J mice demonstrated significantly greater lung volumes compared with age- and weight-matched B6 mice. The Cl was significantly smaller in the B6 strain relative to A/J mice (0.078 ± 0.012 vs. 0.095 ± 0.018 ml/cmH₂O; P < 0.05); however, the P-V hysteresis was the same (68.1 ± 11.1 vs. 69.8 ± 5.4 cm²; P > 0.05). Therefore, differences

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**Fig. 3.** Ventilatory responses between 30- and 90-s reoxygenation with 100% O₂ or air after 5 min of 8% O₂ or 3% CO₂-10% O₂ exposure in B6 and A/J mice. A: representative tracing of respiratory responses to reoxygenation after 8% O₂ or 3% CO₂-10% O₂ exposure in an unanesthetized, unrestrained B6 and A/J mice. B: comparison of respiratory responses to reoxygenation between B6 and A/J mice. Values are means ± SD. Results are presented as a percent change from air baseline in f, Vt, and V̇E. Positive values represent an increase, whereas negative values show a decrease. *Significantly different compared with A/J mice, P < 0.05.
POSTHYPOXIC VENTILATORY BEHAVIOR IN MICE

Table 4. Penh during resting breathing, 5% CO₂, 8% O₂ and 3% CO₂-10% O₂ exposure

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>f, breaths/min</th>
<th>Penh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>10</td>
<td>163 ± 9.2*</td>
<td>0.31 ± 0.04†</td>
</tr>
<tr>
<td>A/J</td>
<td>10</td>
<td>144 ± 9.1</td>
<td>0.48 ± 0.2</td>
</tr>
<tr>
<td>5% CO₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>10</td>
<td>299 ± 32*</td>
<td>0.47 ± 0.08</td>
</tr>
<tr>
<td>A/J</td>
<td>10</td>
<td>219 ± 33</td>
<td>0.51 ± 0.06</td>
</tr>
<tr>
<td>8% O₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>10</td>
<td>177 ± 23*</td>
<td>0.24 ± 0.08</td>
</tr>
<tr>
<td>A/J</td>
<td>10</td>
<td>263 ± 26</td>
<td>0.3 ± 0.08</td>
</tr>
<tr>
<td>3% CO₂-10% O₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>10</td>
<td>236 ± 23</td>
<td>0.36 ± 0.08</td>
</tr>
<tr>
<td>A/J</td>
<td>10</td>
<td>253 ± 33</td>
<td>0.42 ± 0.11</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of animals. Penh, enhanced pause. *Significantly different compared with A/J mice, P < 0.01. †Significantly different compared with A/J mice, P < 0.05.

in total thoracic mechanics are unlikely to explain the differences in posthypoxic ventilatory behavior.

DISCUSSION

This study describes significant strain differences in Vₑ and its components before and during hypoxia-reoxygenation in the mouse. The results are summarized as follows. First, during resting air breathing, there were significant differences in frequency, Vₑ, and Vₑ corrected for the weight of the animal. There were significant differences in the pattern of the ventilatory response to chemical stimuli between the two mice strains. Second, after abrupt termination of brief poikilocapnic hypoxia exposure, B6 mice had a prominent decrease in Vₑ on reoxygenation with both air and 100% O₂, attributed to the significant decline in f. In contrast, the A/J animals showed different ventilatory timing and drive responses to the same environmental gas challenges. Despite the concomitant hypocapnia, ventilatory undershoot was not observed. There were no differences in Vₑ, f, and Vₑ between reoxygenation and resting air breathing levels. During reoxygenation with both air and 100% O₂ after isocapnic hypoxia exposure, B6 mice still exhibited significant decrease in Vₑ and f; however, A/J mice exhibited increased Vₑ, evidence for STP. Third, neither steady-state Crs nor dynamic changes of airway resistance during exposure to different gas mixtures can explain the variability between strains.

