Periodic breathing in the mouse

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Han, Fang, Shyam Subramanian, Edwin R. Price, Joseph Nadeau, Kingman P. Strohl. Periodic breathing in the mouse. J Appl Physiol 92: 1133–1140, 2002; 10.1152/japplphysiol.00785.2001.—The hypothesis was that unstable breathing might be triggered by a brief hypoxia challenge in C57BL/6J (B6) mice, which in contrast to A/J mice are known not to exhibit short-term potentiation; as a consequence, instability of ventilatory behavior could be inherited through genetic mechanisms. Recordings of ventilatory behavior by the plethysmography method were made when unanesthetized B6 or A/J animals were reoxygenated with 100% O2 or air after exposure to 8% O2 or 3% CO2-10% O2 gas mixtures. Second, we examined the ventilatory behavior after termination of poikilocapnic hypoxia stimuli in recombinant inbred strains derived from B6 and A/J animals. Periodic breathing (PB) was defined as clustered breathing with either waxing and waning of ventilation or recurrent end-expiratory pauses (apnea) of ≥2 average breath durations, each pattern being repeated with a cycle number ≥3. With the abrupt return to room air from 8% O2, 100% of the 10 B6 mice exhibited PB. Among them, five showed breathing oscillations with apnea, but none of the 10 A/J mice exhibited cyclic oscillations of breathing. When the animals were reoxygenated after 3% CO2-10% O2 challenge, no PB was observed in A/J mice, whereas conditions still induced PB in B6 mice. (During 100% O2 reoxygenation, all 10 B6 mice had PB with apnea.) Expression of PB occurred in some but not all recombinant mice and was not associated with the pattern of breathing at rest. We conclude that differences in expression of PB between these strains indicate that genetic influences strongly affect the stability of ventilation in the mouse.

ventilation; respiratory control; genetics

METHODS

Animals. Experiments were performed on two strains of inbred B6 and A/J mice (Jackson Laboratory, Bar Harbor, ME) and on groups of recombinant inbred mice strains derived from B6 and A/J parental strains, all raised in the Animal Resource Center at Case Western Reserve University. All animals were housed at the Center under standard conditions of 7 AM to 7 PM light-dark cycles 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. Measurements were made between 10:00 AM and 2:00 PM. All experiments were carried out when the animals were awake, as determined by behavioral observation. Each animal was weighed, placed in the
Fig. 1. Protocols that were used to test parental strains are presented in schematic for the presence and strength of periodic breathing occurring after hypoxic challenge.

Protocol A: (n = 10, group 1)

<table>
<thead>
<tr>
<th>Room air</th>
<th>8% O&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Room air</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room air</td>
<td>8% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>100% O&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

Protocol B: (n = 10, group 2)

<table>
<thead>
<tr>
<th>Room air</th>
<th>10% O&lt;sub&gt;2&lt;/sub&gt;/3% CO&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Room air</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room air</td>
<td>10% O&lt;sub&gt;2&lt;/sub&gt;/3% CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>100% O&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

Fig. 2. Manner in which periodic breathing was quantified for comparisons among protocols is shown in these two examples from B6 mice. M, a value describing the strength of oscillation, is calculated differently for events with obvious apneas (bottom) vs. short pauses (top). V<sub>E</sub><sub>min</sub>, minimum level of instantaneous minute ventilation (VE); V<sub>E</sub><sub>max</sub>, maximal level of instantaneous VE; Tc, cycle length; Ta, apnea length. See text for greater explanation and reference.
periodic pattern and 2) the strength of the oscillation (M), a
measure of how much the ventilation changes as the pattern
goes from its point of maximum ventilation (V_{\text{Emax}}) to its
point of minimum ventilation (V_{\text{Emin}}) defined by Waggoner et
al. (40). For nonapneic oscillations, M was defined as the
ratio of V_{\text{Emax}} – V_{\text{Emin}} divided by V_{\text{Emax}} + V_{\text{Emin}}, and for
apneic oscillations, this same index was calculated as the
ratio of the Tc over the difference between Tc and length of
apnea (43).