Respiratory Traits During Steady-State Exposure

Tankersley et al. (30) first reported significant intrastain differences in f, hypoxic responsiveness, and hypercapnic responsiveness in mice, and more recently there is evidence presented that a limited number of genes may influence such traits as the level of ventilation on hypoxia (29). Strohl et al. (26) showed that, among four strains of rat, strain, more than the effect of body mass or sex, had a major influence on metabolism, the pattern and level of ventilation during air breathing, and ventilation during loading or unloading of chemoreceptor input in the unanesthetized rat. These studies in combination support the notion that there are genetic mechanisms among rodent strains in regard to the transmission of ventilatory behavior and its components, Vₑ and f. Our data in the present study confirm these observations and by and large replicate those in mice (29). Additionally, our results indicated a higher airway resistance that might contribute to the deep and slow breath pattern of the mouse at rest. Yet, B6 and A/J mice demonstrated similar steady-state Crs and dynamic changes of airway resistance during hypoxic and/or hypercapnic challenge. Therefore, the strain differences in ventilatory responsiveness do not appear to be explained only by variations in respiratory mechanics between the two strains.

Ventilatory Behavior During Hypoxia-Reoxygenation

In the present study, after abrupt termination of both isocapnic and poikilocapnic hypoxia, unanesthetized and unrestrained B6 mice showed depression of Vₑ, increase in Vₑ, and posthypoxic frequency decline; a similar finding is reported in anesthetized rats (6). In contrast, using identical protocols, we did not observe ventilatory undershoot in A/J mice after poikilocapnic hypoxia, and A/J mice showed clear evidence for STP of ventilation during reoxygenation after isocapnic hypoxia exposure. These significant differences in the pattern and magnitude of the ventilatory response to reoxygenation in the two strains of mice under study clearly disprove the null hypothesis. Thus genetic influences are strongly seen to influence the posthypoxic ventilatory behavior, consistent with findings on rat strains from our group (27).

Other literature supports a genetic influence on the breathing pattern during reoxygenation. Jacob and Thach (18) studied the spontaneous recovery from hypoxic apnea, i.e., gasping, in mice. Prolonged but ineffective gasping was more prevalent in Swiss Webster-related than in Swiss Webster mice, whereas gasping was absent in Swiss Webster more than Swiss Webster-related mice. Kline et al. (19) examined the effects of reoxygenation after hypoxia (12% O₂) on the phrenic nerve activity in anesthetized mice; respiratory depression in response to brief hyperoxia was pronounced in wild-type mice and nearly absent in nitric oxide synthase-3 mutant mice.

Issues Addressed by the Experimental Protocol

Original observations on posthypoxic frequency decline were made in anesthetized preparations with reduced sensory and behavioral inputs (6, 16). In the conscious animal, however, proprioceptive responses to nonchemical respiratory stimuli could operate in the posthypoxic ventilatory behavior. Therefore, protocols were designed to control for events associated with flushing the chamber and with prior exposure to air or hypoxia. In the “sham” protocol, we could not detect...
any effect that resembled the transition from hypoxia to reoxygenation after a 5-min exposure to hypoxia. We conclude that posthypoxic ventilatory behavior in the present studies does not significantly engage a behavioral response to the testing apparatus or an effect of the order of presentation of gas mixtures.

There exists a circadian rhythm in human ventilatory chemosensitivity even in the absence of sleep and without simultaneous changes in behavior or the environment (25). This could be caused by indirect influences of body temperature or other variables on metabolism and/or respiratory control. We know the body temperature profiles of B6 and A/J mice are different during 24-h test except for the time between 6 and 8 PM (J. Nadeau, unpublished observation). Consistent with the temperature profiles, we found that metabolic traits of the two strains were similar between 6 and 8 PM.