Data analysis. Ventilatory parameters were measured
continuously throughout the testing period, and scored by
computer by using a respiratory-based software program
(LabView programming by I.C.E). The following variables
were analyzed: inspiratory Vt (in μl), f (in breaths /min), Ve
(in ml/min, Vt × f). Sighs or sniffs were excluded in the
analysis. Sighs were signals that exceeded by 150% the
average Vt for the past 4 s and were sometimes accompanied
by a compensatory sigh. Sniffs are identified as rapid, very
shallow oscillations in voltage, which, on video examination,
are temporally related to exploratory behavior when the
animal appears to be awake. During posthypoxic periods, the
breathing patterns reported here could easily be seen as a
regular periodicity in the strip-chart recording of voltage vs.
time; the cycle time and strength of the patterns were mea-
sured directly off the strip-chart recordings and were as-
sisted by the computer program. During 100% O2 reoxygen-
test, Ve, Vt, and f were determined when the concentration of the inspired oxygen was between 40 and
50%.

All results are expressed as means ± SE. Differences
between means were tested by using a Student’s t-test
(paired or unpaired as required). A point biserial correlation
(Pearson correlation) was used to determine the relationship,
if any, between the presence or absence of PB and ventilatory
behavior among all inbred strains studied. P values of <0.05
were considered significant.

RESULTS

Reoxygenation effects on respiration after 8% O2
exposure were characterized on the first group of 10
mice of each parental strain, the average body weight
being 29.8 ± 0.5 g for B6 mice and 26.5 ± 0.4 g for A/J
mice (P < 0.05). Reoxygenation effects on respiration after
3% CO2-10% O2 exposure were determined on 10
other mice of each strain; in this protocol, the average
weights were 29.6 ± 0.4 and 26.6 ± 0.5 g (P < 0.05),
respectively. All of them were male and age matched
(16–17 wk old).

Ventilatory behavior in A/J and B6 mice during
baseline air breathing and during isocapnic and
poikilocapnic hypoxia. At baseline, A/J mice exhibited
a slower, deeper breathing pattern compared with B6
animals (Table 1). Vt and f were fairly regular; no
breathing oscillation with apnea or near apnea could
be visually identified in both strains of mice. Figure 3
shows a typical example of air baseline breathing of a
B6 and an A/J mouse.

Visual interpretation of data during both hypoxia
showed a relatively stable breathing pattern. No PB
was observed in both strains of mice within the 5 min
of hypoxia. During 8% O2 exposure, both B6 and A/J
mice showed hypoxia ventilatory decline or the “roll-
off” phenomenon. From the minutes 1–5, the magni-
tude of decrease in regard to Ve (19 ± 3 vs. 18 ± 3%, B6
vs. A/J), Vt (11 ± 3.3 vs. 10 ± 2%), and f (10 ± 2.5 vs.
9 ± 2.1%) were similar between the two strains. Mea-
surements of ventilatory responsiveness to chemo-
receptor inputs are approximated by the differences in
ventilation and its components between appropriate
baseline values. The two strains differ substantially in
regard to f response to 3% CO2-10% O2 and 8% O2; the
percentage increases in Vt during 3% CO2-10% O2
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
3% CO2-10% O2 and 8% O2 compared with resting
breathing is similar in the two strains. In summary,
the magnitudes of Ve changes to hypoxia between B6
and A/J animals were similar; however, the breathing
pattern to achieve the same Ve change was unequiv-
cally different between the two strains.

Posthypoxia ventilatory behavior in A/J and B6 mice.
With the abrupt return to room air from 8% O2, 100% of
the 10 B6 mice exhibited PB; among them, five showed breathing oscillation with apnea; but none of
the 10 A/J mice exhibited a cyclic or oscillatory pattern of
breathing. Figure 4 shows a representative sample of
strip-chart recording of PB occurring during the
posthypoxic period in a B6 mouse; changes in Ve for the
same animal were directly reflected in the correspond-
ing time-related changes in both Vt and f.

On transition from 8% O2 to 100% O2, the differences
between the two strains in the posthypoxic ventilatory
behavior were similar to those during air reoxygen-
ation, except that now all 10 B6 mice showed PB with
apnea. Figure 5 shows examples of the different post-
poikilocapnic hypoxia ventilatory behavior in a B6
and an A/J mouse. As shown in Table 2, Tc was the same as
that followed by air, but the apnea time was longer
than during air reoxygenation, indicating that the
magnitude of the breathing oscillations was more
prominent during the 100% O2 reoxygenation. This
was also demonstrated by the increased M of the oscil-
lations during 100% O2 reoxygenation.

In protocol B, a second group of mice were studied
in order to determine whether CO2 might affect hypoxic induced
PB. When the animals were abruptly returned to room
air or 100% O2 after 5 min of isocapnic hypoxia chal-
lenge, no PB was observed in all 10 A/J mice. In
contrast, both of these two conditions still induced PB

Table 1. Values for ventilatory behavior during
resting breathing.

<table>
<thead>
<tr>
<th>Strain</th>
<th>C57BL/6J</th>
<th>A/J</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>f, breaths/min</td>
<td>165 ± 4</td>
<td>140 ± 6</td>
<td>0.001</td>
</tr>
<tr>
<td>Vt, μl</td>
<td>73 ± 4</td>
<td>97 ± 4.1</td>
<td>0.0002</td>
</tr>
<tr>
<td>Vt/WT, μl/g</td>
<td>2.6 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Ve, ml/min</td>
<td>11.8 ± 0.7</td>
<td>12.9 ± 0.8</td>
<td>0.15</td>
</tr>
<tr>
<td>Ve/WT, ml/min⁻¹·g⁻¹</td>
<td>0.41 ± 0.02</td>
<td>0.52 ± 0.03</td>
<td>0.001</td>
</tr>
</tbody>
</table>

f, Frequency; Vt, tidal volume; Ve, minute ventilation; wt, body

weight.
in all the 10 B6 mice. During air reoxygenation, 6 of them showed PB with apnea and, during 100% O\textsubscript{2} reoxygenation, all 10 mice had PB with apnea, 4 of them exhibited a breathing pattern, which was referred to as episodic breathing by Wilkinson et al. (43), consisting of periods of breathing alternating with apneas of similar duration and may be analogous to the qualitatively similar pattern known as Biot’s breath-

Fig. 3. Tracings show representative breathing patterns during resting air breathing in animals from each strain. We characterize the ventilatory behavior of the A/J mice as slow [in frequency (f)] and deep [in tidal volume (VT)], whereas the phenotype of the B6 mice is fast and shallow.

Fig. 4. Top: continuous strip chart recording showing ventilatory behavior in a B6 animal in the transition from breathing 8% oxygen to air. The top tracing is when this transition takes place and, starting in the second tracing, there occur 23 cycles of periodic breathing before VT and f become more regular. Bottom: 3 charts represent instantaneous f (left), VT (middle), and VE (right), as calculated for each breath, starting at the beginning of the period of periodic breathing.
Compared with air reoxygenation, the PB during 100% O2 reoxygenation also had the same Tc but stronger oscillation and longer apnea duration (Table 2).

Figure 6 shows examples of postisocapnic hypoxia ventilatory behavior in a B6 and an A/J mouse. Comparison of the PB between the two hypoxia gas mixtures did not disclose any difference in regard to Tc, M, and apnea time when reoxygenated with the same gas (Table 2).

**Segregation of PB with ventilatory behavior.** Table 3 shows the presence or absence of PB in strains of parental and selected recombinant inbred strains, contrasting the presence or absence of PB to ranking of strains in regard to f, relative Vt, and Ve. We conclude that the presence or absence of PB with reoxygenation after 8% hypoxia is not a function of the pattern of breathing or Ve at rest. Although the location of the genetic elements is not yet known through this experimental approach, it is clear that ventilatory behavior and PB segregate as genetically independent traits.

**DISCUSSION**

This study describes significant strain differences in posthypoxic ventilatory behavior in the mouse. First, during resting air breathing, no PB could be visually identified in B6 and A/J mice. Second, after 5 min of poikilocapnic or isocapnic hypoxia challenge, 100% of the behaviorally awake adult B6 mice exhibited PB on reoxygenation with both air and 100% oxygen; in contrast, none of the A/J mice showed discernible oscillations in ventilatory patterns during the same series of environmental gas exposures. The occurrence of PB was independent of sleep, degree of the previous hypoxia and added CO2 during hypoxia, or O2 level of the

**Table 2. Indexes for periodic behavior during post-challenge in the B6 strain**

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>Challenge Gases</th>
<th>CL, s</th>
<th>M*</th>
<th>Apnea Time, s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Study 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>10</td>
<td>8% O2-air</td>
<td>4.6 ± 1.0</td>
<td>1.7 ± 0.4</td>
<td>0.93 ± 0.28</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>10</td>
<td>8% O2-100% O2</td>
<td>2.8 ± 0.5</td>
<td>5.5 ± 0.7</td>
<td>2.0 ± 0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Study 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>10</td>
<td>3% CO2-10% O2-air</td>
<td>3.5 ± 0.5</td>
<td>2.0 ± 0.4</td>
<td>0.90 ± 0.41</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>10</td>
<td>3% CO2-10% O2-100% O2</td>
<td>2.5 ± 0.3</td>
<td>5.5 ± 0.9</td>
<td>2.2 ± 0.29</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 10 mice for each group). CL, cycle length; M, strength of oscillation. *Dimensionless number for M. For CL, no significant difference was observed between studies. After reoxygenation with 100% O2, M was significantly greater than when reoxygenated with air for each group.
reoxygenation gases. The strain differences of PB in B6 and A/J as well as in the recombinant strains indicate a genetic influence on the stability of the posthypoxic ventilation in mice.