Apart from the carotid chemoreceptor excitation, hypoxia is also reported to depress ventilatory activity via direct effects on the central nervous system (31) or via central accumulation and/or release of inhibitory neurotransmitters on respiratory neurons (23). A slow off-time of the central mechanisms that are responsible for respiratory depression during hypoxia may also influence the posthypoxic ventilatory behavior. In sleeping (3) and awake (8) human subjects, decay of the STP was apparent during reoxygenation after 30 s and 1 min isocapnic hypoxia but was absent after 5 min of the same exposure. These studies indicate an important inhibitory influence of the central effects of sustained hypoxia on the mechanisms of STP. We found a roll-off phenomenon during the 5-min hypoxic exposure in both strains of mice, indicating the existence of hypoxia depression. However, there was no difference in regard to the degree of ventilation depression between the two strains. Subramanian et al. (27) reported no correlation between the magnitude of the ventilation depression during reoxygenation with the magnitude of the level of ventilation during hypoxia or the degree of roll-off, in either Sprague-Dawley or Brown Norway rats. All the above suggests that strain differences involve physiological mechanisms that are not directly coupled to events that influence ventilation during the preceding hypoxic exposure.

Hypocapnia could have a significant inhibitory influence on ventilation during the posthypoxic period. In sleeping human (3) and awake goat (11), when hypocapnia was permitted, much of the STP was eliminated. An interaction of the arterial PCO2 stimulus with arterial PCO2 is equally important in determining ventilatory pattern after hyperventilation produced by chemical stimuli. Gleeson and Sweer (13) compared the relief of 45–60 s of poikilocapnic hypoxia by hyperoxia vs. room air breathing during non-rapid eye movement sleep in humans and observed uniform hypoventilation after hypoxia followed by 100% O2, whereas no hypoventilation occurred when the identical stimulus preceded room air. Dahan et al. (8) reported a similar finding in awake humans after 3 min of isocapnic hypoxia exposure. In our protocols, a significant increase in ventilation, or STP, was observed in the A/J mice after isocapnic but not after poikilocapnic hypoxia, consistent with the findings in humans (8, 13); however, ventilation fell in the B6 strain and did so whether or not CO2 was added to the hypoxic challenge. We suspect that variance among humans does exist in STP and that to some degree it could result from genetic factors.

Brief inhalation of 100% O2 (~30 s) can depress respiration, which has been attributed to decreased chemosensory drive from the carotid body and, therefore, reflects the strength of the peripheral chemoreceptor drive (9). Aaron and Powell (1) found that 30% O2 breathing is sufficient to minimize O2-sensitive ventilatory drive in unrestrained unanesthetized rats. When hyperoxia was induced against a background of hypoxia, nadirs of f also occurred in both strains of mice when FIO2 had reached ~40%; ventilation was transiently depressed in B6 mice, but not in A/J mice, a similar finding as transition from room air breathing to hyperoxia. Nadirs of ventilation observed in B6 mice when hyperoxia succeeded hypoxia were lower than nadirs observed after reoxygenation with room air, which is consistent with the finding in humans (17).

Thus a strain difference in regard to ventilatory behavior still exists during transition from hypoxia to hyperoxia. Although the f decline compared with transition from hypoxia to air indicates that posthypoxic ventilatory behavior may be influenced by ambient hypoxic drive in both strains, a strong STP influence on breathing still persists in A/J mice but not in B6.

In summary, whereas the pattern of ventilation after hypoxic stimulation is subject to modification by the arterial blood-gas composition, this effect may not account alone for the strain differences of the ventilatory behavior during reoxygenation after hypoxia. We interpret this difference between the two strains as evidence that genetically determined systems are operative under the conditions present during hypoxia-reoxygenation.

Perspectives

STP may stabilize ventilation during periods of rapidly fluctuating respiratory drive or after hyperventilation, therefore protecting against dysrhythmic breathing, such as apnea and periodic breathing (10, 35). We speculate that the absence of STP in the B6 mouse might have the effect of removal of a stabilizing mechanism and thereby increasing the propensity for apnea or periodic breathing in this strain. Finding such differences in posthypoxic ventilatory behavior between strains is a good evidence for a genetic factor to be operative under these unusual conditions. The questions of relative strength of the genetic components and the identification of the genes remain to be determined.

Understanding the physiological and genetic mechanisms underlying the hypoxia-reoxygenation differences seen in these mice would help gaining more insight into the variability in breathing over time seen...
in normal human sleep. It would also be potentially relevant to clinical disorders involving disorders of respiratory control, including sudden infant death syndrome, and to patients with sleep-disordered breathing, chronic lung disease, and hypventilation syndromes (2, 10).

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