Hypoxia, imposed through either inhalation of low O2 mixtures or ascent to altitude, is well known to induce PB in animals and humans (5, 8, 10, 19, 42). Sleep, increased gain, increased time delay, and decreased damping of the system are all known to promote respiratory instability (23). However, a major finding in this study is that reoxygenation can induce PB in awake B6 mice but not in awake A/J mice. PB has also been observed in awake patients with chronic heart failure (30), and awake normal adults can develop irregular and PB during induction of mild hypoxia produced by nasal administration of nitrogen (10). In awake newborn lambs, it is possible to produce PB on demand when appropriate settings of blood gases are reached to drive the chemoreceptors (9). These reports indicate that sleep was not obligatory in eliciting PB. In the present study, we did not monitor the electroencephalogram and did not have a rigorous measurement of how wakeful or alert animals were during the protocol, although we took care to observe animals and did not observe behaviors indicating sleep during the induction of PB.

A commonly proposed mechanism for PB involves hypoxia-induced increased gain of the carotid chemoreceptors. Mathematical models and studies on humans have demonstrated strong statistical correlations between the incidence of PB and hypoxic sensitivities (2, 28, 42). Posthypoxic PB during sleep occurs more frequently in individuals with higher peripheral chemosensitivity (19, 42). The finding that PB could occur in B6 mice during hypoxia-air reoxygenation is consistent with the evidence for a critical role of peripheral chemoreceptors in the genesis of PB. However, the breathing periodicity during 100% O2 reoxygenation cannot be mediated by the peripheral chemoreceptors only because it occurs against a background of arterial hyperoxia, which is sufficient to minimize O2-sensitive ventilatory drive. Further evidence supports that PB is mediated by mechanisms other than peripheral chemoreceptors, namely the studies on cats and ponies; in both species, hypoxia-induced PB continued after carotid body denervation (7, 41). As proposed by Wilkinson et al. (43), the observation of episodic breathing in some B6 mice during 100% O2 reoxygenation in this study also suggests that this pattern of breathing is mediated via the central chemoreceptor. As reported before (21) and shown in this study, the magnitudes of VE change, to both polikilocapnic and isocapnic hypoxia and hypocapnia (5% CO2) between B6 and A/J animals, were the same. Thus the strain difference in posthypoxic PB cannot be attributed solely to the different chemoresponsiveness.

Apart from the carotid chemoreceptor excitation, hypoxia is also reported to depress ventilatory activity via direct effects on the central nervous system (39) or via central accumulation and/or release of inhibitory neurotransmitters on respiratory neurons (31). Hypoxia was considered a “primary event” inducing PB via its central depressant effect in the neonatal period (32). Prolonged or more severe hypoxia had an important central inhibitory influence on the mechanisms of STP and would predict that ventilatory instabilities, such as PB would be more likely to occur (14, 29). In this study, we identified the roll-off phenomenon during the 5-min hypoxic exposure in both strains of mice, indicating the existence of hypoxic depression. However, there is no evidence suggesting an influence of hypoxic depression on the occurrence of PB in B6 mice.

### Table 3. Presence or absence of PB in mouse strains according to relative ranking in trait values for ventilatory behavior at rest

<table>
<thead>
<tr>
<th>Strain</th>
<th>f vs. PB*</th>
<th>Vt vs. PB</th>
<th>Vv vs. PB</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>+ BXA-14</td>
<td>73 μl</td>
<td>11.7 ml/min</td>
</tr>
<tr>
<td>A/J</td>
<td>– AXB-5</td>
<td>83 μl</td>
<td>11.9 ml/min</td>
</tr>
<tr>
<td>A/J</td>
<td>– AXB-15</td>
<td>95 μl</td>
<td>13.5 ml/min</td>
</tr>
<tr>
<td>A/J</td>
<td>– A/J</td>
<td>98 μl</td>
<td>14.7 ml/min</td>
</tr>
<tr>
<td>B6</td>
<td>+ AXB-22</td>
<td>101 μl</td>
<td>15.3 ml/min</td>
</tr>
<tr>
<td>B6</td>
<td>+ B6</td>
<td>136 μl</td>
<td>16.2 ml/min</td>
</tr>
<tr>
<td>B6</td>
<td>+ AXB-1</td>
<td>138 μl</td>
<td>18.7 ml/min</td>
</tr>
<tr>
<td>B6</td>
<td>– BXA-24‡</td>
<td>150 μl</td>
<td>24 ml/min</td>
</tr>
</tbody>
</table>

Strains listed from lowest to highest value. Periodic breathing (PB) is as defined in the text. +, Presence of PB; –, absence of PB. *Breathing appeared regularly irregular but did not meet criteria for PB.
Human and animal studies have provided evidence supporting the importance of hypocapnia in the genesis of hypoxia-induced PB (11, 38). In the awake goat (16) and sleeping human (4), STP was largely eliminated and PB develops, if posthypoxic hypocapnia is permitted. However, in the present study, the presence or absence of PB in B6 and A/J mice occurred independent of the hypoxic hypocapnia. Therefore, the difference in posthypoxic ventilatory behavior between the two strains cannot be easily attributed to a difference in arterial partial pressure of CO2.

Oxygen administration seems to have a paradoxical effect on breathing stability. First, according to the predicted model, inhalation of oxygen would depress the carotid body activity and, therefore, should stabilize breathing and eliminate or attenuate PB. This is supported by most experimental and clinical studies (3, 13). Second, some researchers reported the persistence of PB despite O2 administration. Wilkinson et al. (43) could induce PB just by switching inspired gas from air to hyperoxia in lambs but could not produce PB during air breathing after passive hyperventilation. This demonstration supports the idea that inhalation of hyperoxia against a background of hypoxia promotes instability in the respiratory controller. In the present study, the difference of M in B6 mice between air and hyperoxia reoxygenation, and the posthypoxic breathing change in A/J mice during different reoxygenation procedures, clearly indicates that the oxygen level of reoxygenation gas had a significant influence on posthypoxic ventilatory behavior in both strains. However, 100% O2 reoxygenation neither made PB in B6 mice, which did so during air reoxygenation disappear, nor provoked PB in A/J mice, which had no PB during air reoxygenation. Thus the occurrence of strain-related posthypoxic PB may not depend on the oxygen level of the reoxygenation gas.

Increased time delay and decrease in damping lung volume may also be involved in the genesis of PB, but these mechanisms are unlikely to play major roles in mice because hypoxia increases cardiac output and shortens circulation time, and end-expiratory lung volume may increase with hypoxia (6).

From the present findings, inheritance is a mechanism that might produce a difference in neural networks, which act to influence ventilatory stability and produce PB. It is known that there are significant intrastrain differences in hypoxic responsiveness in mice (36), and a limited number of genes may influence the level of ventilation on hypoxia (35). Proof of the principle of genetic transmission of posthypoxic ventilatory behavior is also supported by observations by Kline et al. (25), who reported respiratory depression in response to brief hyperoxia was pronounced in wild-type mice but nearly absent in nitric oxide synthase (NOS) III mutant mice. A second study (26) indicated that NOS I might also be involved in an unstable breathing pattern during hypoxia, again in mice. Jacob and Thach (22) reported that, in a spontaneous recovery from hypoxic apnea, prolonged but ineffective gasping was more often seen in SWR mice, whereas gasping was absent in SW mice. The present study used inbred B6 and offspring (B6 × A/J) inbred recombinant mice to confirm an inherited basis for PB in the adult mouse.

Thus genetic influences act to influence the expression of PB in mice. The questions of relative strength of the genetic components and the identification of the genes, proteins, and systems that produce ventilatory dysrhythmia in the B6 warrant further investigation.

Fig. 7. Top: recording from a B6 animal in regard to ventilatory behavior ~30 s after being exposed to 100% oxygen. There was no prior exposure to hypoxia. Bottom: periodic behavior seen in top tracing in regard to f (left), Vr (middle), and Ve (right). Tc = 1.35 ± 0.04 s; M = 1.82 ± 0.03; Ta = 0.75 ± 0.03 s.
PERIODIC BREATHING IN THE MOUSE

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